Chemico-Biological Interactions 297 (2019) 34–43

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

# The soluble curcumin derivative NDS27 inhibits superoxide anion production by neutrophils and acts as substrate and reversible inhibitor of myeloperoxidase

Thierry Franck[a,b,*], Iyas Aldib[d], Karim Zouaoui Boudjeltia[c], Paul G. Furtmüller[e], Christian Obinger[e], Philippe Neven[f], Martine Prévost[g], Jalal Soubhye[d], Pierre Van Antwerpen[d], Ange Mouithys-Mickalad[b], Didier Serteyn[a,b]

[a] Department of Clinical Sciences, Anesthesiology and Equine Surgery, Faculty of Veterinary Medicine, B 41, University of Liege, Sart Tilman, Liège, Belgium
[b] Centre of Oxygen, Research and Development-CIRM, Institute of Chemistry B 6a, University of Liege, Sart Tilman, Liège, Belgium
[c] Laboratory of Experimental Medicine, Université Libre de Bruxelles (ULB 222), CHU de Charleroi, Hôpital Vésale, Montigny-le-Tilleul, Belgium
[d] Laboratory of Pharmaceutical Chemistry & Analytical Platform of the Faculty of Pharmacy, Faculty of Pharmacy, Université Libre de Bruxelles, Brussels, Belgium
[e] Department of Chemistry, Division of Biochemistry, BOKU, University of Natural Resources and Life Sciences, Vienna, Austria
[f] Laboratory of Medicinal Chemistry-CIRM, Faculty of Pharmacy, B36, CHU Liège, Liège, Belgium
[g] Structure and Function of Biological Membranes, Faculty of Sciences, Université Libre de Bruxelles, Brussels, Belgium

ARTICLE INFO

*Keywords:*
Water soluble curcumin
Hydroxypropyl-β-cyclodextrin
Neutrophils
Myeloperoxidase
Reversible inhibitor

ABSTRACT

A water-soluble curcumin lysinate incorporated into hydroxypropyl-β-cyclodextrin (NDS27) has been developed and shown anti-inflammatory properties but no comparative study has been made in parallel with its parent molecule, curcumin on polymorphonuclear neutrophils (PMNs) and myeloperoxidase (MPO) involved in inflammation. The effect of NDS27, its excipients (hydroxypropyl-β-cyclodextrin and lysine), curcumin lysinate and curcumin were compared on the release of superoxide anion by PMNs using a chemiluminescence assay and on the enzymatic activity of MPO. It was shown that curcumin and NDS27 exhibit similar inhibition activities on superoxide anion release by stimulated PMNs but also on MPO peroxidase and halogenation activities. The action mechanism of curcumin and NDS27 on the MPO activity was refined by stopped-flow and docking analyses. We demonstrate that both curcumin and NDS27 are reversible inhibitors of MPO by acting as excellent electron donors for redox intermediate Compound I ($\sim 10^7\ M^{-1}\ s^{-1}$) but not for Compound II ($\sim 10^3\ M^{-1}\ s^{-1}$) in the peroxidase cycle of the enzyme, thereby trapping the enzyme in the Compound II state. Docking calculations show that curcumin is able to enter the enzymatic pocket of MPO and bind to the heme cavity by π-stacking and formation of hydrogen bonds involving substituents from both aromatic rings. Hydroxypropyl-β-cyclodextrin is too bulky to enter MPO channel leading to the binding site suggesting a full release of curcumin from the cyclodextrin thereby allowing its full access to the active site of MPO. In conclusion, the hydroxypropyl-β-cyclodextrin of NDS27 enhances curcumin solubilization without affecting its antioxidant capacity and inhibitory activity on MPO.

## 1. Introduction

Curcumin or diferuloylmethane is the main pigment present in the rhizome of *Curcuma longa*. This polyphenolic lipophilic molecule was used for centuries in Chinese and Indian medicine for the treatment of inflammatory pathologies [1] since it exhibits a large range of biological activities including anti-inflammatory, anti-angiogenic, anti-oxidant, wound healing and anti-cancer properties [2]. Thanks to the beneficial effect of curcumin for health, numerous strategies have been followed to improve its bioavailability and delivery. Indeed, the low solubility of curcumin in water, its rapid metabolization and elimination from the body constitute the major obstacles in the medical application [3]. In addition, curcumin undergoes rapid non-enzymatic degradation in cell culture medium and possibly *in vivo* as well [4] and more and more studies are interested in the degradation products of curcumin that can explain its polypharmacology [4,5]. Moreover,

* Corresponding author. Department of Clinical Sciences, Anesthesiology and Equine Surgery, Faculty of Veterinary Medecine, B 41, University of Liege, Sart Tilman, Liège, Belgium.
*E-mail address:* t.franck@uliege.be (T. Franck).

https://doi.org/10.1016/j.cbi.2018.10.008
Received 14 June 2018; Received in revised form 10 October 2018; Accepted 17 October 2018
Available online 18 October 2018
0009-2797/ 

curcumin does not have the adequate structural and chemical features of a lead compound for further drug optimization. One of the strategies to overcome these disadvantages is complexing of curcumin with other molecules to improve its medical application [3].

We have developed a highly water-soluble lysine salt of curcumin (curcumin lysinate), incorporated into hydroxypropyl-β-cyclodextrin (HPβCD), called NDS27 [6]. Similarly to curcumin [7–9], NDS27 has been shown to inhibit the NADPH oxidase activity and consequently the oxidative burst of neutrophils [10]. We suggested that NDS27 binds to the membrane due to the lipophilic properties of both curcumin and HPβCD and interferes with the assembly of NADPH oxidase [11]. Moreover, we showed that NDS27 inhibited dose dependently the activity of MPO released by cytochalasin B/N-formyl-methionyl-leucyl-phenylalanine (CB/fMLP) stimulated neutrophils whereas HPβCD alone had no effect [10]. Sandersen et al. [12] also observed that NDS27 reduced MPO degranulation and activity in broncho-alveolar lavage fluid of horses with LPS-induced lung neutrophilia.

These promising results in regards to control inflammatory response prompted us to compare and analyse the effect of curcumin, curcumin lysinate, NDS27 and its excipients lysine and HPβCD on superoxide anion production by stimulated neutrophils and MPO activity. In detail, we aimed at analysing: (i) the stability of curcumin and NDS27 in the working buffer condition (PBS, pH 7.4) and NaCl 0.9% (pH 5.5), (ii) the effect of these compounds on the peroxidase and chlorination activity of MPO, (iii) their impact on the interconversion of the redox intermediates Compound I and Compound II of MPO by multi-mixing stopped-flow spectroscopy and (iv) the binding of curcumin and NDS27 to the active site of MPO by SIEFED and molecular docking.

## 2. Material and methods

### 2.1. Chemicals and reagents

Analytical grade phosphate salts, sodium and potassium chloride, sodium hydroxide, sodium acetate, $H_2O_2$ (30%) and Tween 20 were purchased from Merck (VWR International, Leuven, Belgium). Highly purified dimeric leukocyte human myeloperoxidase with a purity index ($A_{428}/A_{280}$) of at least 0.85 was purchased as lyophilized powder from Planta Natural Products (http://www.planta.at). The bovine serum albumin fraction V (BSA) was obtained from Roche Diagnostics (Mannheim, Germany). Catalase, lucigenin, phorbol myristate acetate, sodium nitrite, taurine were from Sigma-Aldrich (Bornem, Belgium). The 96-well microtiter plates (Combiplate 8 EB) were from Thermo Scientific (Breda, The Netherlands), the fluorogenic substrate, Amplex red (10-acetyl-3,7-dihydroxyphenoxazine) from Molecular Probes (Invitrogen) (Merelbeke, Belgium). The PBS solution contained 20 mM phosphate buffer, pH 7.4, 137 mM NaCl, and 2.7 mM KCl. Sterile sodium chloride injection (0.9%), pH 5.5 was from Baxter Belgium SPRL, NDS27, curcumin lysinate, curcumin (99% purity), lysine base and 2-hydroxypropyl-beta-cyclodextrin (HPβCD) were all a gift from Philippe Neven (Faculty of Pharmacy, University of Liege, Belgium).

The preparation of curcumin lysinate and NDS27 were described in Neven et al. [6]. Briefly, curcumin in methanol and lysine base in water both at equimolar concentration (1.36 mM) were mixed together during 5 min then the mixture was evaporated. The non-dissolved residue was taken back with ethanol and was brought to boiling point then filtered. The ethanol-based solution was placed at - 20 °C for 1 hh and the precipitate of curcumin lysinate was collected by filtration. NDS27 was prepared as follows: 4 gg of HPβCD (Roquette SA) were solubilized in 50 mL distilled water then 514 mg of curcumin lysinate were added in one movement and under vigorous stirring. Distilled water (40 mL) was added to the solution and stirred for 60 min. The solution was filtered and the content of curcumin was checked by HPLC. All concentrations of NDS27 are expressed as molar concentration of pure curcumin and contain a 5-fold stoichiometric excess of HPβCD.

