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Streptomyces albus G serine β-lactamase

Probing of the catalytic mechanism via molecular modelling of mutant enzymes

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In previous studies, several amino acids of the active site of class A β-lactamases have been modified by site-directed mutagenesis. On the basis of the catalytic mechanism proposed for the *Streptomyces albus* G β-lactamase [Lamotte-Brasseur, Dive, Dideberg, Charlier, Frère & Ghuysen (1991) Biochem. J. **279**, 213–221], the influence that these mutations exert on the hydrogen-bonding network of the active site has been analysed by molecular mechanics. The results satisfactorily explain the effects of the mutations on the kinetic parameters of the enzyme's activity towards a set of substrates. The present study also shows that, upon binding a properly structured β-lactam compound, the impaired cavity of a mutant enzyme can readopt a functional hydrogen-bonding-network configuration.

INTRODUCTION

The serine β-lactamases of classes A, C and D hydrolyse the β-lactam antibiotics by an acylation–deacylation mechanism. Several β-lactamases of class A have been studied by X-ray crystallography (Dideberg *et al.*, 1987; Herzberg & Moult, 1987; Moews *et al.*, 1990; Herzberg, 1991). They have almost completely superimposable three-dimensional structures. Their active site is located at the junction between an all-α domain and an α/β domain which consists of a five-stranded β-sheet with additional helices on both faces (Ghuysen, 1991).

The structure of the class A *Streptomyces albus* G β-lactamase has been resolved to 0.17 nm (1.7 Å) and optimized by energy minimization (Dideberg *et al.*, 1987; Lamotte-Brasseur *et al.*, 1991). The active site is a dense hydrogen-bonding network in which nine amino acids, namely S70, K73, S130, N132, E166, N170, K234, T235 and A237, and two water molecules, W1 and W2, are involved. The S70VFK73 motif, where S70 is the nucleophilic serine residue, on α2 of the all-α domain, occupies a central position in the cavity; the E166PELN170 motif, on a loop between α6 and α8 of the all-α domain, is at the entrance of the cavity; the S130DN132 motif, between α4 and α5 of the all-α domain, lies on one side of the cavity; the K234T235GA237 motif, on the innermost β3 strand of the α/β domain, forms the other side.

On the basis of these structural data, molecular-mechanics studies have allowed us to propose a mechanism of proton abstraction–donation during hydrolysis of benzylpenicillin by the *Streptomyces albus* G β-lactamase (Lamotte-Brasseur *et al.*, 1991). The same procedure has now been used to study the changes in the enzyme–ligand interactions resulting from changes in amino acids to which a role has been assigned in catalysis. As shown below, the rearrangements undergone by the active-site hydrogen-bonding network are compatible with the effects that these mutations have on the second order-rate constant for the acylation of the essential serine residue, characterized by the $k_{cat.}/K_m$ ratio.

MATERIALS AND METHODS

The active site of the *Streptomyces albus* G β-lactamase, defined by all the amino acids and water molecules present within a 1.5 nm (15 Å) radius around S70Cα, was optimized by energy minimization (Lamotte-Brasseur *et al.*, 1991) using the Amber molecular-mechanics V3 framework (Weiner *et al.*, 1984). As an initial guess for optimization, the torsion angles of the wild-type active site were retained as much as possible to simulate the amino acid changes in the mutant enzymes. Minimization of the structures was carried out until the root-mean-square gradient was < 0.0419 kJ/nm (0.1 kcal/Å). Water molecules were gener-

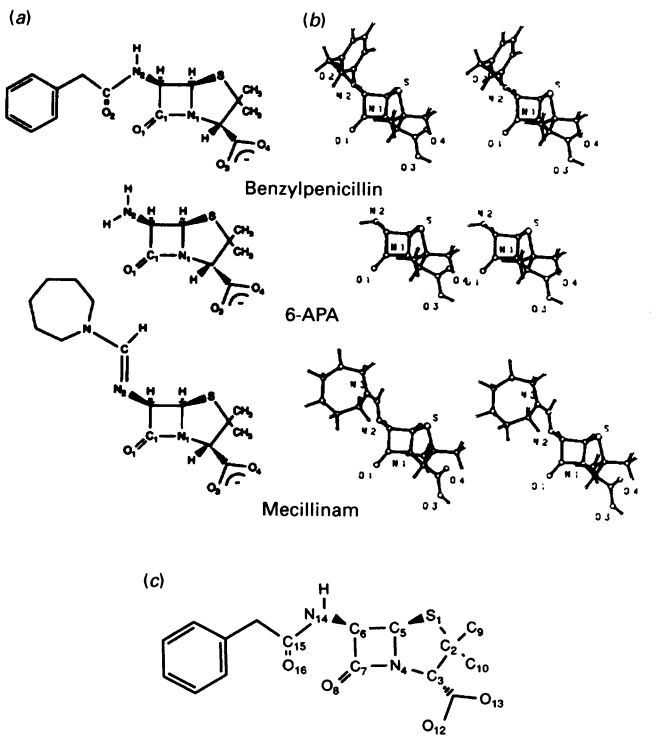

Fig. 1. (*a*) Arbitrary numbering of benzylpenicillin used in the present study, (*b*) optimized structures of benzylpenicillin, 6-APA and mecillinam, and (*c*) standard chemical numbering of penicillins

Abbreviation used: 6-APA, 6-aminopenicillanate.

ated by a Monte Carlo bath (using the Amber procedure) and their co-ordinates were minimized together with the enzyme-mutant co-ordinates.

