Structural and kinetic studies on β -lactamase K1 from *Klebsiella aerogenes*

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β -Lactamase K1 from *Klebsiella aerogenes* 1082E hydrolyses both penicillins and cephalosporins comparably and is inhibited by mercurials but not by cloxacillin. These properties distinguish it from those other β -lactamases that have been allotted to classes on the basis of their amino acid sequences. β -Lactamase K1 has been isolated by affinity chromatography; its composition shows resemblances to class A β -lactamases. Moreover, the *N*-terminal sequence is similar to those of class A β -lactamases: there is about 30% identity over the first 32 residues. Furthermore, a putative active-site octapeptide has been isolated and its sequence is similar to the region around the active-site serine residue in class A β -lactamases. There is one thiol group in β -lactamase K1; it is not essential for activity. The pH-dependence of k_{cat} and k_{cat}/K_m for the hydrolysis of benzylpenicillin by β -lactamase K1 were closely similar, suggesting that the rate-determining step is cleavage of the β -lactam ring.

INTRODUCTION

Many bacteria produce β -lactamases, enzymes that can be important clinically since they may render pathogens insusceptible to penicillins, cephalosporins and related antibiotics. The many different β -lactamases known have been classified on a functional basis (Richmond & Sykes, 1973) and on the basis of their sequences (Ambler, 1980; Jaurin & Grundström, 1981). Although the classification by sequence is the more fundamental, there are only about six complete sequences known, and the functional classification is often preferred (Sykes, 1982). Here the five main classes are denoted by Roman numerals (Richmond & Sykes, 1973). Several members of class I and class III have been assigned to structural classes C and A respectively (Ambler, 1980; Jaurin & Grundström, 1981; Knott-Hunziker *et al.*, 1982; Joris *et al.*, 1984). The most important class IV β -lactamases are those produced by strains of *Klebsiella*. We now show that limited structural studies strongly suggest that the β -lactamase produced by *Klebsiella aerogenes* 1082E (Ross & Boulton, 1973) belongs to structural class A. Earlier work on this enzyme (Hamilton-Miller, 1963; Marshall *et al.*, 1972; Ross & Boulton, 1973; Richmond, 1980; Bush *et al.*, 1982) did not utilize the highly purified enzyme, which is now readily obtained by affinity chromatography (Cartwright & Waley, 1984). Hence the pH-dependence of the steady-state kinetic parameters have now been measured.

MATERIALS AND METHODS

Klebsiella aerogenes (K1082E) was grown in a fermenter (15-litre scale) as described by Marshall *et al.* (1972). After 4 h at 30 °C, the cells were harvested by centrifugation and resuspended in 300 ml of 30 mM-sodium phosphate/10 mM-citric acid buffer, pH 6.3. The suspension was frozen and thawed four times and kept for 48 h at 4 °C after the addition of deoxyribonuclease

(Worthington Biochemical Corp., Freehold, NJ, U.S.A.) (3 $\mu\text{g}/\text{ml}$). The cell debris was removed by centrifugation and the supernatant was dialysed against 0.5 M-NaCl/20 mM-triethanolamine/HCl buffer, pH 7.0 (loading buffer). The β -lactamase was then isolated by affinity chromatography on a column (8 cm $\times$ 2 cm) of the more hydrophobic boronic acid gel (Cartwright & Waley, 1984). The dialysed solution was applied to the column at 30 ml/h. The column was then washed with the loading buffer until the A_{280} of the washings was negligible. The β -lactamase was then eluted with 0.5 M-NaCl/0.5 M-sodium borate buffer, pH 7.0. The relative molecular mass, estimated by electrophoresis in 10% polyacrylamide gels in the presence of SDS (Laemmli & Farve, 1969), was 26 500, and the isoelectric point was 6.5.

Tosylphenylalanylchloromethane ('TPCK')-treated trypsin was obtained from Millipore (Harrow, Middx., U.K.); citraconic anhydride was from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Specific cleavage at the peptide bond of arginine residues by trypsin was achieved by citraconylation of the protein (modification of lysine residues) as described by Perham (1978). Protein was determined by the method of Bradford (1976), with bovine serum albumin as standard.

