Biochem. J. (1986) 235, 177–182 (Printed in Great Britain)

Streptomyces K15 DD-peptidase-catalysed reactions with suicide β-lactam carbonyl donors

Mélina LEYH-BOUILLE, Martine NGUYEN-DISTÈCHE, Suzanne PIRLOT, Angelica VEITHEN, Catherine BOURGUIGNON and Jean-Marie GHUYSEN*
Service de Microbiologie, Faculté de Médecine, Université de Liège, Institut de Chimie, B6, B-4000 Sart Tilman (Liège), Belgium

The values of the kinetic parameters that govern the interactions between the *Streptomyces* K15 DD-peptidase and β-lactam compounds were determined by measuring the inactivating effect that these compounds exert on the transpeptidase activity of the enzyme and, in the case of [^{14}C]benzylpenicillin and [^{14}C]cefoxitin, by measuring the amounts of acyl-enzyme formed during the reaction. K15 DD-peptidase binds benzylpenicillin or cefoxitin at a molar ratio of 1:1. Benzylpenicilloate is the major product released during breakdown of the acyl-enzyme formed with benzylpenicillin. Benzylpenicillin is not a better acylating agent than the amide Ac_2-L-Lys-D-Ala-D-Ala and ester Ac_2-L-Lys-D-Ala-D-lactate carbonyl-donor substrates. β-Lactam compounds possessing a methoxy group on the α-face of the molecule show high inactivating potency.

INTRODUCTION

The *Streptomyces* K15 DD-peptidase is a membrane-bound penicillin-binding protein. This enzyme, purified to 95% homogeneity (Nguyen-Distèche *et al.*, 1982), performs effective transpeptidation reactions on carbonyl-donor and amino-acceptor co-substrates (Nguyen-Distèche *et al.*, 1986). Benzylpenicillin is recognized as carbonyl donor by the K15 DD-peptidase and immobilizes the enzyme in the form of a rather stable penicilloyl-enzyme. The interaction between the K15 DD-peptidase and β-lactam compounds has now been studied with the use of the equations developed in Ghuysen *et al.* (1986). The results thus obtained are described below.

MATERIALS AND METHODS

Enzymes

The *Streptomyces* K15 DD-peptidase, obtained to 95% purity in the presence of 0.05% cetyltrimethylammonium bromide, was that used previously (Nguyen-Distèche *et al.*, 1982). The *Bacillus cereus* penicillinase I was from Whatman Biochemicals, Maidstone, Kent, U.K., and the *Enterobacter cloacae* P99 cephalosporinase was prepared as described by Ross (1975).

β-Lactam compounds

[^{14}C]Benzylpenicillin (50 mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K., and [^{14}C]cefoxitin (2.7 mCi/mmol) was a gift from Merck, Sharp and Dohme Research Laboratories, Rahway, NJ, U.S.A. The non-radioactive β-lactams were from the following companies: carbenicillin, temocillin and ticarcillin, from Beecham, Brussels, Belgium; ampicillin and oxacillin, from Bristol Benelux, Brussels, Belgium; cephalexin, cephaloglycine, cephalothin and moxalactam, from Lilly Laboratories, Indianapolis, IN, U.S.A.; sulfazecin, from Takeda Chemical Industries, Osaka, Japan; mecillinam, from Leo Pharmaceutical Products, Ballerup, Denmark; azthreonam and compounds SQ 81387, SQ 26559, SQ 81427, SQ 26630 and SQ 26324, from Squibb Institute for Medical Research, Princeton, NJ, U.S.A.; piperacillin, from Cyanamid Benelux, Ottignies, Belgium.

Detergent-containing phosphate buffer, pH 7.5

Unless otherwise stated, the reactions were carried out in 5 mM-potassium phosphate buffer, pH 7.5, containing 0.008% cetyltrimethylammonium bromide.

Determination of transpeptidase activity

The reaction catalysed was (Nguyen-Distèche *et al.*, 1986):

$$Ac_2\text{-L-Lys-D-Ala-D-Ala (8 mM)} + \text{Gly-Gly (2 mM)} \rightarrow \text{D-Ala} + Ac_2\text{-L-Lys-D-Ala-Gly-Gly}$$

The assays were carried out by incubating the enzyme and the co-substrates in 30 μl of detergent-containing phosphate buffer at 37 °C and measuring the amount of released D-Ala by the D-amino acid oxidase technique. In some cases [^{14}C]Gly-Gly was used and the transpeptidated product Ac_2-L-Lys-D-Ala[^{14}C]Gly-Gly was determined after separation by paper electrophoresis. For more details see Johnson *et al.* (1975) and Leyh-Bouille *et al.* (1977).