### 2.2. Solubilization of the compounds and preparation of the stock solutions

Curcumin and curcumin lysinate were dissolved in DMSO while NDS27 and its excipients were dissolved in distilled $H_2O$ at the starting stock solution $10^{-2}$ M. From this solution, serial dilutions were performed in the respective solvent to obtain $5.10^{-3}$ M, $10^{-3}$ M, $5.10^{-4}$ M and $10^{-4}$ M. The same volume of these stock solutions was added to PBS or NaCl 0.9% to be used at final concentration of 1 μM, 5 μM, 10 μM, 50 μM and 100 μM during the experiments. HPβCD was provided in a 5-fold stoichiometric excess. Controls were carried out by adding the appropriate solvents (distilled $H_2O$ or DMSO being both at final concentration 1%), which did not contain the tested molecules.

### 2.3. Transformation of curcumin and NDS27

Curcumin degrades quickly at physiological pH [4]. Due to the incubation time of 10 min or 2 h of the molecules with MPO before performing the measurement of MPO activity by the classical or SIEFED assays respectively, the possible transformation of the molecules was followed after 10 min and 2 h in PBS buffer (pH 7.4) or NaCl 0.9% (pH 5.5). The loss of chromophore of curcumin was followed at 430 nm and the possible appearance of an oxidized intermediate (spiroepoxide intermediate) was followed at 265 nm according to Luis et al. [13]. Ten μL of $10^{-2}$ M curcumin in DMSO or NDS27 in water were added to 1.990 mL 50 mM phosphate buffer saline (PBS) at pH 7.4 or NaCl 0.9% to reach a final concentration of 50 μM. The reaction was monitored in a UV-vis spectrophotometer directly after the dilution in PBS or NaCl 0.9% then the spectrum modifications were followed after 2 h incubation at 37 °C in darkness. Absorbances values were recorded simultaneously at 430 nm and 265 nm according to Luis et al. [13].

### 2.4. Measurement of superoxide anion release by activated neutrophils

Blood was obtained from the jugular vein of healthy horses, which were fed and bred under identical conditions and were not under medical treatment (Faculty of Veterinary Medicine, University of Liège, Belgium). Neutrophils were isolated at room temperature using a discontinuous percoll density gradient according to the method of Pycock et al. [14]. The cell pellets were suspended in 20 mM phosphate buffer saline (PBS) at pH 7.4 containing 137 mM NaCl and 2.7 mM KCl. The cell preparation contained 90% neutrophils with a viability of 95% as measured by the Trypan blue exclusion test. Each experiment was repeated three times with different cell batches from different horses.

The superoxide anion production by activated neutrophils was measured by chemiluminescence (CL) according to Franck et al. [8]. Neutrophils ($10^6$ neutrophils/well) were incubated for 10 min in the well of a white microtiter plate (White Combiplate 8, Thermo Labsystems) with various concentrations of curcumin, curcumin lysinate, NDS27, lysine and HPβCD or solvent only. Finally, 25 μL $CaCl_2$ (7.5 μM), 2 μL lucigenin (5 μM) and 10 μL phorbol 12-myristate-acetate (PMA) (16 μM) were added. Just after PMA addition, the CL response was monitored during 30 min and expressed as the integral value of the total CL emission. Controls were performed with neutrophils incubated with the respective solvents which did not contain the tested molecules (distilled $H_2O$ or DMSO being both at final concentration 1% during the assay).

### 2.5. Determination of myeloperoxidase activity

In the SIEFED (Specific Immunologic Extraction Followed by Enzymatic Detection) assay [15], curcumin, curcumin lysinate or NDS27 were pre-incubated for 10 min with MPO (5 mU/ml) in 50 mM PBS pH 7.4. This mixture was loaded (100 μLl/well) onto the microplate coated with rabbit anti-human MPO antibodies and incubated 2 h at 37 °C. For controls only the solvents were added, respectively. Next, the supernatant was carefully removed and the plate drained on a

paper towel to remove any remaining liquid. Captured MPO was washed four times with the PBS solution containing 0.1% Tween 20. Finally, the peroxidase activity of MPO was monitored by adding 100 µL of a 40 µM Amplex red solution freshly prepared in 50 mM phosphate buffer, pH 7.4, supplemented with 10 µM $H_2O_2$ and 10 mM sodium nitrite. The reaction was followed by fluorimetry. Total fluorescence developed during 30 min (37 °C) was monitored using a Fluoroskan Ascent (Thermo Scientific) set at 544 nm and 590 nm for excitation and emission wavelengths, respectively. Total fluorescence was directly proportional to the amount of active MPO present in the sample.

Additionally, the activity of MPO was measured by a classical enzymatic assay at the same concentrations used in the SIEFED assay. Briefly, curcumin, curcumin lysinate or NDS27 were dissolved in their respective solvent and added to MPO (10 mU/mL) in 50 mM phosphate buffer, pH 7.4, to reach the desired final concentrations. After 10 min of incubation, 100 µL of these mixtures were loaded into the wells of a microplate, and the peroxidase activity of MPO was detected by adding 100 µL of the reagent solution containing Amplex red, $H_2O_2$ and nitrite in 50 mM phosphate buffer, pH 7.4. The reaction was followed by fluorimetry as described above.

Due to the suspected transformation of curcumin in PBS buffer at physiological pH, the SIEFED and classical enzymatic assays were also performed with curcumin and NDS27 diluted in sterile NaCl 0.9% having a more acidic pH (pH 5.5) and without a 10 min preincubation time of the molecules with MPO before loading of the microplates. Moreover, for the SIEFED assay, the washing step was performed with the NaCl solution (0.9%) containing 0.1% tween 20.

Finally, the myeloperoxidase-chlorinating activity was determined. The assay is based on the production of taurine chloramine produced by the MPO/$H_2O_2$/$Cl^-$-system in the presence of a selected inhibitor at a defined concentration. The reaction mixture contained the following reagents in a final volume of 200 µL: 10 mM phosphate buffer (pH 7.4, 300 mM NaCl), 15 mM taurine, the compound to be tested at 1, 2, 2.5, 5, 7.5, 9 and 10 µM and a fixed amount of recombinant MPO (40 nM). When necessary, the volume was adjusted with water. This mixture was incubated at 37 °C and the reaction was initiated with 10.0 µL of $H_2O_2$ (100 µM). After 5 min, the reaction was stopped by the addition of 10 µL of catalase (8 units/µL). To determine the amount of taurine chloramine production, 50 µL of 1.35 mM solution of thionitrobenzoic acid (TNB) was added and the volume was adjusted to 300 µL with water. Then the absorbance of the solutions was measured at 412 nm with a microplate reader, and the curve of absorbance as a function of inhibitor concentration was plotted. $IC_{50}$ values were then determined by standard procedures, considering the absence of hydrogen peroxide as 100% inhibition and the absence of inhibitors as 0% inhibition [16,17].

### 2.6. Stopped-flow analysis

Concentrations of myeloperoxidase and hydrogen peroxide were calculated by spectrometry at 428 nm ($\varepsilon\varepsilon_{428}$ = 91 $mM^{-1}$ $cm^{-1}$) and 240 nm ($\varepsilon\varepsilon_{240}$ = 39.4 $M^{-1}$ $cm^{-1}$), respectively [18]. Curcumin stock solutions were prepared in DMSO and stored in dark flasks. Dilution was performed with 100 mM phosphate buffer, pH 7.0. Highest DMSO concentration in stopped-flow measurements was 8% (v/v). NDS27 was resolved in water.

Because of the inherent instability of MPO Compound I, the sequential stopped-flow (multi-mixing) technique was used for determination of apparent bimolecular rate constants of the reaction of Compounds I and II with curcumin or NDS27. For Compound I formation ferric MPO was mixed with a 10-fold excess of hydrogen peroxide. Typically, MPO (8 µM) was premixed with 80 µM $H_2O_2$ in the aging loop for 20 ms (100 mM phosphate buffer, pH 7.0). Finally, MPO Compound I was allowed to react with varying concentrations of curcumin or NDS27. Reactivity of Compound II was investigated by following the reaction of Compound I with curcumin or NDS27 to Compound II and back to the ferric enzyme. In this case the resulting biphasic curves at 456 nm and 430 nm reflected the initial formation of Compound II and then its subsequent reduction. The initial exponential increase and after a short steady-state phase the following exponential decrease in absorbance at 456 nm were fitted to calculate the apparent bimolecular rate constants for Compound I and Compound II reduction.

