Chemistry and Physics of Lipids, 33 (1983) 11–20
Elsevier Scientific Publishers Ireland Ltd.

CALCIUM TRANSPORT BY A β-DIKETONE IN MODEL MEMBRANES

MICHEL DELEERS, ROBERT BRASSEUR and WILLY J. MALAISSE

Laboratories of Experimental Medicine and Macromolecules at Interface, Brussels University, B-1000 Brussels (Belgium)

Received October 14th, 1982 accepted November 26th, 1982

The β-diketone 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dione (FOD) translocates calcium from an aqueous medium into an organic phase. FOD is less efficient than but acts synergistically with A23187 in causing calcium translocation. The FOD-mediated process of calcium translocation is inhibited by NaCl, although the translocation of sodium by FOD is two to three orders of magnitude lower than that of calcium, when expressed relative to the concentration of these cations in the aqueous medium. At pH 7.4, FOD mediates calcium exchange-diffusion in fluid liposomes as efficiently as A23187. The extent of exchange-diffusion depends on the rigidity and cholesterol content of the liposomes. Conformational analysis of the complex formed by two molecules of FOD and one calcium atom at a simulated membrane interface reveals the existence of several interconvertible, asymmetrical and more-or-less planar configurations. The efficiency of FOD-mediated calcium ionophoresis thus appears to be regulated in a multifactorial manner by such factors as the concentration of calcium and monovalent cations, chemical composition and fluidity of the membrane, availability of other ionophoretic molecules and spatial configuration of the calcium complex.

Keywords: calcium transport; liposomes; ionophore.

Introduction

Hunt [1] recently reported that FOD, which is currently used as a ligand of the paramagnetic Pr^{3+} ion, acts as carrier of Pr^{3+} across phospholipid membranes. Gomperts et al. [2] observed that FOD also transports Ca^{2+} across phospholipid bilayers and stimulates Ca^{2+}-mediated cellular processes including K^+ efflux from red blood cells and histamine release by mast cells. The aim of the present study is to further characterize the ionophoretic behaviour of FOD in model membranes, with emphasis on such environmental factors as the concentration of monovalent cations, membrane composition and fluidity, and availability of other ionophoretic molecules. The interaction of the ligand with calcium is also scrutinized by conformational analysis.

0009–3084/83/$03.00 © 1983 Elsevier Scientific Publishers Ireland Ltd.
Published and Printed in Ireland

Materials and methods

The sodium salt of FOD was kindly provided by Dr. R. Bulman (National Radiological Protection Board, U.K.). A23187 was purchased from Calbiochem (La Jolla, CA). Dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC) and cholesterol were purchased from Sigma (St. Louis, MO).

The translocation of calcium from an aqueous medium (0.2 ml) into an organic phase (0.2 ml) formed of toluene and butanol was measured as described in detail elsewhere [3,4]. The aqueous phase consisted of a Tris–HCl buffer (20–50 mM, pH 7.4 or 8.0) containing variable concentrations of $CaCl_2$ and NaCl.

The efflux of ^{45}Ca from multilamellar liposomes was measured as described elsewhere [5], in the absence of Ca^{2+} gradient, in a Tris–HCl buffer (pH 7.4) containing 0.2 mM $CaCl_2$ and 120 mM NaCl. FOD was inserted in the liposomal matrix at the time of formation of the liposomes. The viscosity of the liposomes was measured as described elsewhere [5].

The method used for the conformational analysis of the $(FOD)_2Ca$ complex is based on a strategy described elsewhere [6] and currently used for the study of polypeptides [6–8] and other molecules [9–11]. The total conformational energy of the complex was calculated as the sum of the Van der Waals energy, the torsional potential and electrostatic interaction. The orientation of the complex at a simulated lipid-water interface was established after calculation of the hydrophilic and hydrophobic gravity centers (C) of the complex, based on the following equation:

$$C = \sum_{i=1}^{n} [E_i (x_i^2 + y_i^2 + z_i^2)^{1/2}] / \sum_{i=1}^{n} E_i$$

in which E represents the energy of transfer [12] of either the hydrophobic or hydrophilic parts of the molecule and x_i, y_i and z_i their corresponding coordinates. Calculations were made on a CDC Cyber 170 computer coupled to a Benson drawing table (Brussels University Computing Center).