ID_{50} values

ID_{50} values refer to the concentrations of the β-lactam compounds necessary to decrease the transpeptidase activity of the enzyme by 50%. With all the β-lactam compounds listed in Table 2, except compound SQ 26559, the enzyme (0.79 μM), the co-substrates (at the concentrations aforementioned) and various concentrations of the selected β-lactam compound were mixed together in detergent-containing buffer (30 μl final volume) and

* To whom correspondence should be addressed.

incubated at 37 °C for 30 min. The reaction was stopped by placing the solutions in a boiling-water bath for 1 min, and the released D-alanine was determined. In all cases the reaction was carried out under conditions where [β-lactam compound] was less, or very much less, than [enzyme]. With compound SQ 26559 (which is the most active β-lactam compound examined), in order to fulfil this latter condition, the enzyme (0.011 μM), the co-substrates (at the concentrations aforementioned and with [^{14}C]Gly-Gly) and various concentrations of compound SQ 26559 were incubated for 120 min, after which time the amount of Ac_2-L-Lys-D-Ala-[^{14}C]Gly-Gly was determined.

Acyl-enzyme formation with [^{14}C]benzylpenicillin and [^{14}C]cefoxitin: saturation curves

The enzyme (1.59 μM or 0.73 μM in the experiments involving cefoxitin or benzylpenicillin respectively) and various concentrations of the selected radioactive β-lactam compound were incubated at 37 °C in detergent-containing phosphate buffer (30 μl final volume) for 30 s ([^{14}C]cefoxitin) or 600 s ([^{14}C]benzylpenicillin). The reactions were stopped by adding 5 μl of 0.1 M non-radioactive β-lactam and 30 μl of denaturing buffer (Nguyen-Distèche *et al.*, 1986), and placing the solutions in a boiling-water bath for 60 s. After polyacrylamide-gel electrophoresis at pH 8.3 in the presence of SDS, the amounts of [^{14}C]acyl-enzyme formed were measured by microdensitometry of the fluorograms. For more details see Laemmli & Favre (1973), Laskey (1980) and Nguyen-Distèche *et al.* (1986).

Pseudo-first-order rate constant k_2 of acyl-enzyme formation with non-radioactive β-lactam compounds

The enzyme (1.26 μM) and various concentrations of the non-radioactive β-lactam compounds listed in Table 1 (except cefoxitin) were incubated at 37 °C in detergent-containing phosphate buffer (75 μl final volume). At intervals samples (25 μl) were removed and supplemented with a mixture containing the co-substrates at the aforementioned concentrations and, in order to destroy the excess of β-lactam present, sufficient amounts of P99 β-lactamase (in the case of cephalothin) or *B. cereus* β-lactamase I (in all the other cases). The reaction mixtures were incubated at 37 °C for 30 min (20 min in the case of piperacillin). The reaction was stopped by placing the solutions in a boiling-water bath for 60 s, and the released D-alanine was measured. Since breakdown of the acyl-enzyme (and enzyme recovery) proceeded during incubation with the co-substrates, a correction was introduced as described previously (Frère *et al.*, 1974).

Acyl-enzyme breakdown and enzyme recovery

With compound SQ 26324 and the β-lactam compounds listed in Table 1 except cefoxitin, samples of enzyme (0.95 μM) in detergent-containing phosphate buffer (25 μl final volume) were incubated for 10 min at 37 °C with a concentration of β-lactam compound more than sufficient to inhibit the enzyme activity completely. Excess β-lactam compound was destroyed by incubating the reaction mixtures for 5 min at 30 °C with the P99 β-lactamase (in the case of cephalothin and compound SQ 26324) or with the *B. cereus* β-lactamase I (in all other cases), and the samples, containing the acyl-enzyme, were maintained at 37 °C. At various intervals these incubation mixtures were supplemented with the co-substrates at the aforementioned concentrations, and incubated for an additional 30 min (20 min in the case of piperacillin), after which time the amount of released D-alanine was determined. The first-order rate constant of acyl-enzyme breakdown (and enzyme recovery) was calculated after correction as described previously (Frère *et al.*, 1974).