### 2.7. Docking calculations

The X-ray structure of human myeloperoxidase complexed to 2-{[3,5-Bis (Trifluoromethyl)benzyl]amino}-*N*-Hydroxy-6-Oxo-1,6-Dihydropyrimidine-5-Carboxamide (HX1) (RCSB code 4C1M) was used as the receptor target in docking studies [18]. The X-ray water and other ligand molecules were removed from the active site. The ligand input files were prepared according to the following procedure. The initial 3D structures of curcumin were generated with the LigPrep module from Schrödinger (Schrödinger, LLC, New York, NY, 2017). Docking was performed with the Glide program (version 7.4, Schrödinger, LLC, New York, NY, 2017), which approximates a systematic search of positions, orientations, and conformations of curcumin at the active site of MPO by use of a series of hierarchical filters. The Glide XP docking protocol and scoring function were used. The remaining parameters were set to their default values. Preparation of hydroxypropyl-β-cyclodextrin with LigPrep turned out to be impossible as its atom number is beyond the limit imposed by the module and β-cyclodextrin (CD) was used instead. Docking was computed in regions centered on different locations along the channel connecting the MPO binding site pocket to its molecular surface.

### 2.8. Statistical analysis

Data are presented in relative values (%) in reference to appropriate control groups (DMSO or $H_2O$) defined as 100%. All data are expressed as mean ± standard deviation (SD) of at least three independent experiments carried out at least in duplicate. The statistical analysis was performed with GraphPad 6.0 (GraphPad Software, San Diego California, USA) with one way ANOVA (Dunnett's post test). The results for the tested molecules were compared to control $H_2O$ or DMSO. The $IC_{50}$ values for the enzymatic assay of MPO were calculated with GraphPad Prism 6.0 under application of the function "log (inhibitor) vs. normalized response-variable slope" after converting the values into their decimal logarithms.

## 3. Results

### 3.1. Curcumin and NDS27 transformation

The spectra of curcumin obtained just after the dilution of the molecule in PBS or NaCl 0.9% (T0) showed two major peaks having a maximum of absorbance values at 265 nm and 430 nm. Just after the dilution of curcumin (orange powder) and NDS27 (purple powder) in PBS, both resulting coloured solutions appeared yellow. In contrast, when the saline solution 0.9% was used instead of PBS, the solution obtained finally was yellow and slightly cloudy for 100 µM curcumin and purple for 100 µM NDS27.

In PBS (pH 7.4), the loss of chromophore at 430 nm occurred rapidly. After 10 min at 37 °C in darkness, the loss was −60 ± 3% for curcumin and −49 ± 8% for NDS27 in comparison to T0. After 2 h incubation this loss was accentuated and reached −84 ± 1% for curcumin and −79 ± 3% for NDS27 (n = 3).

In NaCl 0.9% (pH 5.5), the loss of chromophore at 430 nm was slower. After 10 min, the loss was −18 ± 2% for curcumin and −11 ± 4% for NDS27 in comparison to T0, while after 2 h, the loss was more accentuated and reached −57 ± 5% for curcumin and −59 ± 4% for NDS27 (n = 3).

Regarding the absorbance intensity at 265 nm in PBS, after 10 min incubation at 37 °C, an increase of absorbance was observed for

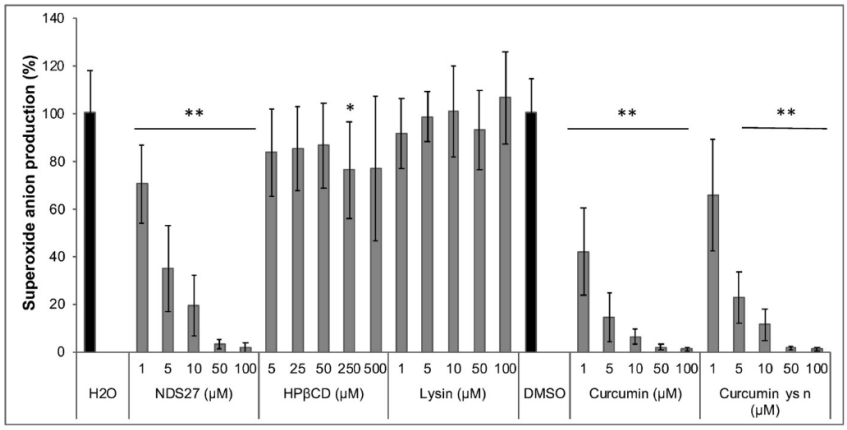


**Fig. 1. NDS27 and curcumin inhibit extracellular superoxide anion production by neutrophils.** Effect of NDS27, curcumin, curcumin lysinate, HPβCD and lysin on superoxide anion release by equine neutrophils stimulated with PMA. 100% activity corresponds to superoxide anion release by PMNs in the presence of solvent ($H_2O$ DMSO) only (mean ± SD, n ≥ 5). **p < 0.01; *p < 0.05 vs $H_2O$ or DMSO control.

curcumin (+28 ± 6%) and NDS27 (+20 ± 3%) in comparison to T0. After 2 h incubation, the intensity of the peak decreased but stayed higher than T0: +11 ± 3% for curcumin and +7 ± 4% for NDS27 (n = 3).

When the molecules were diluted in NaCl 0.9%, a decrease of absorbance at 265 nm was observed for curcumin (−12 ± 2%) and NDS27 (−5 ± 02%) in comparison to T0 while after 2 h the loss of the absorbance value was accentuated for curcumin (−44 ± 8%) and remained quite unchanged for NDS27 (3.6 ± 5.1%) in comparison to T0 (n = 3).

### 3.2. Impact of curcumin and NDS27 on superoxide anion production by PMNs

First we probed the impact of curcumin and NDS27 on the superoxide anion release by neutrophils stimulated by PMA using lucigenin as chemiluminescent probe. The effect of these compounds was related to the response obtained with stimulated PMNs in the presence of the respective solvent (DMSO or $H_2O$) (=100% activity). Fig. 1 shows that NDS27, curcumin and curcumin lysinate induced a dose dependent inhibitory effect on superoxide anion production following the hierarchy curcumin ($IC_{50}$ = 0.72 ± 0.24 µM) < curcumin lysinate ($IC_{50}$ = 1.76 ± 0.41 µM) < NDS27 ($IC_{50}$ = 2.52 ± 0.71 µM). The $IC_{50}$ of curcumin differs significantly from that of curcumin lysinate and NDS27 ($p < 0.05$). HPβCD showed a slight but significant inhibitory effect at 100 µM, whereas lysin had no impact on the superoxide anion formation (Fig. 1).

### 3.3. Curcumin and NDS27 inhibit the peroxidase and chlorination activity of myeloperoxidase

Next, we tested the influence of curcumin and NDS27 on the peroxidase activity of MPO (Fig. 2). The percentage of inhibition was related to the activity tested in the presence of the respective solvent ($H_2O$ or DMSO) added to PBS (=100%). The results obtained with the classic enzymatic test showed a dose dependent inhibition of MPO peroxidase activity by NDS27 ($IC_{50}$ = 4.9 ± 0.5 µM), curcumin ($IC_{50}$ = 4.5 ± 0.5 µM), and curcumin lysinate ($IC_{50}$ = 6.0 ± 0.5 µM). The $IC_{50}$ of NDS27 and curcumin differ significantly from that of curcumin lysinate ($p < 0.05$). No significant difference was observed between the IC50 of curcumin and NDS27. Lysine and HPβCD had no impact on MPO activity (Fig. 2A).

Similarly, the SIEFED assay (that includes immunocapture of MPO and washing steps after incubation of MPO with the potential inhibitors) showed a dose dependent decrease of MPO activity in the presence of NDS27 ($IC_{50}$ = 77.4 ± 16.5 µM) curcumin ($IC_{50}$ = 75.7 ± 32.7 µM) and curcumin lysinate ($IC_{50}$ = 42.17 ± 13.5) respectively. At higher concentrations (> 50 µM) curcumin lysinate exhibited an even higher inhibitory effect than curcumin and NDS27 (Fig. 2B). No significant difference was observed between the IC50 of curcumin and NDS27. Lysine had no impact on MPO activity, whereas in this assay HPβCD at 500 µM significantly ($p < 0.05$) decreased the activity of MPO by about 30% most probably by interfering on the immunocapture step.

In the SIEFED assay, the excess of the tested molecule was eliminated by washing. Thus, the final concentration of the tested molecule after washing was drastically diminished in comparison to the classical assay and only the molecules bound to the active site of MPO can disturb the activity of MPO probably by hindering the access of the substrates to the active site. In the classical assay, the initial concentration of the molecule was always present during the revelation of the enzyme and this assay provides information on the competitive reactions between the drugs and reagents (nitrite, Amplex Red) used for the peroxidase reaction, or on the interactions between the drug, substrates, and the reaction products [15]. The most intense inhibition of curcumin and NDS27 on MPO activity observed with the classical assay suggest that the molecules act mainly as electron donors for Compound I and Compound II, without staying in the enzymatic pocket and thus being in competition with nitrite and Amplex Red for the peroxidase cycle of MPO (Fig. 4). However, at higher concentrations, some molecules of curcumin or NDS27 have the ability to enter into the enzymatic pocket of the enzyme, bind to the active site and resist to the washing step during the SIEFED assay.