All results are expressed as the mean ± S.E.M.

Results

Calcium translocation in a two-phase system

FOD provoked the translocation of calcium from an aqueous medium into an organic phase. At pH 8, the concentration of calcium in the organic phase, which contained 0.1 mM FOD, increased from 8.0 ± 0.3 nM to 529 ± 14 nM as the initial Ca^{2+} concentration of the aqueous phase was raised from 2 μM to 200 μM. Increasing concentrations of NaCl (10–120 mM) caused a dose-related inhibition of FOD-mediated calcium translocation, the relative extent of such an inhibition being more marked at low (2 μM) than at high (200 μM) Ca^{2+} concentration (Fig. 1). At the same pH (8) but in the absence of $CaCl_2$, FOD (0.1 mM) caused sodium transloca-

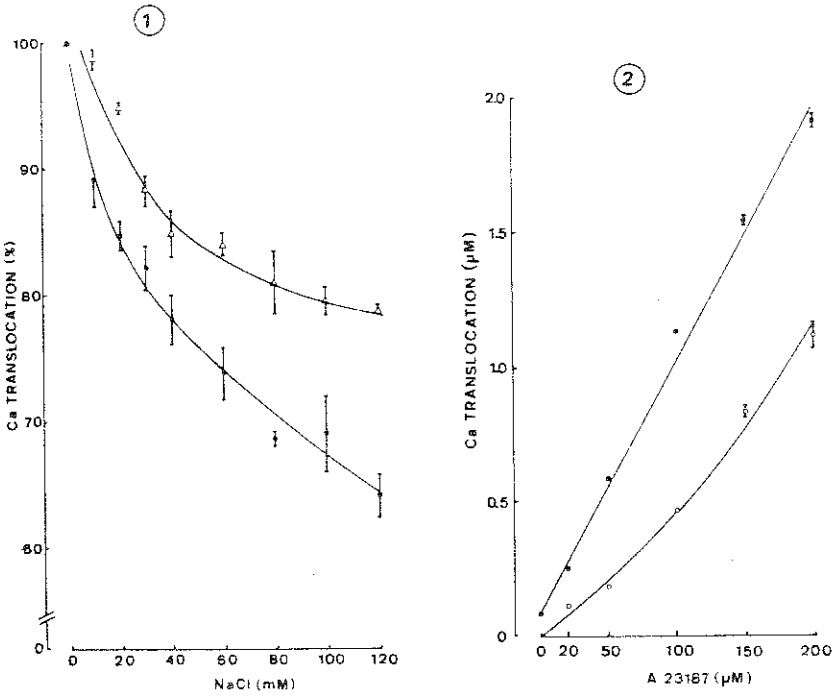

Fig. 1. Influence of increasing concentrations of NaCl upon the translocation of calcium from an aqueous medium (Tris–HCl 50 mM; pH 8) containing either 2 μM (•) or 200 μM (△) $CaCl_2$ into an organic phase containing 0.1 mM FOD. Mean values (± S.E.M. $n = 4$) are expressed in percent of the control value found in the absence of NaCl. Such a control value averaged 8.0 ± 0.3 nM (2 μM $CaCl_2$) and 530 ± 14 nM (200 μM $CaCl_2$).

Fig. 2. Effect of increasing concentrations of A23187 upon the translocation of calcium from an aqueous medium (Tris–HCl 20 mM; pH 7.4) containing 2.5 μM $CaCl_2$ and 120 mM NaCl into an organic phase deprived (○) or containing 1.0 mM FOD (•). Mean values (± S.E.M.) refer to three individual observations.

tion, which averaged 97 ± 17, 547 ± 70 and 891 ± 75 nM in the presence of 10, 60 and 120 mM NaCl, respectively. Thus, relative to the concentration of cation in the aqueous phase, the amount of sodium translocated was two to three orders of magnitude lower than the amount of calcium translocated. In both series of experiments, the amount of FOD complexing calcium and/or sodium represented less than 2% of the total amount of ionophore present in the system.