With cefoxitin (which is not susceptible to the *B. cereus* or P99 β-lactamase), the enzyme (17 μM) and an excess of cefoxitin were incubated for 10 min at 37 °C in detergent-containing buffer (25 μl final volume). The reaction mixture was supplemented with an equal volume of acetone precooled to −20 °C and maintained at −20 °C for 1 h. The suspension was centrifuged (20800 *g* for 20 min) at −10 °C and the pellet was dissolved in 25 μl of buffer. Samples, containing the acyl-enzyme, were incubated at 37 °C for increasing times and the rate of enzyme recovered was determined as described above.

[^{14}C]Benzylpenicilloyl-enzyme breakdown: release of the acyl moiety

The enzyme (26 μM) was incubated with 0.2 mM-[^{14}C]benzylpenicillin for 15 min at 37 °C in 30 mM-potassium phosphate buffer, pH 7.5, containing 0.05% cetyltrimethylammonium bromide (60 μl final volume). Acetone (60 μl) precooled to −20 °C was added and the reaction mixture was maintained at −20 °C for 1 h. The suspension was centrifuged at 20800 *g* for 20 min at −10 °C and the pellet was dissolved in 117 μl of the same buffer as above. The yield in [^{14}C]benzylpenicilloyl-enzyme was 86%. Spontaneous breakdown, at 37 °C, of the radioactive acyl-enzyme gave rise to a mixture of [^{14}C]phenylacetylglycine and [^{14}C]benzylpenicilloate. The two reaction products were isolated and assayed (i) by paper electrophoresis at pH 6.5 (after 1 h and at 60 V/cm, phenylacetylglycine and benzylpenicilloate migrated 25 cm and 32 cm towards the anode respectively), and (ii) by chromatography on thin-layer Polygram Sil G (Macherey and Nagel Co., Düren, Germany) with either chloroform/methanol/acetic acid (44:5:1, by vol.) (under which conditions the R_F values of phenylacetylglycine and benzylpenicilloate were 0.49 and 0.0 respectively) or a water/butan-1-ol/acetic acid (5:4:1, by vol., upper phase) (under which conditions the corresponding R_F values were 0.67 and 0.60).

Table 1. Second-order rate constant k_{+2}/K of acyl-enzyme formation during reaction between the K15 DD-peptidase and β-lactam compounds and first-order rate constant k_{+3} of acyl-enzyme breakdown (at 37 °C and in detergent-containing potassium phosphate buffer, pH 7.5)

	k_{+2}/K ($M^{-1} \cdot s^{-1}$)	k_{+3} (s^{-1})	Half-life of acyl-enzyme (min)
Cephalothin	2	$< 0.25 \times 10^{-4}$	> 460
Oxacillin	8	1.2×10^{-4}	95
Ticarcillin	21	0.69×10^{-4}	165
Carbenicillin	37.5	0.26×10^{-4}	440
Benzylpenicillin	150	1×10^{-4}	115
Piperacillin	122	2.6×10^{-4}	45
Cefoxitin	860	0.17×10^{-4}	670

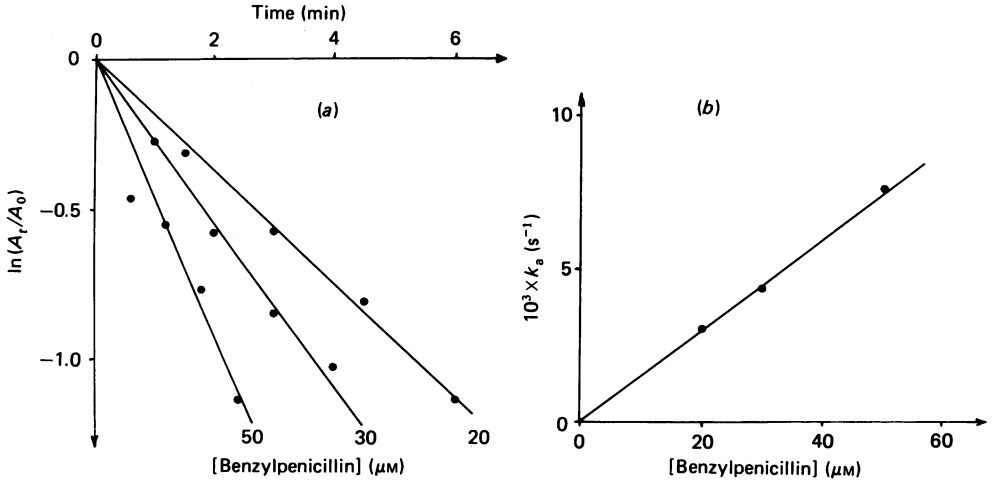

Fig. 1. Determination of the second-order rate constant k_{+2}/K of acylation of the K15 DD-peptidase by benzylpenicillin