The structures of benzylpenicillin, 6-aminopenicillanate (6-APA) and mecillinam, used as ligands, were optimized (Fig. 1) by the AM1 quantum-chemistry method (Dewar *et al.*, 1985). Each optimized molecule was docked in the cavity of the wild-type and mutant β-lactamases. The β-lactam carbonyl carbon C-1 was placed close to the O-γ of S70 and the oxygen atoms O-3 and O-4 of the carboxylate were oriented towards the bottom of the cavity, i.e. K234. The structures of the Michaelis complexes and, in one case, of the tetrahedral intermediate leading to enzyme acylation, were optimized as described for the wild-type enzyme, using CNDO charges on the ligand. The bond lengths, bond angles and ring dihedral angles of the β-lactam ligands were constrained to the AM1 values.

RESULTS

The atom numbering of the β-lactam ligands is arbitrary (see Fig. 1). The amino acids numbering of the wild-type *Streptomyces* β-lactamase and the enzyme mutants is that described by Ambler *et al.* (1991).

Selected β-lactamase mutants

The goal of the present study was to seek an answer to questions regarding the relationships between the alterations in the activity of the *Streptomyces albus* G β-lactamase caused by single amino acid changes and the modifications that these amino acid changes induce in the configuration of the hydrogen-bonding network of the enzyme cavity.

Benzylpenicillin binds to the active site of the wild-type β-lactamase (Fig. 2) via formation of hydrogen bonds between: O-3 of penicillin and the γ-OH of S130; O-4 of penicillin and the γ-OH of T235; O-1 of penicillin and the backbone NH groups of S70 and A237; N2-H of penicillin and the backbone carbonyl group of A237; and O-2 of penicillin and the side-chain NH_2 group of N132. Formation of the Michaelis complex has no or little effect on the pre-existing hydrogen-bonding network of the ligand-free active site, except that the hydrogen atom of S130γOH no longer interacts with W2, but is oriented towards N-1 of penicillin [as shown in Fig. 2; 0.311 nm (3.11 Å); 168.5°]. The Michaelis complex has other remarkable features. The oxygen atom of S70OγOH is 0.28 nm (2.8 Å) from C-1 of penicillin; the angle Cβ–Oγ (of S70)···C-1 (of penicillin) is 108°; the hydrogen atom of S70γOH is in interaction with Oε1E166 via W1 (the triad S70OγH→W1H→Oε1E166) and, more loosely, with Oε2E166. Hence the conditions are fulfilled to allow: proton abstraction of the S70γOH by Oε1E166 via W1; attack of C-1 of penicillin by O-γ of S70; polarization of C-1=O-1 of penicillin located in the oxyanion hole formed by the backbone NH groups of S70 and A237; and proton back-delivery to N-1 of penicillin by the W2, NζK73, NζK234 and S130γOH hydrogen-bonding subnetworks, thus achieving formation of the serine-ester-linked penicilloyl-enzyme.

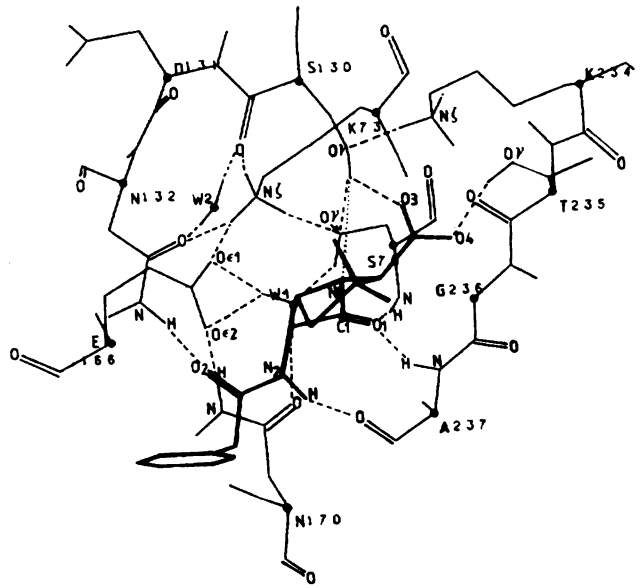

Fig. 2. Diagram of the hydrogen-bonding network of the Michaelis complex formed between benzylpenicillin and the active site of the wild-type *S. albus* G β-lactamase

From the foregoing it follows that the E166D mutation affects the presumed proton abstractor, whereas the S130G, S130A, N132S and K73R mutations affect, one way or another, the presumed machinery of proton transfer from E166 to S130. The hydrogen-bonding rearrangements induced by these mutations (in the ligand-free active sites and Michaelis complexes) are expressed in terms of X···H bond distances and YH···X bond-angle values (X and Y are heteroatoms) in Tables 1 and 2. These rearrangements, especially the fate of W1 and W2, are briefly described in the ensuing sections. They are illustrated in Figs. 3–7. A 0.25 nm (2.5 Å) distance is regarded as the upper limit for a hydrogen bond.