The Classical and SIEFED assays were also performed with curcumin, NDS27 and its excipients diluted in NaCl 0.9% (Fig. 3). The percentage of inhibition was related to the activity tested in the presence of the respective solvent ($H_2O$ or DMSO) added to NaCl 0.9% (=100%). The results obtained with the classic enzymatic test showed a dose dependent inhibition of MPO peroxidase activity by NDS27 ($IC_{50}$ = 4.0 ± 0.3 µM) and curcumin ($IC_{50}$ = 3.3 ± 0.3 µM). The $IC_{50}$ of NDS27 and curcumin differ significantly ($p < 0.01$). Lysine and HPβCD had no impact on MPO activity (Fig. 3A).

The SIEFED showed a dose dependent decrease of MPO activity in the presence of NDS27 ($IC_{50}$ = 4.8 ± 1.4 µM) diluted in NaCl 0.9% (Fig. 3B). This inhibition appeared more intense than that observed in PBS buffer. In comparison to the control with DMSO, curcumin showed a significant inhibition from the concentration of 10 µM but the inhibition profile was not dose dependent and a strong inhibition (~90%) was only observed at 100 µM. Lysine and HPβCD had no impact on MPO activity.

Finally, we probed the impact of curcumin and NDS27 on the chlorination activity of MPO. A dose dependent inhibition of this activity was observed with calculated $IC_{50}$ values of 4.2 ± 0.03 µM (curcumin) and 4.9 ± 0.05 µM (NDS27), respectively (data not shown).

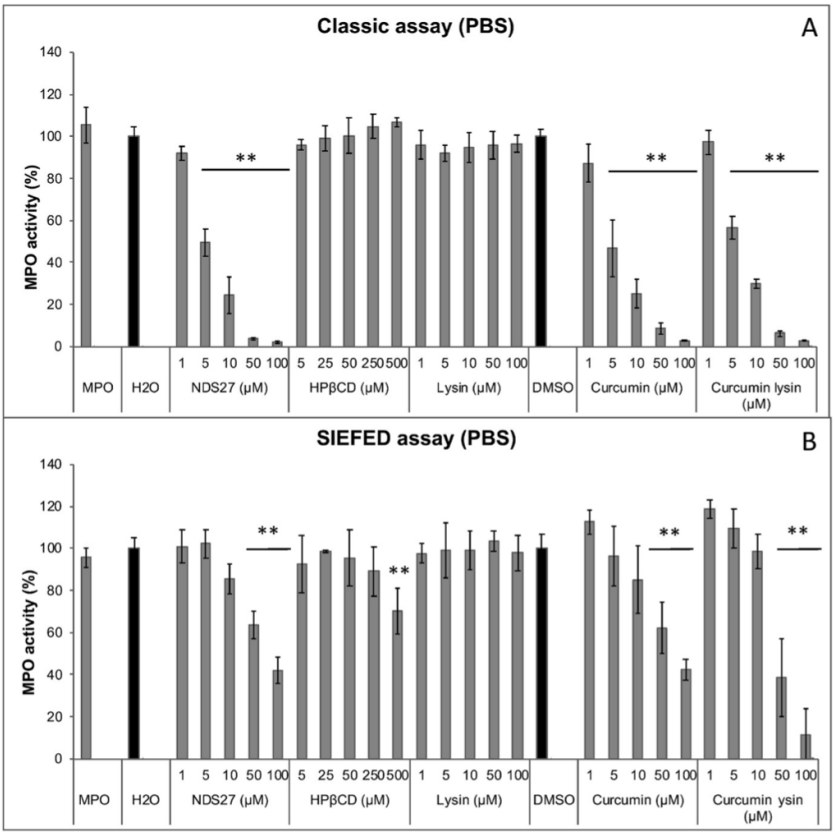


**Fig. 2. NDS27 and curcumin diluted in PBS (ppHH 7.4) inhibit the peroxidase activity of MPO.** Effect of NDS27, curcumin, curcumin lysinate, HPβCD and lysin on myeloperoxidase activity, measured by (A) a classic enzymatic assay and (B) by SIEFED (with MPO immunocapture and washing steps). 100% activity refers to MPO activity in the presence solvent ($H_2O$ or DMSO) only (mean ± SD, n ≥ 5). **p < 0.01 vs $H_2O$ or DMSO control.

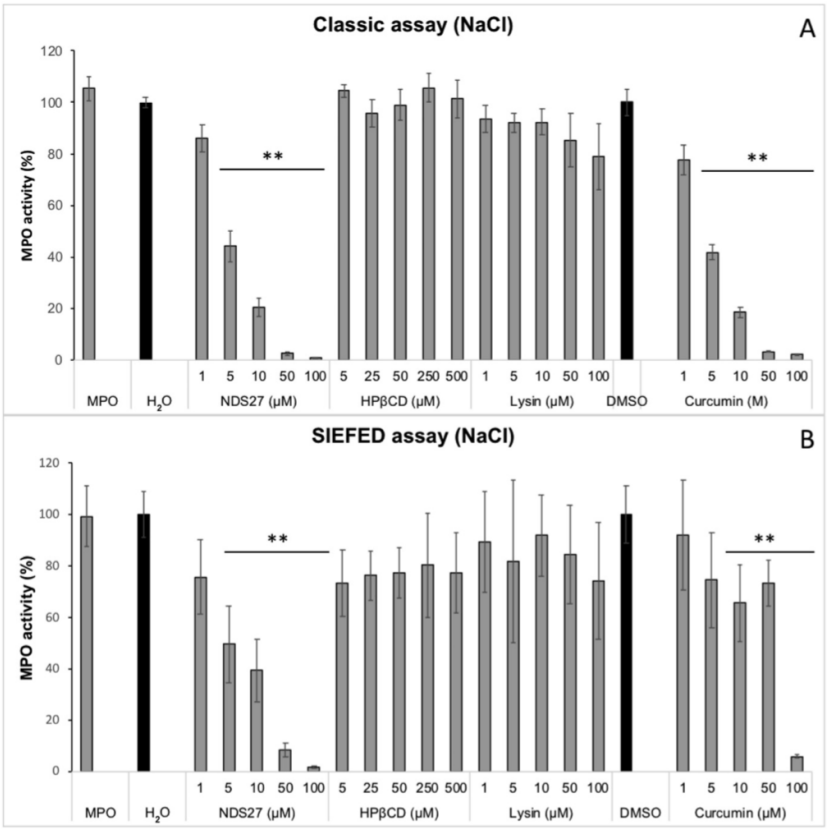


**Fig. 3. NDS27 and curcumin diluted in 0.9% NaCl (ppHH 5.5) inhibit the peroxidase activity of MPO.** Effect of NDS27, curcumin, HPβCD and lysin on myeloperoxidase activity, measured by (A) a classic enzymatic assay and (B) by SIEFED (with MPO immunocapture and washing steps). 100% activity refers to MPO activity in the presence solvent ($H_2O$ or DMSO) only (mean ± SD, n = 5). **p < 0.01 vs $H_2O$ or DMSO control.

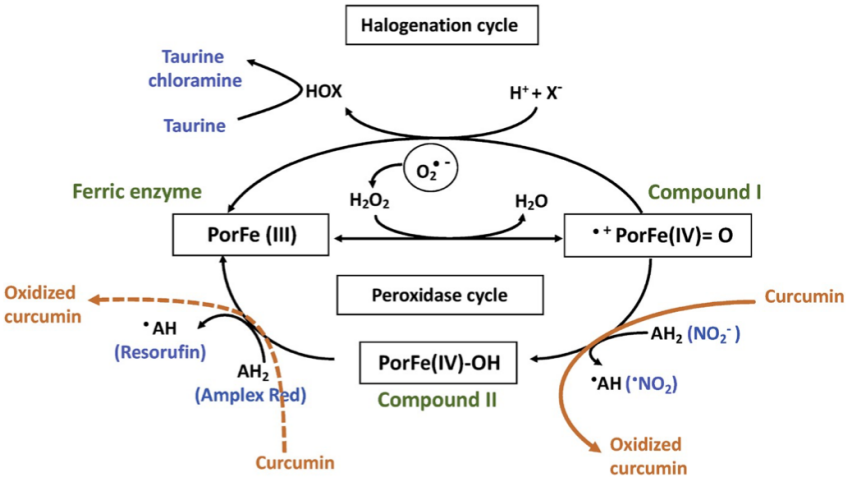


**Fig. 4. Scheme of the chlorination and peroxidase cycle of myeloperoxidase (MPO).** In the halogenation cycle MPO is oxidized by hydrogen peroxide to Compound I which catalyzes the two-electron oxidation of halides ($X^-$) to the corresponding hypohalous acids (HOX). In the peroxidase cycle Compound I catalyzes the one-electron oxidation of typically aromatic substrates ($AH_2$) to the corresponding radicals ($^{\bullet}AH$). Compound I is reduced to Compound II which is reduced to the resting state by oxidizing another $AH_2$ molecule. In blue colour, the substrates used in this study for chlorination (taurine) and peroxidase activity (nitrite and Amplex red) of MPO and their resulting oxidized product: taurine chloramine, nitrogen dioxide ($^{\bullet}NO_2$), and the fluorescent compound, resorufin. In orange, curcumin (or NDS27) competes with the substrates used for measuring the peroxidase activity and it acts as a good electron donor for Compound I (unbroken orange arrow) but as a poor electron donor for Compound II (dashed orange arrow).