For amino acid analysis, samples (40 nmol) of the β -lactamase were hydrolysed with 6 M-HCl under vacuum at 110 °C for 24, 48 and 72 h. Peptides were hydrolysed with 6 M-HCl at 110 °C for 18 h. Amino acid composition was determined with an LKB Biochrom 4101 analyser. The ratio of tyrosine to tryptophan was determined spectrophotometrically (as described by Bicknell *et al.*, 1985) and thiol groups were determined with 5,5'-dithiobis-(2-nitrobenzoic acid) in the presence of 1% SDS (Habeeb, 1972).

Reductive *S*-carboxymethylation of the protein with iodoacetic acid was carried out as described by Bibring & Baxandall (1978). Automatic liquid-phase sequencing of the *S*-carboxymethylated protein and isolated peptides

was performed with a Beckman 890C Sequenator, and the phenylthiohydantoins were identified by h.p.l.c. (Campbell *et al.*, 1981).

Reverse-phase h.p.l.c. purification of peptide was carried out on a Lichrosorb RP (5 μ m) column (4.6 mm $\times$ 250 mm). Buffer A was 0.1% trifluoroacetic acid, and buffer B was acetonitrile/propan-1-ol/0.1% trifluoroacetic acid (4:2:1, by vol.). The gradient was linear over 60 min.

N-Terminal residues were identified by the dansyl procedures of Bruton & Hartley (1970) and Gray (1972) for peptides and proteins respectively.

Progress curves for the hydrolysis of benzylpenicillin by β -lactamase K1 were measured spectrophotometrically at 232 nm (Waley, 1974), with a Cary model 219 spectrophotometer, with a pathlength of 10 mm. The following buffers were used, all containing 0.5 M-NaCl: 50 mM-Mes, pH 5–6.5; 50 mM-Mops, pH 7–8; 20 mM-NaHCO₃/NaCO₃, pH 8.5–10. No loss of enzymic activity was detected at the extreme pH values by Selwyn's (1965) method. The concentration of the substrate and enzyme were 0.1–1 mM and 2 nM respectively. The progress curves were analysed by the half-time method of Wharton & Szawelski (1982).

RESULTS

Amino acid composition

β -Lactamase K1 had M_r 26 500, in agreement with a previously reported value (23 000 $\pm$ 4000; Ross & Boulton, 1973); class A β -lactamases have M_r values in the range 25 000–30 000 (Ambler, 1980), whereas class C β -lactamases have M_r values of about 40 000 (Jaurin & Grundström, 1981). β -Lactamase K1 has one cysteine residue/molecule; the similar enzyme from *Aerobacter cloacae* has two or three cysteine residues/molecule and is inactivated by *p*-chloromercuribenzoate (Jack, 1971). Comparison of the amino acid compositions of β -lactamase K1 (Table 1) and a class A β -lactamase, namely β -lactamase I from *Bacillus cereus*, by the method of Cornish-Bowden (1983) suggested that they were related (Table 2); the extent of similarity in composition corresponded to 23–62% identity of sequence, on the assumptions given by Cornish-Bowden (1983).

N-Terminal sequence

The N-terminal sequence of β -lactamase K1 was determined by the Edman degradation. There was considerable similarity with class A β -lactamases (Fig. 1), about 30% identity over the first 32 residues, confirming, at least for this region, the predictions from the composition; there was no similarity of structure with β -lactamases of other structural classes.

Putative active-site peptide

Class A β -lactamases have a conserved arginine residue at position 65, the active-site serine being residue 70 (Ambler, 1980). There is also a conserved phenylalanine residue at position 66 (Fig. 2); there are four other phenylalanine residues in β -lactamase K1. These features suggested the possibility of searching for a putative active-site peptide by cleaving the polypeptide chain after arginine residues and screening for peptides with N-terminal phenylalanine. As there is a conserved lysine residue at position 73, a second cleavage after lysine

Table 1. Amino acid compositions of β -lactamase K1 and the putative active-site tryptic peptide

The values given in the Table are the residues/molecule of: β -lactamase K1, based on M_r 26 500 (column 1) and the integral values (column 2) and the composition of peptide T2A from amino acid analysis (column 4) and from the sequence (column 5). The composition of β -lactamase I in column 3 was obtained from its sequence (Sloma & Gross, 1983). N-terminal residues were identified by the dansyl procedure (Bruton & Hartley, 1970; Gray, 1974).