The effects caused by the selected mutations on the enzyme activity are expressed in terms of $k_{cat.}/K_m$ values (Table 3). It is assumed that the K73R and E166D mutations have effects on the *Streptomyces* β-lactamase comparable with those for the highly homologous β-lactamase I of *Bacillus cereus* 569/H (Gibson *et al.*, 1990), whose assumed catalytic residues are conserved, except for T235, which is replaced by S235. The $k_{cat.}/K_m$ values (equivalent to the second-order rate constant of enzyme acylation) show that the S130A and K73R mutations and, to a still greater extent, the E166D mutation, are profoundly detrimental, irrespective of the β-lactam ligand used (benzylpenicillin or 6-APA). The S130G and N132S mutations decrease selectively the enzyme activity towards 6-APA (to various extents, the S130G mutation being more deleterious than the N132S mutation), but do not affect significantly the activity towards benzylpenicillin.

Wild-type β-lactamase

Unlike benzylpenicillin, 6-APA and mecillinam have no exocyclic amide bond (Fig. 1). Thereby binding of 6-APA and mecillinam to the wild-type β-lactamase active site generates Michaelis complexes that lack the bond N132Nδ H···O-2 (in the case of 6-APA) and bonds N132Nδ H ...O−2 and N2-H···O=CA237 (in the case of mecillinam). Apart from the fact that these bonds are absent, the hydrogen-bonding networks have features comparable with those found in the Michaelis complex formed with benzylpenicillin (Fig. 2; Table 1, rows 1–4). Consistently, benzylpenicillin, 6-APA and mecillinam have almost identical substrate activity ($k_{cat.}/K_m \approx 3000\ mM^{-1}\cdot s^{-1}$).

S130G and S130A enzyme mutants

In the wild-type enzyme, the S130C=O interacts via W2 with the side-chain carbonyl group of N132 (Fig. 2), and the S130γOH is thought to be involved in proton back-donation. Hence the S130G and S130A mutations are expected to eliminate the

Table 1. X···H distances and YH···X bond angles (X and Y are heteroatoms) of hydrogen bonds within the active sites of the wild-type (WT) *Streptomyces albus* G β-lactamase, mutant enzymes and the Michaelis complexes formed with benzylpenicillin (pen), 6-APA and mecillinam (mec)

Bond distance (Å; note 1 Å≡0.1 nm) or bond angle (°)