### 3.4. Curcumin and NDS27 promote compound II accumulation

In order to determine the mechanism of action of curcumin and formulated curcumin on MPO activity, a pre-steady-state kinetic study was performed using multi-mixing stopped-flow spectroscopy. During turnover native ferric MPO [PorFe(III)] is oxidized by $H_2O_2$, thereby producing water and Compound I {oxoiron(IV) combined with a porphyrin (Por) π-cation radical: [$^{+\bullet}$PorFe(IV) = O].

In the halogenation cycle Compound I is reduced directly back to the resting state by chloride [or other (pseudo-) halides] thereby releasing hypochlorous acid. Alternatively, in the presence of one-electron donors the peroxidase pathway is followed, including Compound I reduction to Compound II [PorFe(IV)—OH] and Compound II reduction to ferric MPO (Fig. 4).

In the present study Compound I was preformed and probed for reaction with curcumin or NDS27. Results obtained with NDS27 are illustrated in Fig. 5. With both compounds there was a direct and fast transition of Compound I to Compound II (Soret maximum at 456 nm) with clear isosbestic points (Fig. 5A). The reactions were monophasic (inset to Fig. 5A). At high curcumin concentrations the absorbance of curcumin did not allow determination of reliable $k_{obs}$ values. The apparent bimolecular rate constants of Compound I reduction has been estimated to be $9 \times 10^6\,M^{-1}\,s^{-1}$ (curcumin) and $9.5 \times 10^6\,M^{-1}\,s^{-1}$ (NDS27) suggesting that both compounds are excellent electron donors for Compound I. By contrast, both curcumin ($7 \times 10^2\,M^{-1}\,s^{-1}$) and NDS27 ($1.7 \times 10^3\,M^{-1}\,s^{-1}$) are only slowly oxidized by Compound II (Fig. 5B). This big difference of more than four orders of magnitude between the apparent rate constants of Compound I and Compound II reduction is also observed by inspecting the time traces at 456 nm (inset to Fig. 5B) that show a lag-phase that strictly depended on the concentration of $H_2O_2$ in the system. After complete hydrogen peroxide consumption (third phase) Compound II was converted back to the resting state (Fig. 5B) in a concentration dependent manner.

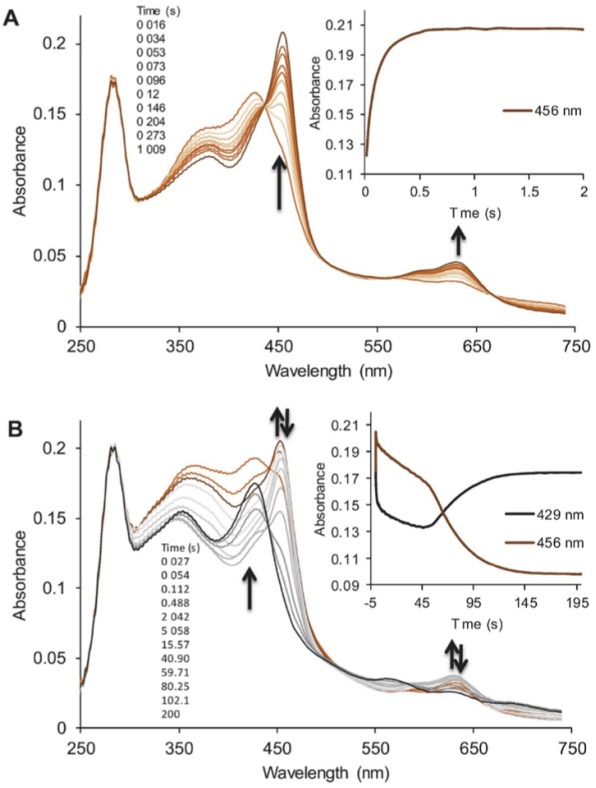


**Fig. 5. NDS27 and curcumin promote Compound II accumulation.** Reaction of MPO Compound I and Compound II with NDS27. (A) Spectral changes upon mixing 2 μM MPO Compound I with 5 μM NDS27 (pH 7.0 and 25 °C). Inset shows a typical monophasic time trace at 456 nm. (B) Spectral changes upon mixing 2 μM Compound I with 25 μM NDS27 (pH 7.0 and 25 °C). Inset shows typical multiphasic time traces followed at 456 nm and 429 nm respectively that indicates Compound II formation followed by Compound II reduction to ferric enzyme. Arrows show the direction of the spectral changes.

### 3.5. Both aromatic rings of curcumin are involved in binding to MPO

Finally, we probed the mode of binding of curcumin to the active site of MPO by molecular docking (Fig. 6). The docking calculations were performed in the crystal structure of MPO in complex with HX1, a trifluoromethyl-substituted aromatic hydroxamate, which is known to inhibit MPO [18]. HX1 forms different non-covalent interactions including a π-stacking of its pyridine ring onto the heme pyrrole ring D. The pyrimidine also forms an interaction via one of its nitrogens with the heme propionate group and hydrogen bond to Arg239 and His95 with its hydroxamic acid group [19]. The trifluoromethyl aromatic ring of HX1 occupies a hydrophobic pocket located at the entrance of the active site.

Curcumin showed a similar stacking position between one of its aromatic rings and the pyrrole ring D (Fig. 6). In the diketone structure

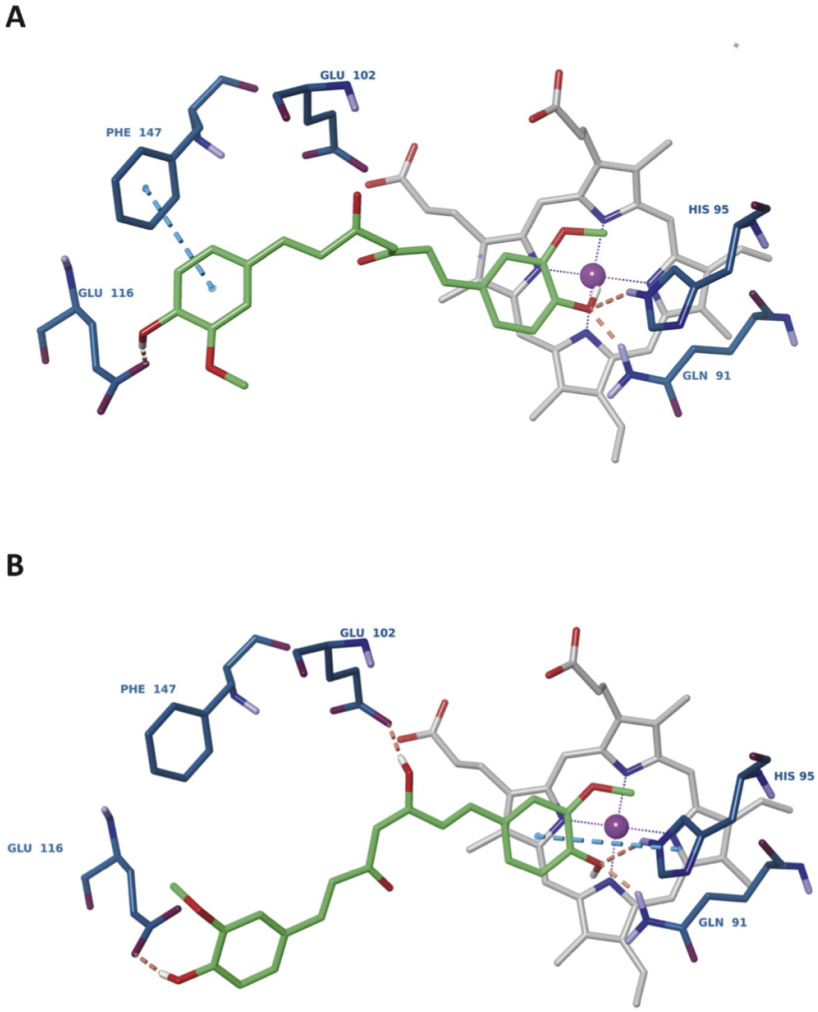


**Fig. 6. Both aromatic rings of curcumin are involved in binding to MPO.** Molecular docking of the diketo (A) and keto-enol (B) forms of curcumin to the active site of MPO. Stacking of a hydroxyl-methoxyphenyl on the pyrrole ring D of the heme is observed for both forms. Hydrogen bonds are shown in orange dotted line and $\pi-\pi$ interactions are shown in cyan dashed line.