Amino acid	Composition (residues/molecule)				
	Column . . . (1)	(2)	(3)	(4)	(5)
Asp	24.7	25	32	1.23	1
Thr	21.7	22	21	0.94	1
Ser	14.2	14	16	1.93	2
Glu	24.0	25	23		
Pro	9.9	10	9		
Gly	19.3	19	21		
Ala	26.1	26	28	1.14	1
Val	12.9	13	15		
Met	4.3	4	4	0.79	1
Ile	8.8	9	21		
Leu	22.6	23	19		
Tyr	4.0	4	8		
Phe	5.2	5	7	0.95	1
His	4.3	4	4		
Lys	16.6	17	23	1.01	1
Arg	22.2	22	15		
Trp	3.0	3	3		
Cys	1.0	1	—		
N-Terminal		Ser	Lys	Phe	

Table 2. Matrix of differences in amino acid compositions of β -lactamases

The values tabulated are the normalized difference index = 100(SDn/N), calculated by the method of Cornish-Bowden (1983), from the amino acid composition of the enzymes; K1, β -I, β -III, Lich and OXA-2 refer to β -lactamase K1, β -lactamase II and β -lactamase III from *Bacillus cereus* (569/H) (Connolly & Waley, 1983), β -lactamase from *Bacillus licheniformis* 749/C and the β -lactamase from the R46 plasmid of *Escherichia coli* (Holland & Dale, 1984).

	K1	β -I	β -III	Lich	OXA-2
K1	0	52	96	189	52
β -I		0	52	44	65
β -III			0	186	94
Lich				0	186
OXA-2					0

residues was carried out. This search, carried out by tryptic cleavage of citraconylated protein, removal of citraconyl groups and a second tryptic cleavage, is now described. β -Lactamase K1 (10 mg) in 5 ml of 0.2 M-N-ethylmorpholine/HCl buffer, pH 8.5, containing 5 M-guanidinium chloride was incubated with 30 mM-citraconic anhydride (20 molar proportions based

Row	β-Lactamase	
1	<i>K. aerogenes</i> (K1)	S T D A I H Q K L T D L E K W S G A T L G V A L I T T A D T
2	<i>B. cereus</i> (β-1)	K H K N Q A T H K E F S Q L E K K F D A R L G V Y A I D T G T N
3	<i>B. licheniformis</i>	S Q P A E K N E K T E M K D D F A K L E E Q F D A K L G I F A L D T G T N
4	<i>E. coli</i> (RTEM)	H P E T L V K V K D A E D ^K _Q L G A R V G Y I E L D L N S G
5	<i>S. aureus</i> (PC1)	K E L N D L E K K Y N A H I G V Y A L D T K S G
6	<i>ampC</i>	A P Q Q I N D I V H R T I T P L I E Q Q K I P G M A V A V I
7	<i>Ps. aeruginosa</i>	G Q A P A D R L K A L V D A A V Q P V M T A N T I P G T A V A F S
8	P99	T P V S E K Q L A E V V A N T I T P L M K A Q S V P G(M) E V A V I

Fig. 1. Comparison of the *N*-terminal sequence of β-lactamase K1 with those of class A β-lactamases (rows 2–5) and class C β-lactamases (rows 5–8)

The *N*-terminal sequences in rows 2–5 are from Ambler (1980), in row 6 from Jaurin & Grundström (1981), in row 7 from E. L. Emanuel, J. Gagnon & S. G. Waley (unpublished work), and in row 8 from Joris *et al.* (1985).

β-Lactamase	
<i>S. aureus</i>	⁶⁵ Arg-Phe-Ala-Tyr-Ala-Ser-Thr-Ser- ⁷³ Lys
<i>B. licheniformis</i>	Arg-Phe-Ala-Phe-Ala-Ser-Thr-Ile- Lys
<i>B. cereus</i> (β-1)	Arg-Phe-Ala-Phe-Ala-Ser-Thr-Tyr- Lys
<i>E. coli</i> (RTEM)	Arg-Phe-Pro-Met-Met-Ser-Thr-Phe-Lys
<i>K. aerogenes</i> (K1)	Phe-Ala-Met-Asn-Ser-Thr-Ser- Lys