		S70Oγ	W1			W2		S130Oγ				K73Nζ				K234Nξ	N170Nδ
		H···	H1···		H2···	H1···	H2···	H···				H1···		H2···	H3···	H1···	H···
Row	Enzyme or Michaelis constant	W1	Oε1E166 *Oδ1D166	Oε2E166 *Oδ2D166	Oδ1N170	Oδ1N132 *OγS132	OCS130 *OCG130 #OCA130	W2	OγS70	C-3	N-1	Oε1E166 *Oδ1D166	Oδ1N132 *OγS132	OCS130 *OCG130 #OCA130	OγS70	OγS130	Oε2E166 *Oδ2D166
1	WT	1.863	1.792	2.526	1.895	1.929	1.947	1.936	2.394	–	–	1.668	2.19	2.082	1.732	1.719	1.661
		148.5	164.0	136.3	156.2	144.5	142.1	164.8	106.9	–	–	145.4	114.1	121.1	148.5	147.2	161.3
2	+pen	2.07	1.703	2.194	1.794	1.836	1.816	4.223	2.409	1.962	3.11	1.698	2.031	1.695	1.686	1.685	1.669
		133.4	160.7	132.2	165.9	154.9	158.0	104.1	104.1	131.3	168.5	139.4	122.2	166.0	169.1	174.6	171.4
3	+6-APA	2.1	1.719	2.142	1.801	1.818	1.817	3.072	2.168	2.044	3.44	1.700	2.048	1.696	1.707	1.684	1.672
		126.7	155.2	136.7	167.7	150.4	151.0	117.4	114.6	128.6	177.1	140.7	122.7	171.0	167.2	173.9	170.5
4	+mec	2.083	1.706	2.203	1.801	1.832	1.819	4.317	2.310	1.953	3.130	1.699	2.031	1.694	1.687	1.694	1.672
		135.4	161.3	131.5	165.3	154.6	157.1	103.6	111.0	129.1	170.4	139.8	121.4	166.9	169.7	178.0	168.4
5	S130G	3.221	1.767	3.437	3.224	1.896	*1.877	–	–	–	–	1.665	2.174	1.694	1.713	–	1.68
		97.7	143.3	127.1	103.6	152.4	*168.2	–	–	–	–	149.8	104.1	159.9	161.0	–	158.9
6	+pen	2.098	1.731	2.103	1.794	1.846	*1.814	–	–	–	–	1.684	2.041	*1.684	1.697	–	1.674
		124.0	154.4	136.9	163.2	153.3	*158.1	–	–	–	–	140.2	119.8	*166.1	160.3	–	168.8
7	+6-APA	3.998	1.679	3.28	3.191	1.865	*1.815	–	–	–	–	1.687	2.137	*1.695	1.687	–	1.665
		100.1	164.6	129.4	148.9	149.8	*155.4	–	–	–	–	145.7	109.3	*160.1	154.4	–	163.1
8	S130A	2.173	1.662	3.164	1.866	1.829	#4.411	–	–	–	–	1.68	2.068	#1.688	1.669	–	1.672
		127.8	166.4	149.7	156.5	159.1	#53.2	–	–	–	–	145.1	111.8	#154.6	165.3	–	163.2
9	+pen	2.076	1.675	2.289	1.782	1.888	#4.308	–	–	–	–	1.667	2.037	#1.696	1.685	–	1.672
		132.4	161.3	133.5	170.1	142.4	#39.5	–	–	–	–	143.4	117.8	#151.9	165.3	–	167.9
10	+6-APA	2.221	1.638	2.75	1.816	1.835	#4.586	–	–	–	–	1.685	1.972	1.693	1.671	–	1.672
		126.3	167.4	139.4	174.1	146.0	#70.3	–	–	–	–	138.4	118.8	152.5	167.9	–	166.0
11	N132S	3.293	1.78	3.485	3.261	*3.537	2.094	1.934	3.142	–	–	1.765	*1.905	1.706	1.727	1.753	1.667
		99.5	140.1	120.5	108.6	*95.1	133.8	156.3	114.3	–	–	137.3	*134.3	172.5	162.6	145.5	168.4
12	+pen	2.026	1.695	2.177	1.761	*1.829	1.815	4.528	1.952	2.453	3.02	1.673	*2.159	1.707	1.709	1.672	1.678
		131.1	159.9	130.2	167.4	*151.8	167.7	112.3	128.6	98.7	155.9	148.9	*128.1	158.6	167.9	170.5	165.9
13	+6-APA	4.53	1.685	3.240	5.772	*1.876	1.846	3.76	3.423	2.055	2.80	1.909	*1.825	1.698	1.696	1.722	1.683
		100.0	172.2	146.1	16.5	*141.7	142.7	98.3	94.4	124.3	164.0	129.3	*145.0	170.0	156.0	170.6	175.6
14	+mec	2.053	1.716	2.167	1.766	*1.841	1.822	>5.0	2.364	2.029	3.22	1.690	*2.047	1.714	1.686	1.701	1.667
		139.0	161.4	131.1	167.1	*154.5	167.7	–	118.4	123.9	174.6	145.6	*128.9	155.2	163.3	169.3	163.6
15	K73R	1.777	2.38	1.701	1.84	3.76	1.834	1.829	4.189	–	–	–	see Table 2		–	1.72	1.665
		164.6	132.1	163.5	162.3	74.4	145.4	146.1	121.4	–	–	–	–	–	–	160.1	173.2
16	+pen	2.069	2.255	1.7	1.787	1.812	1.79	4.119	3.023	1.905	3.36	–	–	–	–	1.716	1.694
		139.5	133.1	160.8	175.6	164.8	166.4	103.8	89.4	130.3	142.1	–	–	–	–	163.6	172.6
17	E166D	2.768	*3.306	*1.681	2.694	1.892	3.788	1.817	3.325	–	–	*1.673	2.28	1.705	1.736	1.701	*1.675
		88.8	*144.6	*174.4	127.8	136.9	33.2	171.3	109.1	–	–	*148.1	103.3	170.4	158.7	165.5	*162.9
18	+pen	2.567	*2.794	*1.65	3.282	1.887	3.55	2.1	3.319	2.883	3.36	*1.655	2.403	1.699	1.737	1.692	*1.692
		91.1	*129.0	*170.6	83.6	132.4	40.8	157.4	110.6	89.7	139.7	*150.8	104.8	176.2	158.1	161.2	*166.2

Table 2. X···H distances and YH···X bond angles (X and Y are heterotoms) of hydrogen bonds around R73 within the active site of the K73R *Streptomyces albus* G β-lactamase mutant and the Michaelis complex formed with benzylpenicillin ('+pen')