At close-to-physiological pH (7.4) and low Ca^{2+} concentration (2.5 μM), A23187 (20–200 μM) caused a dose-related translocation of calcium into the organic phase (Fig. 2). In good agreement with prior observations [3], the amount of calcium translocated in the organic phase appeared as a power function of A23187 concentration. FOD (1.0 mM) acted synergistically with A23187. Thus the FOD-induced increment in calcium translocation increased from 80 ± 5 nM to 796 ± 25 nM as the concentration of A23187 was raised from zero to 200 μM (Fig. 2). The syner-

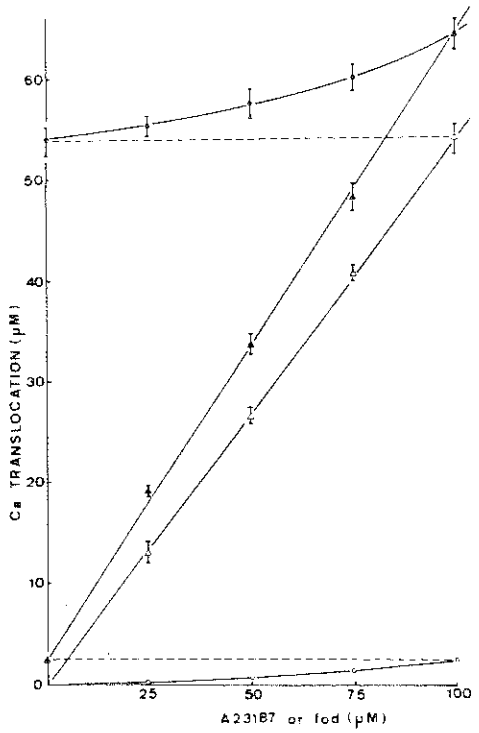

Fig. 3. Effect of increasing concentrations of FOD (○,●) or A23187 (△,▲) upon the translocation of calcium from an aqueous medium (Tris–HCl 50 mM; pH 8.0) containing 1.0 mM $CaCl_2$ into an organic phase deprived (△,○) or containing 0.1 mM of the other ionophore (▲,●). Mean values (± S.E.M.) refer to six individual observations.

gistic behaviour of the two ionophores was confirmed in a second series of experiments performed at a higher Ca^{2+} concentration (1.0 mM). Under the latter condition, the translocation of calcium was virtually proportional to the concentration of A23187, with a calcium/A23187 molar ratio close to 0.5 (Fig. 3). FOD (0.1 mM) further increased calcium translocation, such an increase averaging 2.54 ± 0.33 μM and 10.02 ± 1.46 μM, respectively, in the absence and presence of A23187 (100 μM). Whether in the presence or absence of A23187, the FOD-induced increment in calcium translocation appeared as a power function of the FOD concentration, but the apparent calcium/FOD molar ratio did not exceed 0.1.

Calcium exchange-diffusion in liposomes

In fluid liposomes formed of DMPC (viscosity 1.0 P) and examined at 37°C, i.e. above transition temperature, FOD (1–10 mol/1000 mol lipid) provoked a dose-related stimulation of calcium exchange-diffusion (Fig. 4, left panel). In some-