Fig. 2. Active-site sequences of class A β-lactamases

The sequences around the active-site serine (marked with an asterisk) of class A β-lactamases are shown in the first four rows. The part of the sequence shown comprises residues 65–73, with the serine being residue 70 (Ambler, 1980). The last row shows the sequence deduced from the tryptic octapeptide (T2A) from β-lactamase K1; the sequence similarity is clear.

on lysine) for 4 h at 25 °C, the pH being maintained by the addition of 0.1 M-NaOH. The reaction mixture was dialysed against 1% NH₄HCO₃, 0.1 mg of trypsin was added to the dialysed solution and digestion was allowed to proceed for 16 h at 37 °C. The digest was freeze-dried, resuspended in 0.5 ml of 5% (v/v) acetic acid, incubated at 37 °C for 2 h to remove the citraconyl group, and fractionated on a column (150 cm long × 0.9 cm) of Sephadex G-25 (superfine grade) in 10% (v/v) acetic acid at 4 °C. *N*-Terminal analysis of the elute revealed two fractions, T1 (*K_D* 0.2) and T2 (*K_D* 0.6), containing peptides with *N*-terminal phenylalanine. Fraction T1 contained less than 5% of the amount of *N*-terminal phenylalanyl-peptides that were present in fraction T2 and was not studied further. Fractionation of fraction T2 on SP-Sephadex 50 gave only one fraction containing peptides with *N*-terminal phenylalanine, which on further digestion with trypsin followed by fractionation by reverse-phase h.p.l.c. gave only one peptide, T2A, with *N*-terminal phenylalanine. Peptide T2A was an octapeptide (Table 1). The amino acid sequence was determined, and the results (Fig. 2) showed the expected similarity to active-site peptides from class A β-lactamases.

Substrate profile and pH-dependence

Benzylpenicillin has a higher *k_{cat}*/*K_m* than cephaloridine or cephalothin, but the differences are not very large (Table 3). Carbenicillin was hydrolysed somewhat more slowly, and cephalexin considerably more slowly. The substrate profile had previously been based only on *k_{cat}*. (Ross & Boulton, 1973), but gave a similar picture.

The pH-dependence of the kinetic parameters *k_{cat}* and *k_{cat}*/*K_m* for the hydrolysis of benzylpenicillin were similar (Fig. 3). Thus *K_m* does not vary appreciably with pH, and may well be a true dissociation constant (Cornish-Bowden, 1976), as was reported earlier for β-lactamase I (Waley, 1975). [Hardy & Kirsch (1984) concluded that *K_m* for the hydrolysis of benzylpenicillin by β-lactamase I did vary with pH; the reason for this discrepancy is not clear.] The instability of β-lactamase K1 below pH 5 means that the lower *pK* (4.5) is likely to be less reliable than the higher one (8.5). These results suggest that opening the β-lactam ring is the rate-determining step in the hydrolysis (see below).

Role of thiol group in β-lactamase K1

One thiol group was found by titration with 5,5'-dithiobis-(2-nitrobenzoic acid), and this was in

Table 3. Kinetic parameters for hydrolysis of some penicillins and cephalosporins by β-lactamase K1

The hydrolysis of the β-lactams in 0.5 M-NaCl/50 mM-Mops buffer, pH 7, at 30 °C was measured spectrophotometrically. The kinetic parameters were calculated from progress curves by the half-time method of Wharton & Szawelski (1982).

Substrate	10 ⁻⁴ × <i>k_{cat}</i> (min ⁻¹)	<i>K_m</i> (μM)	<i>k_{cat}</i> / <i>K_m</i> (μM ⁻¹ ·min ⁻¹)
Benzylpenicillin	4.35	100	435
Carbenicillin	0.412	167	24.7
Cephaloridine	2.58	417	61.9
Cephalexin	0.0337	897	0.376
Cephalothin	1.41	225	62.7
Cephaloram	2.7	856	31.5

Fig. 3. pH-dependence of k_{cat}/K_m (a) and k_{cat} (b) for the hydrolysis of benzylpenicillin by β -lactamase K1

The rate of hydrolysis was measured spectrophotometrically at 30 °C. The curves are theoretical: (a) pK_1 4.47, pK_2 8.45; (b) pK_1 4.47, pK_2 8.46.