	Bond distance (Å; note 1 Å≡0.1 nm) or bond angle (°)				
	R73Nε	R73Nη1	R73Nη2		
Enzyme or Michaelis complex	H··· Oε1E166	H··· Oγ S130	H1··· Oε1E166	H1··· Oδ N132	H2··· Oγ S70
K73R	1.707	1.707	1.72	2.176	3.007
	144.8	155.0	141.8	110.0	100.4
+pen	1.759	1.688	1.679	2.327	1.784
	144.2	158.4	146.1	101.6	134.8

Table 3. Catalytic efficiency ($k_{cat.}/K_m$) of the *S. albus* G and *B. cereus* wild-type β-lactamases and β-lactamase mutants

The catalytic-centre activities of the *Streptomyces* and *Bacillus* wild-type β-lactamases are identical with respect to benzylpenicillin ($k_{cat.}$ 2800 s^{-1} as against 2200 s^{-1}) and of the same order of magnitude with respect to 6-APA (720 s^{-1} as against 260 s^{-1}). The differences in the $k_{cat.}/K_m$ values are mainly due to differences in the K_m values. Results for *S. albus* G are from Jacob *et al.* (1990*a*,*b*); those for *B. cereus* are from Gibson *et al.* (1990).

		$k_{cat.}/K_m$ ($mM^{-1}\cdot s^{-1}$)		
Enzyme	Substrate...	Benzylpenicillin	6-APA	Mecillinam
S. albus G				
Wild-type		2800	3700	2600
S130G		480	4	
S130A		70	3	
N132S		1500	100	2500
B. cereus				
Wild-type		34000	170	
K73R		550	0.4	
E166D		77	2.2	

proton donor and to perturb the W2 hydrogen-bonding subnetwork.

Molecular modelling of the S130G mutant enzyme (Figs. 3*a* and 3*b*; Table 1, row 5) shows that W2 still closely interacts with Oδ N132 and forms a W2H···O=CG130 bond (similar to the W2H···O=CS130 bond in the wild-type enzyme), but W2 is displaced 0.29 nm (2.9 Å) towards the G130DN132 motif. Replacement of S130 by G also affects the W1 subnetwork. Though still closely interacting with Oε1E166, W1 is disconnected from Oε2E166 and Oδ N170 as well as from S70Oγ, thus damaging the S70Oγ H→W1H→Oε1E166 proton-abstractor triad.

Molecular modelling of the S130A mutant enzyme (Figs. 4*a* and 4*b*; Table 1, row 8) shows that the triad S70Oγ H→W1H→Oε1E166 remains as strongly hydrogen-bonded as it is in the wild-type enzyme but, as a result of the steric effect of the A130 methyl group, W2 is displaced 0.14 nm (1.4 Å) so that it forms, with N132 and W1, a strongly hydrogen-bonded N132δO···HW2H···W1 triad.

Docking 6-APA in the cavity of both mutant enzymes leads to optimized structures in which the configuration of the proton-abstractor machinery (Table 1, rows 7 and 10) remains incompatible with any significant catalytic activity.

By contrast, docking benzylpenicillin in the active site of the S130G mutant enzyme shows that, in the Michaelis complex, a strongly hydrogen-bonded S70Oγ H→W1H→Oε1E166 triad is reformed (Fig. 3*c*; Table 1, row 6). Moreover, in the tetrahedral intermediate, W2 undergoes such a displacement that it is located at approximately the same position as that occupied by S130γOH in the wild-type enzyme and has one of its hydrogen atoms oriented towards N-1 of penicillin (Fig. 3*d*). These induced configuration changes are probably able to restore a functional proton-abstraction–donation network, though its configuration differs, at least in part, from that of the wild-type β-lactamase. With reference to the wild-type enzyme, the S130G mutant enzyme hydrolyses benzylpenicillin effectively, with a 6-fold-decreased $k_{cat.}/K_m$ value.

Conversely, the presence of the additional methyl group in the S130A mutant results in a less efficient positioning of the W2 molecule, explaining an additional decrease of the rate of acylation by benzylpenicillin.

N132S mutant enzyme

Changing N132 is another way to perturb the S130C=O···HW2H···Oδ N132 triad and, as a consequence of this, the W2 and W1 hydrogen-bonding subnetworks. Modelling of the N132S mutant enzyme (Figs. 5*a* and 5*b*; Table 1, row 11) shows that W2 is in interaction with O=CS130 (as in the wild-type enzyme), but not with OγS132, and that W1 is in interaction with Oε1E166 (as in the wild-type enzyme), but not with S70Oγ. Docking benzylpenicillin (Fig. 5*c*; Table 1, row 12) and mecillinam (Fig. 5*d*; Table 1, row 14) in the mutant enzyme generates Michaelis complexes whose W1 and W2 hydrogen-bonding subnetworks re-adopt a configuration similar to that formed in the benzylpenicillin-, or mecillinam-, wild-type-enzyme Michaelis complex. 6-APA (Table 1, row 13), however, fails to restore a functional configuration. These observations are consistent with the data of Table 3.