the hydroxyl group of this aromatic moiety forms hydrogen bonds with His95 and Glu91 in the distal cavity. The other aromatic ring extends further to the entrance of the active site to make a hydrogen bond with Glu116 and in the best-score poses even forms a stacking with Phe147. A similar hydrogen bonding network of both aromatic rings can be found in the keto-enol form of curcumin. Additionally, in the keto-enol form a hydrogen bond is established between the enolic hydroxyl group and Glu102. The lowest energy of the diketo form pose (−7.1 kcal/mol) is slightly more favourable than that of the keto-enol form (−6.6 kcal/mol) although the difference might not be significant.

### *3.6. β-cyclodextrin cannot enter within the active site of MPO*

β-cyclodextrin was docked instead of HPβCD (see Material and methods). No docked poses were found neither in the binding pocket nor in the channel. Docked poses were produced at the MPO surface at the entrance of the channel (Fig. 7). Unless a conformational change would occur upon interaction of the ligand with MPO (which has not been considered here), β-cyclodextrin is too bulky to position itself in the binding site. One may conjecture that this would be true as well for HPβCD.

## 4. Discussion

Curcumin belongs to the polyphenol family and is well known for its anti-oxidant, anti-inflammatory and anti-cancer activities [20,21]. Consequently, it is expected that curcumin has therapeutic potency to prevent various lifestyle-related diseases. In order to increase its bioavailability and enable its solubility in water, our group developed NDS27 having the lysine salt of curcumin incorporated into hydroxypropyl-β-cyclodextrin [6]. In horse, the efficiency of NDS27 has already been demonstrated *in vivo* on recurrent airway obstruction and in a LPS-induced lung neutrophilia. NDS27 was able to reduce PMNs influx in respiratory airway thereby leading to decreased MPO concentration and activity in bronchoalveolar lavages [12,22]. Furthermore, in an *in vitro* study, we demonstrated the potential of curcuminoids and tetrahydrocurcuminoids to inhibit both the oxidant response of horse neutrophils and the release of total and active MPO [6]. Later it was shown that NDS27 has the potential to inhibit the release of ROS and active MPO by stimulated neutrophils [10].

In the present study we aimed at comparing the *in vitro* activity of curcumin, curcumin lysinate, NDS27 and its excipients (lysine and HPβCD) and at elucidating of their mode of action on MPO. Regarding

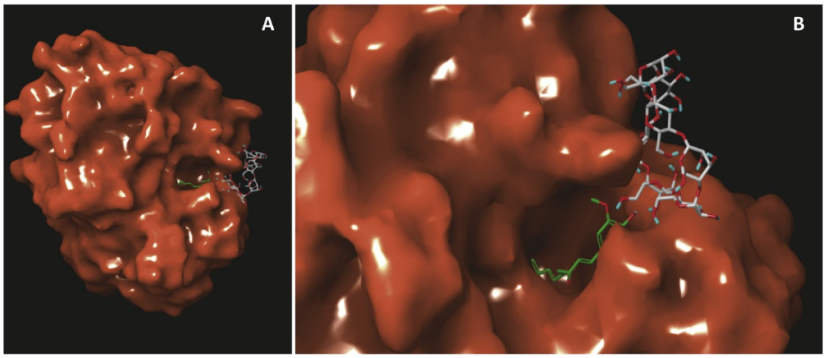


**Fig. 7. β-cyclodextrin cannot enter within the active site of MPO**. Two views of MPO structure with the docked pose of β-cyclodextrin at the entrance of the channel leading to the binding site pocket (A and B). The β-cyclodextrin was docked in absence of any other ligand: curcumin docked pose is shown only to highlight the binding site pocket.

interference with superoxide anion release on an ex-vivo activation model with equine PMNs, we observed a slightly better inhibitory effect of curcumin in comparison with curcumin lysinate and NDS27, while no evident effect was observed with lysine and HPβCD alone (Fig. 1). Coordination of lysine to the enolic hydroxyl group of curcumin seems to slightly reduce the antioxidant properties of the curcumin salt. Indeed, the electron-donating groups such as the phenolic and enolic hydroxyl groups are essential to the antioxidant activity of curcumin [23,24]. Morales et al. [25] suggested that curcumin and its derivatives principally act as chain breaking antioxidants rather than as direct free radical scavengers. Our data also show that HPβCD alone does not affect the antioxidant potential of the salt which is in line with previous studies, which demonstrated that the complexation of antioxidant molecules like resveratrol or tocopherol by cyclodextrin does not or only slightly modifies the antioxidant activity of the complex in comparison to the free molecule [26]. Beside their antioxidant activity, curcumin and NDS27 have an inhibitory effect on the superoxide anion production by neutrophils via the direct inhibition of the activity of NADPH oxidase and the inhibition of MPO released by PMNs [10,11].

Another aspect of this study was to elucidate the mechanism of inhibition of MPO. MPO is the only human enzyme that is able to oxidize chloride to hypochlorous acid which acts as antimicrobial agent [27,28]. It can also mediate the two-electron oxidation of bromide and thiocyanate to hypobromous acid and hypothiocyanite, respectively [29,30]. Beside their role in innate immunity, hypohalous acids has been shown to contribute to inflammatory diseases. As a consequence, MPO was identified as a therapeutic target [31–33].

By enzymatic assay we showed that curcumin, curcumin lysinate and NDS27 inhibited very similarly the peroxidase activity of MPO without interferences of lysine and HPβCD (Fig. 2A). These results suggest that the excipients of NDS27 do not (i) interact specifically with MPO, (ii) disturb the electron transfer reactions to Compound I and Compound II, nor (iii) interfere with the oxidation of Amplex Red and nitrite in the used peroxidase assay.

Previous studies showed the ability of the peroxidase to react and oxidize curcumin with the difficulty to distinguish between the oxidation due to the enzyme and the spontaneous auto-oxidation of curcumin in PBS buffer at physiological pH [4,13]. In the present study, we observed in PBS buffer either for curcumin and NDS27 a rapid loss of the curcumin chromophore at 430 nm and after 10 min incubation the appearance of a peak at 265 nm corresponding to spiroepoxide intermediate an earliest oxidized product in the oxidation pathway of curcumin [4]. Under these conditions, aware that the observed inhibition could be partly attribute to the oxidized products, we performed a similar experiment with NaCl 0.9% (pH 5.5) that did not induce the appearance of the spiroepoxide intermediate. It was shown that curcumin was more stable in acidic pH, but tended to crystallize out of aqueous acid solutions [34]. As well as for PBS, a similar inhibition profile (with similar $IC_{50}$) was observed for the two molecules in NaCl 0.9% (Fig. 3A). Therefore, the progressive oxidation of curcumin and NDS27 in our experimental conditions did not impair the inhibition potential of curcumin and NDS27 on MPO activity. Here we demonstrate that both compounds act as one-electron donors of Compound I and Compound II thus following the peroxidase cycle of MPO (Fig. 4). In contrast to the halogenation cycle, where Compound I is directly reduced to the ferric state by chloride, bromide or thiocyanate, in the peroxidase cycle Compound I is step-wise reduced to Compound II and further to ferric MPO. The presented stopped-flow analysis (Fig. 5) clearly showed that both curcumin and NDS27 induced a direct and fast transition of Compound I to Compound II with apparent bimolecular rate constants of almost $10^7\,M^{-1}\,s^{-1}$, which is three orders of magnitude faster than the two-electron oxidation of chloride and about 4.5-fold faster than the one-electron oxidation of nitrite by Compound I at pH 7 [29,35]. By contrast, Compound II reduction by curcumin and NDS27 is more than four orders of magnitude slower than Compound I reduction. As a consequence, both compounds promote Compound II accumulation. Since this redox intermediate does not participate in the halogenation cycle, curcumin and NDS27 dampen the production of hypohalous acid as was demonstrated by using the taurine chlorination assay. Similar effects are seen in the presence of reversible MPO inhibitors [36]. The differences in reactivity towards Compound I and Compound II can easily be explained by a comparison of the involved reduction potentials with $E°'$(Compound I/Compound II) $\gg >$ (Compound II/ferric MPO) [27,37]. Nevertheless, MPO Compound I and Compound II can contribute to the one-electron oxidation of curcumin which may lead to the formation of bicyclopentadione as end product [5] that could also further acts as an electron donor to the peroxidase cycle and thus compete with Amplex Red and nitrite. In our enzymatic system, curcumin can also scavenge nitrogen dioxide issue from the oxidation of nitrite [38].