agreement with the amount of cysteic acid in a hydrolysate of the protein oxidized by performic acid. The enzyme was not readily inactivated by iodoacetic acid, and the thiol group did not react with 5,5'-dithiobis-(2-nitrobenzoic acid) unless denaturant was present. After removal of the denaturant by dialysis, the modified enzyme regained activity, but had altered kinetic properties (Table 4). When dialysis was carried out in the presence of dithiothreitol, the 2-nitrothiobenzoate was removed and the enzyme had the same kinetic properties as the native enzyme. The relationship between reaction at the active-site serine residue and modification of the thiol group was investigated as follows. After the enzyme had been inactivated by reaction with 6 β -iodopenicillanic acid, the thiol group still reacted with 5,5'-dithiobis-(2-nitrobenzoic acid), and the refolded 5,5'-dithiobis-(2-nitrobenzoic acid)-modified enzyme was readily inactivated by 6 β -iodopenicillanic acid. Thus the thiol group in β -lactamase K1 is inaccessible, and inessential.

Table 4. Effect of modification of the thiol group of β -lactamase K1 on recovery of activity after denaturation

β -Lactamase K1 (4 mg) was incubated with 0.25 mM-5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in 80 mM-sodium phosphate buffer, pH 8, containing 5 M-guanidinium chloride (GuCl), at 25 °C for 30 min. The reaction was dialysed against 50 mM-sodium phosphate buffer, pH 7. In the last row, dithiothreitol (DTT) (40 mM) was present during dialysis. The kinetic parameters refer to benzylpenicillin as substrate, at pH 7 at 30 °C, as described in the Materials and methods section.

Treatment	$10^{-3} \times k_{cat}$ (min^{-1})	K_m (μM)
None	36	94
GuCl, then dialysis	36	96
GuCl+DTNB, then dialysis	1.5	302
GuCl+DTNB, then dialysis with DTT	36	96

This is similar to β -lactamase L1 from *Pseudomonas maltophilia* (Bicknell *et al.*, 1985), but different from β -lactamase II from *Bacillus cereus*, where the thiol group is essential (Davies & Abraham, 1974).

DISCUSSION

The structural classification of β -lactamases helps to throw light on their origin. Thus the existence of two, apparently unrelated, classes of serine β -lactamase suggests convergent evolution (Knott-Hunziker *et al.*, 1982). There are, moreover, practical aspects, e.g. class A β -lactamases are inactivated much more effectively than class C ones are by an inhibitor that is used clinically. The rigorous structural characterization of a β -lactamase requires the complete sequence; thus there are obvious advantages in short cuts, as long as they lead to the right destination. The amino acid composition of related proteins can provide a guide to the relatedness of their sequences (Cornish-Bowden, 1983). This method suggested that β -lactamase K1 was related to class A β -lactamases (Table 2), a suggestion borne out by the structural work reported here. Similarly, the suggestion by Holland & Dale (1984) that the plasmid-encoded OXA-2 β -lactamase is a good candidate for class A is consistent with the amino acid composition, which has similarities to that of β -lactamase K1 (Table 2).

Commonly, the most readily obtained sequence information relates to the N-terminus. If a sequence of about 30 residues can be determined, this appears to provide a reliable guide to the structural classification (Emanuel *et al.*, 1985), at least in the eight examples studied (Fig. 1). Previously, we have used the characteristic active-site sequences of β -lactamases for classification (Knott-Hunziker *et al.*, 1982; Joris *et al.*, 1984), as have others (Joris *et al.*, 1985). In the present work we were able to base a strategy for isolating a putative active-site peptide on its expected structure. In the event, the isolated octapeptide T2A showed close similarities to its counterparts: seven of the eight residues were present in corresponding positions in active-site peptides from class A β -lactamases (Fig. 2). Thus limited structural studies appear to be useful in classifying β -lactamases.

Both class A and class C β -lactamases function by an

acyl-enzyme mechanism. In this mechanism there are two bond-breaking steps. The first step consists of cleavage of the β -lactam ring and formation of the acyl-enzyme; the second step consists of hydrolysis of the acyl-enzyme. The rate-determining step (with benzylpenicillin as substrate) is hydrolysis of the acyl-enzyme for class C β -lactamases (Knott-Hunziker *et al.*, 1982), but for class A β -lactamases it may well be cleavage of the β -lactam ring. The results described for the pH-dependence of k_{cat} and k_{cat}/K_m suggest the latter alternative for β -lactamase K1.