K73R mutant enzyme

In the wild type β-lactamase, K73Nζ is central to the W2–W1 hydrogen-bonding subnetworks through the bonds that it forms with O=CS130, Oδ N132, Oε1E166 and OγS70 (Fig. 2; Table 1, row 1). Replacement of K73 by R73 perturbs the original configuration (Fig. 6*a* and 6*b*; Table 1, row 15, and Table 2). In the newly formed configuration, W2 still interacts with O=CS130, but it is connected to S130γOH, not to Oδ N132. W1 still interacts with S70γOH, but it is linked much more closely to Oε2E166 than to Oε1E166. Upon benzylpenicillin binding (Table 1, row 16, and Table 2), the N132δO···HW2H···O=CS130 triad is regenerated (as it occurs in the wild-type β-lactamase), but W1 remains in close interaction with Oε2E166, thus preventing restoration of the 'normal' S70Oγ H→W1H→Oε1E166 proton-abstractor triad. Consistently the K73R mutant enzyme has a very weak activity towards benzylpenicillin.

E166D mutant enzyme

Shortening the side chain of the carboxylic acid at position 166, i.e. the proton abstractor, has drastic effects (Figs. 7*a* and 7*b*; Table 1, row 17). W1 is disconnected from S70γOH and is hydrogen-bonded to Oδ2D166, not Oδ1D166; W2 still interacts with Oδ N132, but it is disconnected from OγS130 and forms with W1 a hydrogen-bonded W2-H···W1 dyad. Benzylpenicillin binding fails to regenerate a configuration compatible with proton abstraction–donation (Table 1, row 18). The E166D

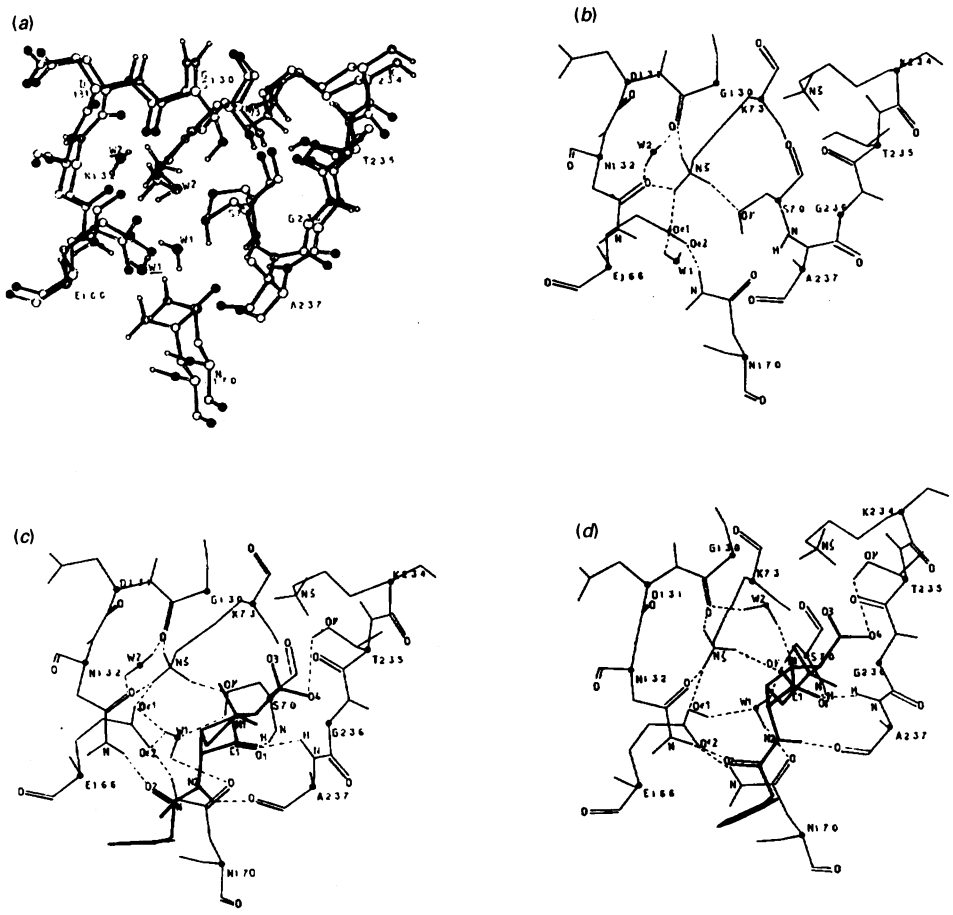

Fig. 3. *S. albus* G β-lactamase S130G mutant

(*a*) Superimposition of the cavity of the mutant (black polypeptide backbone) and the active site of the wild-type enzyme (white polypeptide backbone). Key to atoms: ○, oxygen; ⊘, nitrogen atoms. The underlined W1 and W2 belong to the mutant. (*b*) Diagram of the hydrogen-bonding network of the ligand-free cavity of the mutant. (*c*) Michaelis complex formed with benzylpenicillin. (*d*) Tetrahedral intermediate leading to penicilloylation of S70.