(*a*) Plots of $\ln(A_t/A_0)$ versus time. (*b*) Secondary plots of k_a versus [benzylpenicillin]. A_t = residual enzyme activity after incubation in the presence of benzylpenicillin; A_0 = enzyme activity in the absence of benzylpenicillin. For experimental details see the Materials and methods section.

Reaction model

Inactivation of the K15 DD-peptidase by β-lactam compounds was interpreted on the basis of the reaction model:

$$E + D \overset{K}{\rightleftharpoons} E\cdot D \overset{k_{+2}}{\rightarrow} E\text{–}D^* \overset{k_{+3}}{\rightarrow} E + P$$

where E = DD-peptidase, D (for carbonyl donor) = β-lactam, K = dissociation constant of the Michaelis complex E·D, E–D* = acyl-enzyme, P = degradation product, k_{+2} and k_{+3} = first-order rate constants and k_{+2}/K = second-order rate constant of enzyme acylation. Reference to the equations developed in Ghuysen *et al.* (1986) is made with use of the original numbering.

RESULTS

Stoichiometry of the reaction and determination of the first-order rate constant k_{+3} of acyl-enzyme breakdown

Isolation of the [^{14}C]benzylpenicilloyl-enzyme formed by reaction with an excess of [^{14}C]benzylpenicillin showed that 1 mol of the 26000-M_r DD-peptidase bound 1 equivalent of penicilloyl moiety. Breakdown of the [^{14}C]benzylpenicilloyl-enzyme proceeded with a k_{+3} value of 0.91×10^{-4} s^{-1} and generated primarily [^{14}C]benzylpenicilloate ($k'_{+3} = 7.6 \times 10^{-5}$ s^{-1}) and, to a lesser extent, [^{14}C]phenylacetylglycine ($k''_{+3} = 1.1 \times 10^{-5}$ s^{-1}).

The k_{+3} values reported in Table 1 were calculated on the basis of the recovery of the enzyme activity as described in the Materials and methods section.

Determination of the second-order rate constant k_{+2}/K of acyl-enzyme formation

Two procedures were used, indicated below.

(1) The values of the pseudo-first-order rate constant k_a of protein acylation were determined by monitoring the disappearance of the enzyme activity at three β-lactam compound concentrations and three incubation times. In all cases the k_a values were at least 10 times larger than the k_{+3} values [showing that eqn. (17) in Ghuysen *et al.* (1986) applied] and the plots of k_a versus [β-lactam compound] gave rise to straight lines (showing that the condition [β-lactam compound] $\ll K$ also applied). Consequently the k_{+2}/K values reported in Table 1 (except for cefoxitin) were calculated from the slopes of the lines thus obtained [eqn. (18) in Ghuysen *et al.* (1986)]. Figs. 1(*a*) and 1(*b*) illustrate the data obtained with benzylpenicillin. Note that at the lowest (20 μM) benzylpenicillin concentration used the k_a value (3.1×10^{-3} s^{-1}) is 31-fold larger than the k_{+3} value (1×10^{-4} s^{-1}).