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REFERENCES

- Ambler, R. P. (1980) *Philos. Trans. R. Soc. London Ser. B* **289**, 321–331
- Bibring, T. & Baxandall, J. (1978) *Anal. Biochem.* **85**, 1–15
- Bicknell, R., Emanuel, E., Gagnon, J. & Waley, S. G. (1985) *Biochem. J.* **229**, 791–797
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Bruton, C. J. & Hartley, B. S. (1970) *J. Mol. Biol.* **52**, 165–178
- Bush, K., Freudenberger, J. S. & Sykes, R. B. (1982) *Antimicrob. Ag. Chemother.* **22**, 414–420
- Campbell, D. C., Gagnon, J., Reid, K. B. M. & Williams, A. F. (1981) *Biochem. J.* **195**, 15–30
- Cartwright, S. J. & Waley, S. G. (1984) *Biochem. J.* **221**, 505–512
- Connolly, A. K. & Waley, S. G. (1983) *Biochemistry* **22**, 4647–4651
- Cornish-Bowden, A. (1976) *Biochem. J.* **153**, 455–461
- Cornish-Bowden, A. (1983) *Methods Enzymol.* **25**, 333–344
- Davies, R. B. & Abraham, E. P. (1974) *Biochem. J.* **143**, 129–135
- Emanuel, E. L., Gagnon, J. & Waley, S. G. (1985) *Biochem. Soc. Trans.* **13**, 769–770
- Gray, W. R. (1972) *Methods Enzymol.* **25**, 121–128
- Habeeb, A. F. S. A. (1972) *Methods Enzymol.* **25**, 457–464
- Hamilton-Miller, J. M. T. (1963) *Biochem. J.* **87**, 209–214
- Hardy, L. W. & Kirsch, J. F. (1984) *Biochemistry* **23**, 1282–1287
- Holland, S. & Dale, J. W. (1984) *Biochem. J.* **224**, 1009–1013
- Jack, G. W. (1971) *Biochim. Biophys. Acta* **250**, 428–436
- Jaurin, B. & Grundström, T. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4897–4901
- Joris, B., Dusart, J.-M., Frère, J.-M., van Beeumen, J., Emanuel, E. L., Petursson, S., Gagnon, J. & Waley, S. G. (1984) *Biochem. J.* **223**, 271–274
- Joris, B., De Meester, F., Galleni, M., Reckinger, G., Coyette, J. & Frère, J.-M. (1985) *Biochem. J.* **228**, 241–248
- Knott-Hunziker, V., Petursson, S., Waley, S. G., Jaurin, B. & Grundström, T. (1982) *Biochem. J.* **207**, 315–322
- Laemmli, U. K. & Favre, M. (1969) *J. Biol. Chem.* **244**, 4406–4410
- Marshall, M. J., Ross, G. W., Chanter, K. V. & Harris, A. M. (1972) *Appl. Microbiol.* **23**, 765–769
- Perham, R. N. (1978) *Techniques in Protein and Enzyme Biochemistry B110*, pp. 1–39, Elsevier/North-Holland, Amsterdam
- Richmond, M. H. (1980) *J. Antimicrob. Chemother.* **6**, 445–453
- Richmond, M. H. & Sykes, R. B. (1973) *Adv. Microb. Physiol.* **9**, 31–88
- Ross, G. W. & Boulton, M. G. (1973) *Biochim. Biophys. Acta* **309**, 430–439
- Selwyn, M. J. (1965) *Biochim. Biophys. Acta* **105**, 193–195
- Sloma, A. & Gross, M. (1983) *Nucleic Acids Res.* **11**, 4997–5004
- Sykes, R. B. (1982) *J. Infect. Dis.* **145**, 762–765
- Waley, S. G. (1974) *Biochem. J.* **139**, 789–790
- Waley, S. G. (1975) *Biochem. J.* **149**, 547–551
- Wharton, C. W. & Szawelski, R. J. (1982) *Biochem. J.* **203**, 351–360

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