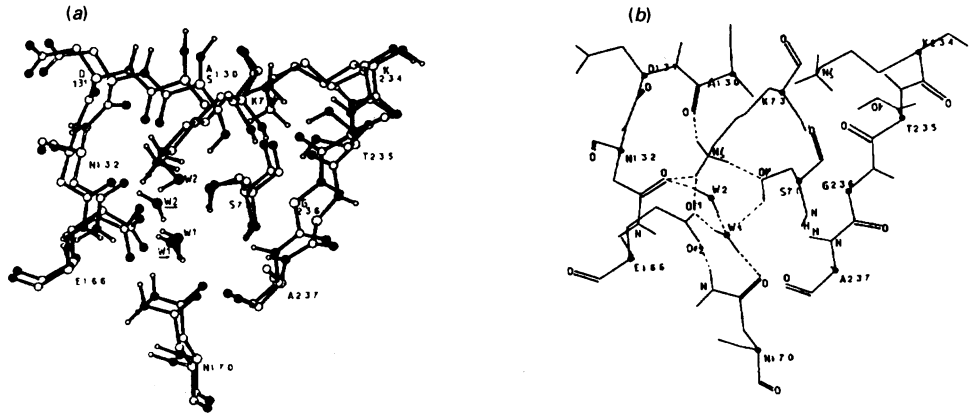

Fig. 4. *S. albus* G β-lactamase S130A mutant

(*a*) Superimposition of the cavity of the mutant (black polypeptide backbone) and the active site of the wild-type enzyme (white polypeptide backbone). Key to atoms: ○, oxygen; ⊘, nitrogen. The underlined W1 and W2 belong to the mutant. (*b*) Diagram of the hydrogen-bonding network of the ligand-free cavity of the mutant.

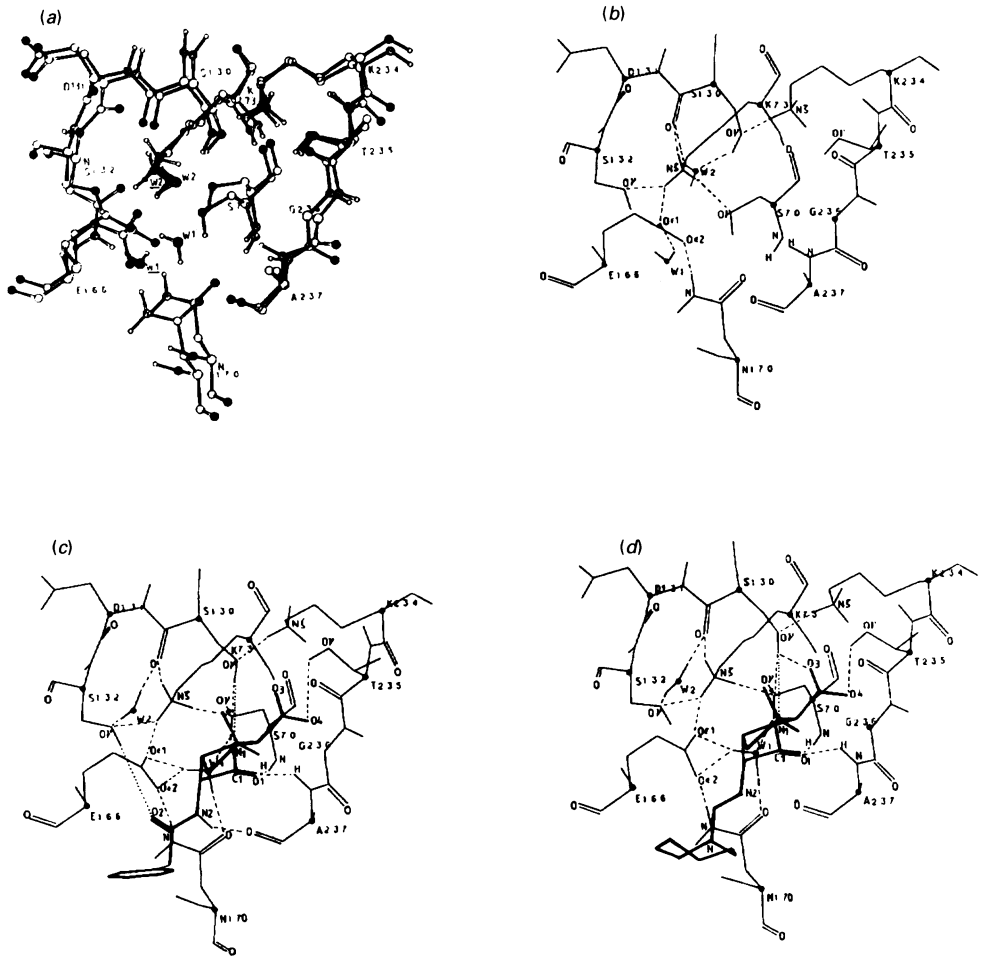

Fig. 5. *S. albus* G β-lactamase N132S mutant

(*a*) Superimposition of the cavity of the mutant (black polypeptide backbone) and the active site of the wild-type enzyme (white polypeptide backbone) Key to atoms: ○, oxygen; ⊘, nitrogen. The underlined W1 and W2 belong to the mutant. (*b*) Diagram of the hydrogen-bonding network of the ligand-free cavity of the mutant. (*c*) Michaelis complex formed with benzylpenicillin. (*d*) Michaelis complex formed with mecillinam.