(2) The second procedure was based on saturation curves obtained by measuring the amounts of radioactive

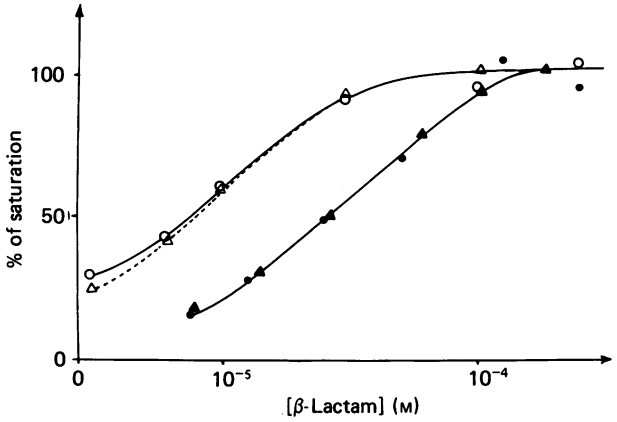

Fig. 2. Saturation curves of the K15 DD-transpeptidase by [^{14}C]benzylpenicillin (○) and [^{14}C]cefoxitin (●)

For experimental details see the Materials and methods section. △ and ▲, Theoretical curves calculated on the basis of a k_{+2}/K value of 150 M^{-1}·s^{-1} for benzylpenicillin and 850 M^{-1}·s^{-1} for cefoxitin, and of a k_{+3} value of 1×10^{-4} s^{-1} for benzylpenicillin and 1.7×10^{-5} s^{-1} for cefoxitin (for more details see Ghuysen *et al.*, 1986).

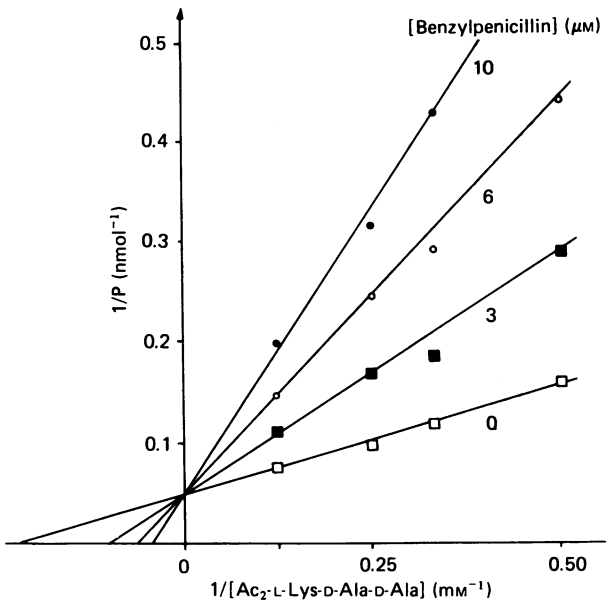

Fig. 3. Lineweaver–Burk plots of 1/P versus 1/[Ac_2-L-Lys-D-Ala-D-Ala] in the presence of a fixed (2 mM) Gly-Gly concentration and in the absence and in the presence of 3 μM-, 6 μM- and 10 μM-benzylpenicillin

The enzyme (0.47 μM) and the various reactants were incubated for 60 min at 37 °C in detergent-containing phosphate buffer (30 μl final volume). P = reaction product Ac_2-L-Lys-D-Ala-Gly-Gly.

acyl-enzyme formed by reaction between the enzyme and various concentrations of [^{14}C]benzylpenicillin for 600 s or of [^{14}C]cefoxitin for 30 s. Note that the incubation times were 11.5-fold smaller (in the case of benzylpenicillin) and very small (in the case of cefoxitin) when compared with the half-lives of the corresponding acyl-enzyme derivatives. As shown in Fig. 2, [^{14}C]benzylpenicillin concentrations of 3.4, 7.6 and 17 μM, and [^{14}C]cefoxitin concentrations of 13.5, 26 and 58 μM, acylated the enzyme by 30%, 50% and 75% respectively. From the extents of protein acylation and the corresponding β-lactam compound concentrations, eqn. (18) in Ghuysen *et al.* (1986) yielded k_{+2}/K values of 150, 175 and 135 $M^{-1} \cdot s^{-1}$ in the case of benzylpenicillin (average value 150 $M^{-1} \cdot s^{-1}$) and 885, 880 and 800 $M^{-1} \cdot s^{-1}$ in the case of cefoxitin (average value 860 $M^{-1} \cdot s^{-1}$). On the basis of the k_{+2}/K values thus obtained, and the known k_{+3} values, eqn. (11) in Ghuysen *et al.* (1986) showed that, at the steady state of the reactions, benzylpenicillin at 3, 6, 10 and 30 μM concentrations would acylate the enzyme by 82%, 90%, 94% and 98% respectively. At the lowest cefoxitin concentration used (7.5 μM) and at the steady state of the reaction, cefoxitin would acylate the enzyme by 99.7%. These examples illustrate cases where the range of β-lactam compound concentrations and incubation times used justify satisfactorily (benzylpenicillin) or perfectly (cefoxitin) the use of eqn. (18) in Ghuysen *et al.* (1986).