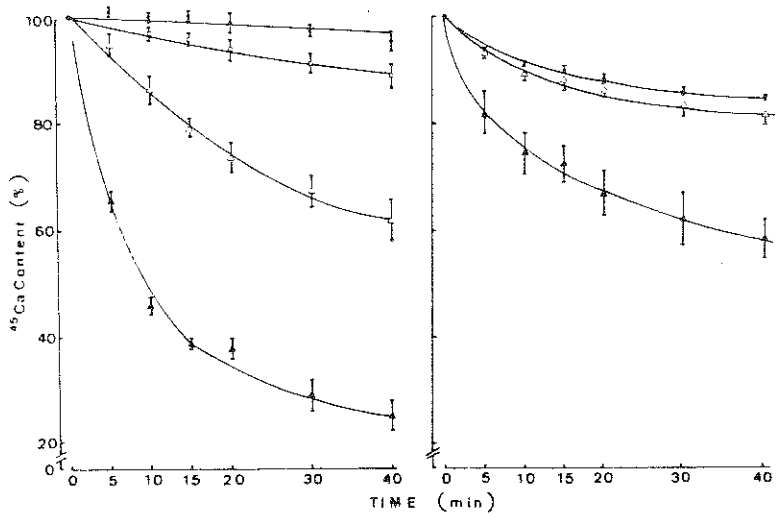

Fig. 4. Time course for the decrease in ^{45}Ca content of DMPC (left panel) or DMPC-cholesterol (right panel) liposomes deprived of FOD (●) or containing FOD in increasing concentrations, namely 1 (○), 3 (△) and 10 (▲) mol FOD/1000 mol lipid. Mean values (± S.E.M. $n = 8$) are expressed in percent of the initial ^{45}Ca content.

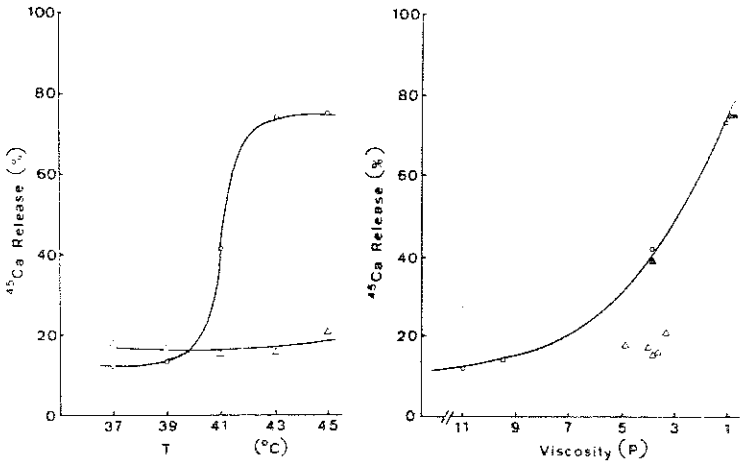

Fig. 5. Left panel: influence of increasing temperatures upon the release of ^{45}Ca from DPPC (○) or DPPC-cholesterol (△) liposomes containing FOD (10 mol/1000 mol lipid). Mean values for the amount of ^{45}Ca released after 30 min incubation at the stated temperature are expressed in percent of the initial ^{45}Ca content of the liposomes, and refer to four individual experiments in each case. Right panel: relationship between the release of ^{45}Ca (at the 30th min of incubation) and the viscosity of DMPC (●), DMPC-cholesterol (▲), DPPC (○) and DPPC-cholesterol (△) liposomes incubated at variable temperatures.

what more rigid liposomes (viscosity 3.8 P) formed of DMPC and cholesterol (2:1, molar ratio), only the highest concentration of FOD (10 mol/1000 mol lipid) was able to significantly increase the rate of ^{45}Ca efflux from the liposomes (Fig. 4, right panel).

The influence of membrane fluidity upon the efficiency of FOD-mediated exchange-diffusion was also evident when DPPC liposomes were incubated at increasing temperature (Fig. 5, left panel). At a relatively high concentration of FOD (10 mol/1000 mol lipid), the amount of ^{45}Ca released after 30 min incubation increased from 12.3 ± 0.8% to 73.7 ± 0.4% of the liposome initial ^{45}Ca content, as the temperature was raised from 37°C to 45°C. The sigmoidal curve relating ^{45}Ca outflow to temperature was similar to that characterizing the changes in viscosity of the DPPC liposomes as a function of the temperature, with a phase transition temperature close to 41°C. We have previously shown that changes in temperature do not affect ^{45}Ca outflow from DPPC or DPPC:cholesterol liposomes deprived of ionophore [13,14].

When FOD (10 mol/1000 mol lipid) was incorporated in liposomes formed of DPPC and cholesterol (2:1, molar ratio), the release of ^{45}Ca occurred at a low rate, whatever the temperature (Fig. 5, left panel). In this case, the rate of calcium exchange-diffusion was no more tightly related to the viscosity of the lipid matrix (Fig. 5, right panel).