By using the SIEFED assay, we demonstrated that curcumin and NDS27 can bind to the active site of MPO (Fig. 2B). This is reflected by the fact that inhibition remains after washing step of the tested molecules that block the access of the substrates to the active site. In PBS buffer, a similar binding of molecules to the active site of MPO is suspected at high initial concentrations (before washing) with $IC_{50}$ near 75 μM. Interestingly, in NaCl 0.9% a strong dose dependent inhibition was observed with NDS27 but not with curcumin (Fig. 3B). With NDS27 the inhibition appeared even more intense than that observed with PBS and showed a similar $IC_{50}$ (4.8 ± 1.4 μM) with the classical assay (Fig. 3A). These results suggest the important role of the buffer in the interaction of the molecule with the enzyme. Our hypothesis is that, in PBS, the curcumin from NDS27 is rapidly released from cyclodextrin, which explains similar transformation and inhibitory action compared to free curcumin (with DMSO). In PBS, ionic, oxidation or solubility constraints could disturb the access of the molecule to the enzymatic pocket. In NaCl 0.9%, NDS27 remains more stable and more soluble than free curcumin which may offer to curcumin released by the complex a more efficient binding to the active site of MPO thus acting more by blocking the access of the substrates than by as an electron donor.

Docking studies showed that curcumin can bind to the active site by adopting a stacked position on the pyrrole ring D of the heme and by forming hydrogen bonds with His95 and Glu91, residues of the distal cavity and with Glu102 (Fig. 6). The importance of these specific interactions has been demonstrated for both MPO substrates and inhibitors [17,19,39,40].

Remains the question on how curcumin complexed by HPβCD can interact with MPO. Curcumin lysinate might interact with HPβCD in two ways. Either in a stoichiometry of curcumin:cyclodextrin of 1:1 having one aromatic ring included in the cyclodextrin cavity or a stoichiometry of 1:2 having each of the two aromatic moieties included in two cyclodextrin cavity [41,42]. According to Shitiyakov et al. [43], a 1:1 binding isotherm for the curcumin:cyclodextrin complex seems more likely, although some 1:2 complexation was suspected between curcumin and HPβCD [44]. A stoichiometry 1:1 would be more favourable to a better interaction of curcumin with MPO since at least one aromatic moiety could interact with hydrophobic residues from the substrate channel of the enzyme leading to active site. On the other hand, the molecular docking clearly suggested first, that both aromatic rings are involved in binding of curcumin to MPO (Fig. 6) and second, that β-cyclodextrin and thus HPβCD would be too large to enter the channel (Fig. 7) unless the channel undergoes a conformational change upon interaction with the complex. Thus, when the solubilizsation of NDS27 is optimized, we hypothesize that in the curcumin-lysine-HPβCD complex a free aromatic moiety of curcumin interacts with the MPO surface at the entrance of the channel leading to the active site. As a consequence, this interaction weakens binding to cyclodextrin and leads to dissociation of the complex. This, finally, enables curcumin to fully enter into the heme cavity and establish non-covalent interactions with several amino acids of the enzymatic pocket as described in Fig. 6. Nevertheless, further studies are needed to confirm this hypothesis, to precise the role of lysine during the complex dissociation and to understand the more efficient access of curcumin into the active site of MPO, when solubilized in NaCl 0.9%.

In conclusion, this study demonstrates that the water-soluble complex of curcumin (NDS27) is as effective as its parent molecule curcumin in inhibiting both superoxide anion production by activated neutrophils and the peroxidase and halogenation activity of MPO. Both curcumin and NDS27 show almost similar inhibitory activities, suggesting an easy dissociation of the curcumin-lysine-cyclodextrin without interference of lysine and HPβCD. The solubilizsation of NDS27 in NaCl 0.9% having a more acidic pH (pH 5.5) rather than in PBS (pH 7.4) allows a better access of curcumin to the active site of the MPO. Curcumin acts as a reversible MPO inhibitor by specific binding to the heme cavity and as an electron donor promoting the formation of Compound II but not its reduction.

## Acknowledgements

This work was supported by a research grant (1610151, CURSTEM) from the Region Wallonne: Programme de Recherche “WaLinnov 2016” (Belgium). Martine Prévost is a “Maître de recherches” at the FRS/FNRS (Belgium).

## Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.cbi.2018.10.008.

## References

[1] A. Unlu, E. Nayir, M. Dogukan Kalenderoglu, O. Kirca, M. Ozdogan, Curcumin (Turmeric) and cancer, J. Buon. 21 (2016) 1050–1060.
[2] R.K. Maheshwari, A.K. Singh, J. Gaddipati, R.C. Srimal, Multiple biological activities of curcumin: a short review, Life Sci. 78 (2006) 2081–2087.
[3] Z. Stanić, Curcumin, a compound from natural sources, a true scientific challenge - a review, Plant Foods Hum. Nutr. 72 (2017) 1–12.
[4] C. Schneider, O.N. Gordon, R.L. Edwards, P.B. Luis, Degradation of curcumin: from mechanism to biological implications, J. Agric. Food Chem. 63 (2015) 7606–7614.
[5] F. Cavaleri, W. Jia, The true nature of curcumin's polypharmacology, J. Prev. Med. 2 (2017) 5.
[6] P. Neven, D. Serteyn, J. Delarge, R. Kiss, V. Mathieu, D. Cataldo, N. Rocks, World intellectual property organization, International Publication number WO/2009/144220 A1: Water soluble curcumin compositions for use in anticancer and anti-inflammatory therapy http://www.wipo.int/patentscope/search/en/WO2009144220, (2009) , Accessed date: 13 July 2011.
[7] Y. Kato, A. Nagao, J. Terao, T. Osawa, Inhibition of myeloperoxidase-catalyzed tyrosylation by phenolic antioxidants in vitro, Biosci. Biotechnol. Biochem. 67 (2003) 1136–1139.
[8] T. Franck, S. Kohnen, S. Grulke, P. Neven, Y. Goutman, F. Péters, B. Pirotte, G. Deby-Dupont, D. Serteyn, Inhibitory effect of curcuminoids and tetrahydrocurcuminoids on equine activated neutrophils and myeloperoxidase activity, Physiol. Res. 57 (2008) 577–587.
[9] K. Drábiková, T. Perečko, R. Nosáľ, J. Harmatha, J. Smidrkal, V. Jančinová, Polyphenol derivatives - potential regulators of neutrophil activity, Interdiscipl. Toxicol. 5 (2012) 65–70.
[10] S. Derochette, T. Franck, A. Mouithys-Mickalad, G. Deby-Dupont, P. Neven, D. Serteyn, Intra- and extracellular antioxidant capacities of the new water-soluble form of curcumin (NDS27) on stimulated neutrophils and HL-60 cells, Chem. Biol. Interact. 201 (2013) 49–57.
[11] S. Derochette, A. Mouithys-Mickalad, T. Franck, S. Collienne, J. Ceusters, G. Deby-Dupont, P. Neven, D. Serteyn, NDS27 combines the effect of curcumin lysinate and hydroxypropyl-β-cyclodextrin to inhibit equine PKCδ and NADPH oxidase involved in the oxidative burst of neutrophils, FEBS Open Bio. 4 (2014) 1021–1029.
[12] C. Sandersen, D. Bienzle, S. Cerri, T. Franck, S. Derochette, P. Neven, A. Mouytis-Mickalad, D. Serteyn, Effect of inhaled hydrosoluble curcumin on inflammatory markers in broncho-alveolar lavage fluid of horses with LPS-induced lung neutrophilia, Multidiscip. Respir. Med. 10 (2015) 16.
[13] P.B. Luis, O.N. Gordon, F. Nakashima, A.I. Joseph, T. Shibata, K. Uchida, C. Schneider, Oxidative metabolism of curcumin-glucuronide by peroxidases and isolated human leukocytes, Biochem. Pharmacol. 132 (2017) 143–149.
[14] J.F. Pycock, W.E. Allen, T.H. Morris, Rapid, single-step isolation of equine neutrophils on a discontinuous Percoll density gradient, Res. Vet. Sci. 42 (1987) 411–412.
[15] T. Franck, G. Minguet, C. Delporte, S. Derochette, K. Zouaoui Boudjeltia, P. Van Antwerpen, O. Gach, G. Deby-Dupont, A. Mouithys-Mickalad, D. Serteyn, An immunological method to combine the measurement of active and total myeloperoxidase on the same biological fluid, and its application in finding inhibitors which interact directly with the enzyme, Free Radic. Res. 49 (2015) 790–799.
[16] P. Van Antwerpen, M. Prévost, K. Zouaoui-Boudjeltia, S. Babar, I. Legssyer, P. Moreau, N. Moguilevsky, M. Vanhaeverbeek, J. Ducobu, J. Nève, F. Dufrasne, Conception of myeloperoxidase inhibitors derived from flufenamic acid by computational docking and structure modification, Bioorg. Med. Chem. 16 (2008) 1702–1720.
[17] J. Soubhye, I. Aldib, B. Elfving, M. Gelbcke, P.G. Furtmüller, M. Podrecca, R. Conotte, J.M. Colet, A. Rousseau, F. Reye, A. Sarakbi, M. Vanhaeverbeek, J.M. Kauffmann, C. Obinger, J. Nève, M. Prévost, K. Zouaoui Boudjeltia, F. Dufrasne, P. Van Antwerpen, Design, synthesis, and structure-activity relationship studies of novel 3-alkylindole derivatives as selective and highly potent myeloperoxidase inhibitors, J. Med. Chem. 56 (2013) 3943–3958.
[18] P.G. Furtmüller, U. Burner, C. Obinger, Reaction of myeloperoxidase compound I with chloride, bromide, iodide, and thiocyanate, Biochemistry 37 (1998) 17923–17930.
[19] L.V. Forbes, T. Sjögren, F. Auchère, D.W. Jenkins, B. Thong, D. Laughton, P. Hemsley, G. Pairaudeau, R. Turner, H. Eriksson, J.F. Unitt, A.J. Kettle, Potent reversible inhibition of myeloperoxidase by aromatic hydroxamates, J. Biol. Chem. 288 (2013) 36636–36647.
[20] J.S. Jurenka, Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research- A review, Altern. Med. Rev. 14 (2009) 141–153.
[21] X. Fan, C. Zhang, D.B. Liu, J. Yan, H.P. Liang, The clinical applications of curcumin: current state and the future- A review, Curr. Pharmaceut. Des. 19 (2013) 2011–2031.
[22] C. Sandersen, D. Olejnik, T. Franck, P. Neven, D. Serteyn, T. Art, Inhalation with NDS27 attenuates pulmonary neutrophilic inflammation in recurrent airway obstruction, Vet. Rec. 169 (2011) 100.
[23] J.Y. Feng, Z.Q. Liu, Phenolic and enolic hydroxyl groups in curcumin: which plays the major role in scavenging radicals? J. Agric. Food Chem. 57 (2009) 11041–11046.
[24] Q.T. Zheng, Z.H. Yang, L.Y. Yu, Y.Y. Ren, Q.X. Huang, Q. Liu, X.Y. Ma, Z.K. Chen, Z.B. Wang, X. Zheng, Synthesis and antioxidant activity of curcumin analogs, J. Asian Nat. Prod. Res. 19 (2017) 489–503.
[25] N.P. Morales, S. Sirijaroonwong, P. Yamanont, C. Phisalaphong, Electron paramagnetic resonance study of the free radical scavenging capacity of curcumin and its demethoxy and hydrogenated derivatives, Biol. Pharm. Bull. 38 (2015) 1478–1483.
[26] J.M. López-Nicolás, P. Rodríguez-Bonilla, F. García-Carmona, Cyclodextrins and antioxidants, Crit. Rev. Food Sci. Nutr. 54 (2014) 251–276.
[27] J. Arnhold, E. Monzani, P.G. Furtmüller, M. Zederbauer, L. Casella, C. Obinger, Kinetics and thermodynamics of halide and nitrite oxidation by mammalian heme peroxidases, Eur. J. Inorg. Chem. 19 (2006) 3801–3811.
[28] G. Battistuzzi, J. Stampler, M. Bellei, J. Vlasits, M. Soudi, P.G. Furtmüller, C. Obinger, Influence of the covalent heme-protein bonds on the redox