Competition between benzylpenicillin and the carbonyl-donor substrate Ac_2-L-Lys-D-Ala-D-Ala

The presence of 3 mM- and 6 mM-Ac_2-L-Lys-D-Ala-D-Ala in the reaction mixture decreased the value of the second-order rate constant of enzyme acylation by benzylpenicillin from 150 $mM^{-1} \cdot s^{-1}$ to 90 and 65 $M^{-1} \cdot s^{-1}$ respectively. In addition, the Lineweaver–Burk plots of 1/[P] versus 1/[Ac_2-L-Lys-D-Ala-D-Ala] (Fig. 3) as obtained in the presence of 2 mM-Gly-Gly, both in the absence and in the presence of various concentrations of benzylpenicillin, indicated an apparent competitive phenomenon between benzylpenicillin and the carbonyl-donor substrate Ac_2-L-Lys-D-Ala-D-Ala. However, given I = benzypenicillin and S = Ac_2-L-Lys-D-Ala-D-Ala and assuming that the dissociation constant of the ternary complex (ESI⇌ES+I) was of the same order of magnitude as K, both competitive and non-competitive models would give rise, for [I] ≪ K and within the limits of experimental errors, to the same competitive plot (Frère & Joris, 1985). Therefore, under the experimental conditions used, it was not possible to distinguish between the two models.

Effect of Gly-Gly

At 2 mM concentration the amino acceptor Gly-Gly had no effect on the value of the second-order rate constant of enzyme acylation by benzylpenicillin. Similarly, it had no effect on the values of the first-order rate constant of breakdown of the acyl-enzyme formed with benzylpenicillin and with compound SQ 26324 ($k_{+3} = 0.2 \times 10^{-4}$ s^{-1}; half-life 575 min; results not shown in Table 1).

Specificity profile of the K15 DD-peptidase for β-lactam compounds

The profile of the enzyme was investigated by determining the concentration of the β-lactam compounds (ID_{50} values) necessary to inhibit the transpeptidase activity by 50% when the enzyme, the co-substrates Ac_2-L-Lys-D-Ala-D-Ala and Gly-Gly, and the selected β-lactam were mixed together and incubated for a given period of time. Comparison of the data of Tables 1 and 2 showed that there was at least an approximate parallelism between the k_{+2}/K values and the corresponding ID_{50} values.

DISCUSSION

The 26000-M_r protein that is present in the plasma membrane of *Streptomyces* K15 is a powerful transpeptidase on well-defined co-substrates (Nguyen-Distèche *et al.*, 1986) and binds penicillin in a 1:1 molar ratio. In these two respects this protein differs from the high-M_r penicillin-binding proteins isolated from bacterial membranes (in particular the proteins 1A, 1B, 2 and 3 of *Escherichia coli*), which show very low or no detectable transpeptidase activity and bind much less than 1 mol of penicillin per mol of protein (Frère & Joris, 1985).

The K15 DD-peptidase is a target of benzylpenicillin of moderate sensitivity. Acylation of the enzyme by benzylpenicillin ($k_{+2}/K = 150$ $M^{-1} \cdot s^{-1}$) is hardly more effective, whether Gly-Gly is present or not in the reaction mixture, than acylation by the amide carbonyl donor Ac_2-L-Lys-D-Ala-D-Ala in the presence of Gly-Gly ($k_{+2}/K = 92$ $M^{-1} \cdot s^{-1}$) and is less effective than acylation by the ester carbonyl donor Ac_2-L-Lys-D-Ala-D-Lac ($k_{+2}/K = 625$ $M^{-1} \cdot s^{-1}$) (Nguyen-Distèche *et al.*, 1986). Kinetically benzylpenicillin and the carbonyl donor Ac_2-L-Lys-D-Ala-D-Ala seem to compete for the same