Conformational analysis of the calcium/FOD complex

In conducting the conformational analysis of the calcium/FOD complex at a simulated membrane interface, the following assumptions were made. The complex was formed of one calcium atom and two FOD molecules. The formation of an enolate function on C_4, with the H on C_5 in the *trans* position, allowed the second O (on C_6) to participate in the stabilization of the complex. The chemical structure of the complex is illustrated in Fig. 6, which indicates the numbering of all torsional

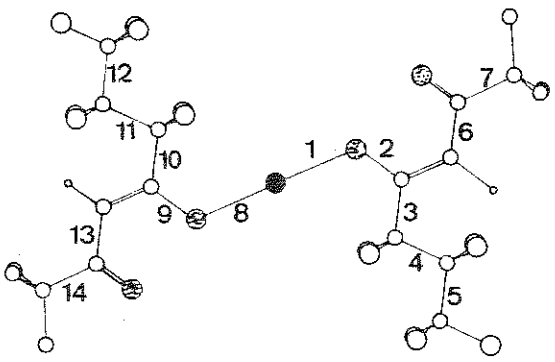

Fig. 6. Structural formula of the $(FOD)_2Ca$ complex, with numbering of the 14 torsional angles. The closed circle refers to the calcium atom, the large open circles to F atoms, the small open circles to C atoms (>C<, –CH=, $-CH_3$), and the dotted circles to O atoms.

TABLE I

CONFORMATIONAL ANALYSIS OF THE $(FOD)_2Ca$ COMPLEX

The table indicates from top to bottom the probability of existence of the five most abundant conformers of $(FOD)_2Ca$ as derived from a first systematic study on angles 1, 2, 3, 6, 9, 10 and 13 (see Fig. 6). the energy above minimal value, the values of all torsional angles after application of a function minimization procedure, and the distance between the hydrophobic and hydrophilic gravity centers. The energy of transfer of the hydrophobic and hydrophilic parts of the complex amounted to 195.0 kcal and 14.2 kcal, respectively.

Probability (%)		49.39	19.08	6.65	6.20	5.37
Energy (kcal/mol)		0	0.56	1.19	1.24	1.31
Torsion angles (°)	1	143.6	81.9	159.4	171.8	16.6
	2	46.7	71.5	53.9	303.1	58.3
	3	23.9	37.2	26.1	26.6	25.8
	4	165.7	166.1	165.9	165.9	168.1
	5	166.7	166.4	166.7	167.2	168.2
	6	211.4	141.6	218.2	154.6	199.8
	7	181.8	209.1	169.7	193.6	188.9
	8	163.5	180.0	160.2	158.7	172.0
	9	18.3	41.1	22.1	24.3	72.8
	10	3.8	357.1	19.8	29.3	356.7
	11	169.2	169.5	167.8	166.4	175.7
	12	167.6	164.4	167.9	166.8	175.8
	13	100.0	91.2	90.8	90.5	89.7
	14	174.1	293.0	189.7	193.2	194.6
Distance (A)		1.41	1.62	1.79	1.06	0.69

angles. In a first systematic study, seven of these torsional angles underwent changes of 60° yielding 6^7 conformers. In a medium with a dielectric constant equal to 3.5 (chosen as representative of a bulk hydrophobic phase), five conformers were selected with an individual probability in excess of 5%. The characteristics of these five conformers, after application of a function minimization procedure [15] bearing on all torsional angles, are listed in Table I. Figure 7 illustrates the orientation of the three most probable conformers at a simulated membrane interface, taking into account the calculation of the hydrophilic and hydrophobic gravity centers.