thermodynamics of human myeloperoxidase, Biochemistry 50 (2011) 7987–7994.
[29] P.G. Furtmüller, U. Burner, C. Obinger, Reaction of myeloperoxidase compound I with chloride, bromide, iodide, and thiocyanate, Biochemistry 37 (1998) 17923–17930.
[30] M. Zederbauer, P.G. Furtmüller, S. Brogioni, C. Jakopitsch, G. Smulevich, C. Obinger, Heme to protein linkages in mammalian peroxidases: impact on spectroscopic, redox and catalytic properties-A review, Nat. Prod. Rep. 24 (2007) 571–584.
[31] E. Malle, P.G. Furtmüller, W. Sattler, C. Obinger, Myeloperoxidase: a target for new drug development? Br. J. Pharmacol. 152 (2007) 838–854.
[32] P. Van Antwerpen, K. Zouaoui Boudjeltia, Rational drug design applied to myeloperoxidase inhibition, Free Radic. Res. 49 (2015) 711–720.
[33] C. Nussbaum, A. Klinke, M. Adam, S. Baldus, M. Sperandio, Myeloperoxidase: a leukocyte-derived protagonist of inflammation and cardiovascular disease, Antioxidants Redox Signal. 18 (2013) 692–713.
[34] M. Kharat, Z. Du, G. Zhang, D.J. McClements, Physical and chemical stability of curcumin in aqueous solutions and emulsions: impact of pH, temperature, and molecular environment, J. Agric. Food Chem. 65 (2017) 1525–1532.
[35] U. Burner, P.G. Furtmuller, A.J. Kettle, W.H. Koppenol, C. Obinger, Mechanism of reaction of myeloperoxidase with nitrite, J. Biol. Chem. 275 (2000) 20597–20601.
[36] J. Nève, N. Parij, N. Moguilevsky, Inhibition of the myeloperoxidase chlorinating activity by non-steroidal anti-inflammatory drugs investigated with a human recombinant enzyme, Eur. J. Pharmacol. 417 (2001) 37–43.
[37] P.G. Furtmüller, J. Arnhold, W. Jantschko, H. Pichler, C. Obinger, Redox properties of the couples compound I/compound II and compound II/native enzyme of human myeloperoxidase, Biochem. Biophys. Res. Commun. 301 (2003) 551–557.
[38] M.K. Unnikrishnan, M.N. Rao, Curcumin inhibits nitrogen dioxide induced oxidation of hemoglobin, Mol. Cell. Biochem. 146 (1995) 35–37.
[39] I. Aldib, J. Soubhye, K. Zouaoui Boudjeltia, M. Vanhaeverbeek, A. Rousseau, P.G. Furtmüller, C. Obinger, F. Dufrasne, J. Nève, P. Van Antwerpen, M. Prévost, Evaluation of new scaffolds of myeloperoxidase inhibitors by rational design combined with high-throughput virtual screening, J. Med. Chem. 55 (2012) 7208–7218.
[40] I. Aldib, M. Gelbcke, J. Soubhye, M. Prévost, P.G. Furtmüller, C. Obinger, B. Elfving, I.C. Alard, G. Roos, C. Delporte, G. Berger, D. Dufour, K. Zouaoui Boudjeltia, J. Nève, F. Dufrasne, P. Van Antwerpen, Novel bis-arylalkylamines as myeloperoxidase inhibitors: design, synthesis, and structure-activity relationship study, Eur. J. Med. Chem. 123 (2016) 746–762.
[41] R. Singh, H.H. Tønnesen, S.B. Vogensen, T. Loftsson, M. Masson, Studies of curcumin and curcuminoids. XXXVI. The stoichiometry and complexation constants of cyclodextrin complexes as determined by the phase-solubility method and UV–Vis titration, J. Inclusion Phenom. Macrocycl. Chem. 66 (2010) 335–348.
[42] H.Z. Ouyang, L. Fang, L. Zhu, L. Zhang, X.L. Ren J He, A.D. Qi, Effect of external factors on the curcumin/2-hydroxypropyl- b-cyclodextrin: in vitro and in vivo study, J. Inclusion Phenom. Macrocycl. Chem. 73 (2012) 423–433.
[43] S. Shityakov, R.E. Salmas, S. Durdagi, N. Roewer, C. Förster, J. Broscheit, Solubility profiles, hydration and desolvation of curcumin complexed with γ-cyclodextrin and hydroxypropyl-γ-cyclodextrin, J. Mol. Struct. 1134 (2017) 91–98.
[44] A.B. Hegge, M. Másson, S. Kristensen, H.H. Tønnesen, Investigation of curcumin-cyclodextrin inclusion complexation in aqueous solutions containing various alcoholic co-solvents and alginates using an UV-VIS titration method. Studies of curcumin and curcuminoides, XXXV, Pharmazie 64 (2009) 382–389.