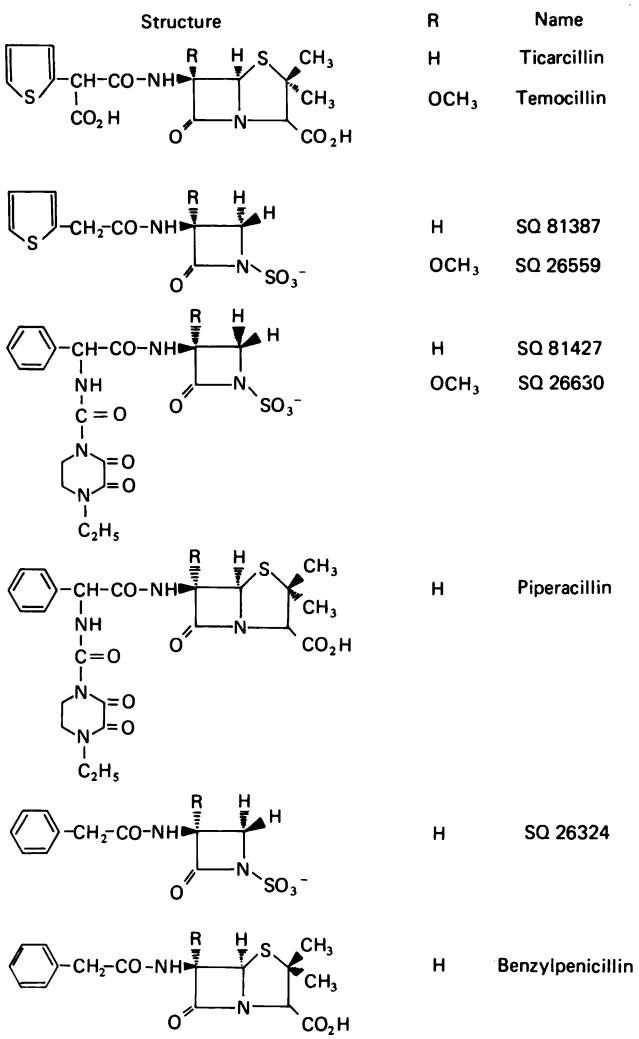

Fig. 4. Structure of comparable pairs of β-lactams

enzyme active site, but a choice between competitive and non-competitive model would need further experiments. The amino acceptor Gly-Gly has no effect on the rate of breakdown of the acyl-enzyme formed with benzylpenicillin or the monocyclic β-lactam compound SQ 26324. It has been suggested (Waxman *et al.*, 1983) that the presence of a thiazolidine (dihydrothiazine) ring in the penicilloyl-(cephalosporoyl-)enzyme immobilizes the DD-peptidases by preventing access of the nucleophilic acceptor to or functioning of the enzyme acceptor site. The present studies show that a monocyclic β-lactam causes the same effect.

Some of the β-lactam compounds studied in the course of the present work form comparable pairs (Fig. 4), from which, on the basis of the data of Table 2, the following conclusions can be drawn. (i) As shown by the pair benzylpenicillin–compound SQ 26324 and the pair piperacillin–compound SQ 81427, replacement of a penam framework by a monocyclic one causes a 30–40-fold decreased inactivating potency of the compounds towards the K15 DD-peptidase. A similar observation has been made with the exocellular R61 serine-active-site DD-peptidase (Georgopapadakou *et al.*, 1982). (ii) As shown by the pair ticarcillin–temocillin, the occurrence of a methoxy group on the α-face of the penam has little effect. (iii) In contrast, and as shown by

Table 2. Specificity profile of the K15 DD-peptidase for β-lactam compounds as expressed by the β-lactam concentrations (ID_{50}) necessary to inhibit the transpeptidase activity by 50% (at 37 °C and in detergent-containing potassium phosphate buffer, pH 7.5)

Temocillin, cefoxitin, compound SQ 26630, sulfazecin, moxalactam and compound SQ 26559 have a methoxy group on the α-face of the molecule. The structures of compounds SQ 81387, SQ 26559, SQ 81427, SQ 26630 and SQ 26324, ticarcillin, temocillin and benzylpenicillin are shown in Fig. 4.