Discussion

The present findings confirm that FOD acts as a calcium-ionophore [2]. In the two-phase bulk system, FOD was less potent than A23187, at the same molar concentration, although both ionophores complex calcium in the same 2:1 stoichiometry [2,3]. We have previously shown that such a stoichiometry accounts for the exponential relationship between the amount of calcium translocated and concen-

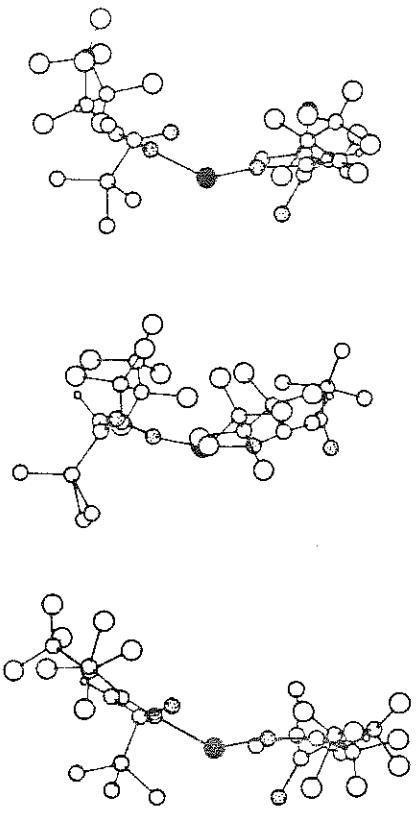

Fig. 7. Frontal view of the three most probable conformers of the $(FOD)_2Ca$ complex at a simulated membrane interface. Same symbols as in Fig. 6.

tration of ionophore, whenever the pH of the aqueous medium and/or its Ca^{2+} concentration are too low to allow saturation of the calcium-complexing capacity of the ionophoretic molecule [3]. The fact that, under identical experimental conditions, namely at the same high Ca^{2+} concentration (1.0 mM), such a relationship remained exponential with FOD, whilst being converted to a rule of proportionality with A23187, indicates that FOD displays a lesser affinity for calcium than A23187.

Several analogies exist between the ionophoretic behaviour of FOD and A23187, respectively. First, although neither of these ionophores display any great affinity for sodium, the phenomenon of calcium translocation is inhibited in both cases by NaCl [16]. We have shown previously that such an inhibition is not attributable to changes in ionic strength of the aqueous medium [16]. The most simple explanation could be that Na^+ competes with Ca^{2+} for the cationic binding site at the interface between the hydrophobic and aqueous phases, even if little sodium is eventually translocated by the ionophores into the organic phase. The concept

that certain ions (e.g. Na^+) may inhibit ionophore-mediated calcium translocation without being themselves extensively transported by the ionophoretic molecule provides a model for the asymmetrical distribution of Ca^{2+} across membranes in the virtual absence of sodium counter-transport [17,18].

A second analogy between the two ionophores, FOD and A23187, consists of their capacity to form hybrid complexes with other ionophoretic molecules and, hence, to act synergistically in mediating calcium translocation or transport. First disclosed in the case of A23187 and Br-X537A [19,20], the formation of such hybrid complexes was recently extended to a number of other ionophores, including hypoglycemic sulfonylureas [9], monensin and etheromycin [21]. In our opinion, the existence of hybrid complexes may again have physiological implications in that exogenous molecules could conceivably be better able to display their ionophoretic potential when acting in synergism with native ionophores [9].

In multilamellar liposomes, the efficiency of FOD-mediated calcium transport was tightly dependent on the viscosity of the lipid matrix. This phenomenon was already observed with such ionophores as Br-X537A [22], A23187 [5] and ionomycin [23]. Whenever calcium is complexed by two molecules of ionophores, as it appears to be the case with FOD [2], a decrease in viscosity seems to facilitate not solely the transverse mobility of the calcium-ionophore complex but also the lateral mobility of the ionophoretic molecules and, hence, the formation of the calcium-complex [24]. The data here obtained in liposomes formed of DPPC and cholesterol confirm, however, that the lipid composition of the artificial bilayer may affect the ionophoretic process independently of any marked changes in membrane fluidity [13].

Although much less efficient than A23187 in the two-phase bulk system, FOD was almost as potent as A23187 in mediating calcium exchange-diffusion in DPPC liposomes incubated at the same pH and temperature (see Ref. 14 for comparison). This is not the first example of a dissociation between the affinity for calcium of a given ionophore and its efficiency in artificial membranes [23], and stresses the view that the ionophoretic response in model or biological membranes is regulated in a multifactorial manner.