β-Lactam	ID_{50} (M)
Azthreonam	$> 10^{-3}$
Cephalexin	$> 10^{-3}$
Mecillinam	$> 10^{-3}$
Cephaloglycine	6×10^{-4}
Compound SQ 26324	4.5×10^{-4}
Cephalothin	4.3×10^{-4}
Compound SQ 81387	2.7×10^{-4}
Compound SQ 81427	2.6×10^{-4}
Oxacillin	1.6×10^{-4}
Ticarcillin	8×10^{-5}
Carbenicillin	3.5×10^{-5}
Temocillin	2×10^{-5}
Ampicillin	1.8×10^{-5}
Benzylpenicillin	1.1×10^{-5}
N-Formimidoylthienamycin	9.6×10^{-6}
Piperacillin	9×10^{-6}
Cefoxitin	2.6×10^{-6}
Compound SQ 26630	1.5×10^{-6}
Sulfazecin	1.4×10^{-6}
Moxalactam	1.3×10^{-6}
Compound SQ 26559	2.5×10^{-7}

the pair compound SQ 81427–compound SQ 26630 and the pair compound SQ 81387–compound SQ 26559, the occurrence of an α-methoxy group at C-3 of the monobactams causes a 200–300-fold increased inactivating potency. Cefoxitin (7α-methoxylated cephalosporin) is also an efficient inactivator. Similarly, the occurrence of an α-methoxy group at C-3 confers on certain monobactams a 10–100-fold increased efficacy of binding to the R61 exocellular DD-peptidase (Georgopapadakou *et al.*, 1982) and the penicillin-binding proteins 4 of *Staphylococcus aureus* and 4, 5 and 6 of *Escherichia coli* (Georgopapadakou *et al.*, 1983). Cefoxitin also has a high affinity for these latter proteins (Curtis *et al.*, 1979). (iv) Among the various β-lactam compounds tested, cefoxitin, compound SQ 26630, sulfazecin, moxalactam and compound SQ 26559 are the most potent inactivators of the K15 DD-peptidase; they all possess an α-methoxy group in an equivalent position. In this respect, the K15 DD-peptidase differs markedly from the exocellular R61 and R39 serine-active-site DD-peptidases, for which benzylpenicillin is one of the most efficient inactivators tested (Georgopapadakou *et al.*, 1982; Frère & Joris, 1985).

This work was supported in part by the Fonds de la Recherche Scientifique Médicale, Brussels, Belgium (Contract no. 3.4507.83), an Action Concertée with the Belgian Government (Convention 79/84-I1) and a Convention Tripartite between the Région Wallonne, Continental Pharma and the University of Liège.

REFERENCES

Curtis, N. A. C., Orr, D., Ross, G. W. & Boulton, M. G. (1979) Antimicrob. Ag. Chemother. **16**, 325–328
Frère, J. M. & Joris, B. (1985) CRC Crit. Rev. Microbiol. **11**, 299–396
Frère, J. M., Leyh-Bouille, M., Ghuysen, J. M. & Perkins, H. R. (1974) Eur. J. Biochem. **50**, 203–214
Georgopapadakou, N. H., Smith, S. A. & Cimarusti, C. M. (1982) Eur. J. Biochem. **124**, 507–512
Georgopapadakou, N. H., Smith, S. A., Cimarusti, C. M. & Sykes, R. B. (1983) Antimicrob. Ag. Chemother. **23**, 98–104
Ghuysen, J. M., Frère, J. M., Leyh-Bouille, M., Nguyen-Distèche, M. & Coyette, J. (1986) Biochem. J. **235**, 159–165
Johnson, K., Duez, C., Frère, J. M. & Ghuysen, J. M. (1975) Methods Enzymol. **43**, 687–698
Laemmli, U. K. & Favre, M. (1973) J. Mol. Biol. **80**, 575–599
Laskey, R. A. (1980) Methods Enzymol. **65**, 363–371
Leyh-Bouille, M., Dusart, J., Nguyen-Distèche, M., Ghuysen, J. M., Reynolds, P. & Perkins, H. R. (1977) Eur. J. Biochem. **81**, 19–28
Nguyen-Distèche, M., Leyh-Bouille, M. & Ghuysen, J. M. (1982) Biochem. J. **207**, 109–115
Nguyen-Distèche, M., Leyh-Bouille, M., Pirlot, S., Frère, J. M. & Ghuysen, J. M. (1986) Biochem. J. **235**, 167–176
Ross, G. W. (1975) Methods Enzymol. **43**, 678–687
Waxman, D. J., Yocum, R. R. & Strominger, J. L. (1983) Philos. Trans. R. Soc. London Ser. B **289**, 257–271

Received 16 July 1985/25 September 1985; accepted 26 November 1985