The conformational analysis of the 3–4 *trans* $(FOD)_2Ca$ complex indicates that calcium is sandwiched between two FOD molecules, without any marked folding around the calcium atom. The existence of several conformers of high probability, usually with an asymmetrical configuration, further suggests that the complex represents a rather flexible structure with easy interconversion between distinct configurations. The rather small size and planar configuration of the $(FOD)_2Ca$ complex could represent favourable attributes for its transport across bilayer formed solely of fluid phospholipids, a process which could be hindered when cholesterol, in relatively high concentration, is present in the bilayer and interacts with the phospholipid molecules [25].

In conclusion, the present work indicates that FOD is a suitable calcium-ionophore in model membranes, and suggests that it could be used instead of or in

combination with A23187 as a tool in further studies on the relevance of ionophoretic processes in biological systems.

Acknowledgements

This work was supported in part by a grant from the Belgian Ministry of Scientific Policy. The authors are grateful to M. Mahy for technical assistance and C. Demesmaeker for secretarial help.

References

1 G.R.A. Hunt, Chem. Phys. Lipids, 27 (1980) 353.
2 B.D. Gomperts, J.P. Bennett and D. Allan, Eur. J. Biochem., 117 (1981) 559.
3 W.J. Malaisse, I. Valverde, G. Devis, G. Somers and E. Couturier, Biochimie, 61 (1979) 1185.
4 E. Couturier and W.J. Malaisse, Biochimie, 62 (1980) 117.
5 M. Deleers and W.J. Malaisse, Biochem. Biophys. Res. Commun., 95 (1980) 650.
6 E. Ralston and J.L. Decoen, J. Mol. Biol., 83 (1974) 393.
7 E. Ralston, J.L. Decoen and R. Walter, Proc. Natl. Acad. Sci. U.S.A., 71 (1974) 1142.
8 J.L. Decoen, C. Humblet and M.H.J. Koch, FEBS Lett., 73 (1977) 38.
9 M. Deleers, R. Brasseur, M. Gelbcke and W.J. Malaisse, J. Inorg. Biochem., 16 (1982) 215.
10 R. Brasseur, E. Goormaghtigh and J.M. Ruysschaert, Biochem. Biophys. Res. Commun., 103 (1981) 301.
11 R. Brasseur, M. Deleers, W.J. Malaisse and J.M. Ruysschaert, Proc. Natl. Acad. Sci. U.S.A., 79 (1982) 2895.
12 C. Tanford, The Hydrophobic Effect. Formations of Micelles and Biological Membranes, John Wiley & Sons, NY, 1973.
13 M. Deleers and W.J. Malaisse, Chem. Phys. Lipids, 31 (1982) 1.
14 M. Deleers, F. Defrise-Quertain, J.M. Ruysschaert and W.J. Malaisse, Res. Commun. Chem. Pathol. Pharmacol., 34 (1981) 423.
15 J.A. Nelder and R. Mead, Comput. J., 7 (1965) 308.
16 E. Couturier, M. Deleers and W.J. Malaisse, Cell Calcium, 1 (1980) 171.
17 W.J. Malaisse and E. Couturier, Nature, 275 (1978) 664.
18 W.J. Malaisse, K. Anjaneyulu, R. Anjaneyulu and E. Couturier, Mol. Cell. Biochem., 30 (1980) 67.
19 E. Couturier, M. Deleers and W.J. Malaisse, Pharmacol. Res. Commun., 12 (1980) 651.
20 M. Deleers, M. Gelbcke and W.J. Malaisse, Proc. Natl. Acad. Sci. U.S.A., 78 (1981) 279.
21 J. Grandjean and P. Laszlo, Biochem. Biophys. Res. Commun., 104 (1982) 1293.
22 M. Deleers and W.J. Malaisse, Biomed. Res., 1 (1980) 400.
23 M. Deleers, E. Couturier and W.J. Malaisse, Cell Calcium, 2 (1981) 159.
24 M. Deleers and W.J. Malaisse, Chem. Phys. Lipids, 31 (1982) 227.
25 M.C. Phillips, Prog. Surf. Membr. Sci., 5 (1972) 139.