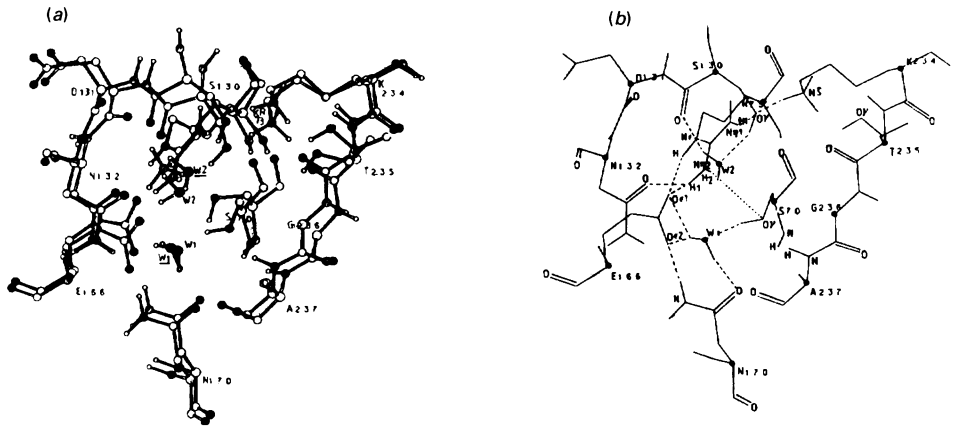

Fig. 6. *S. albus* G β-lactamase K73R mutant

(*a*) Superimposition of the cavity of the mutant (black polypeptide backbone) and the active site of the wild-type enzyme (white polypeptide backbone). Key to atoms: ○, oxygen; ⊘, nitrogen. The underlined W1 and W2 belong to the mutant. (*b*) Diagram of the hydrogen-bonding network of the ligand-free cavity of the mutant.

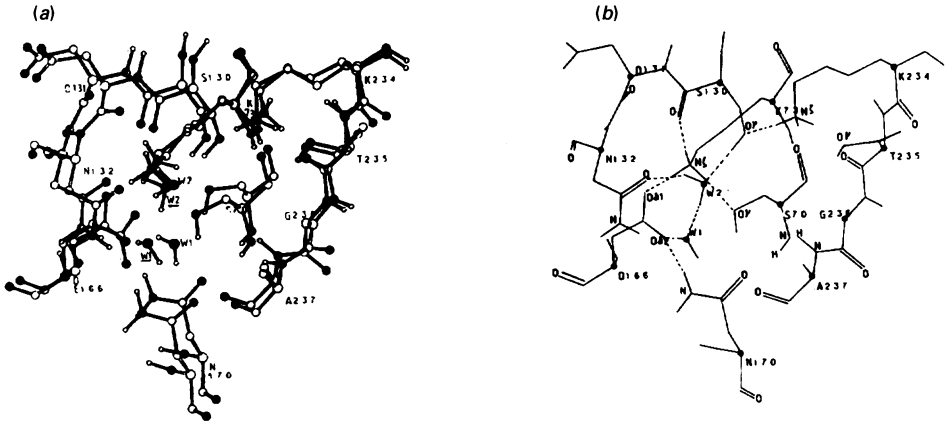

Fig. 7. *S. albus* G β-lactamase E166D mutant

(*a*) Superimposition of the cavity of the mutant (black polypeptide backbone) and the active site of the wild-type enzyme (white polypeptide backbone). Key to atoms: ○, oxygen; ⊘, nitrogen. The underlined W1 and W2 belong to the mutant. (*b*) Diagram of the hydrogen-bonding network of the ligand-free cavity of the mutant.

mutant enzyme has a 500-fold-decreased $k_{cat.}/K_m$ value with respect to benzylpenicillin.

DISCUSSION

It has been proposed (Lamotte-Brasseur *et al.*, 1991) that three amino acids, namely S70, E166 and S130, and two water molecules, W1 and W2, are central to the *Streptomyces albus* G β-lactamase-catalysed hydrolysis of penicillin. W1 transfers the proton from S70γOH to the abstractor Oε1E166, allowing attack of the carbonyl carbon atom of the β-lactam ring by S70Oγ. W2 is an integral component of the relay system through which a proton is delivered back to the nitrogen atom of the β-lactam ring, via S130γOH (thus achieving rupture of the amide bond and formation of the serine-ester-linked acyl-enzyme). To test the mechanism, the effects of mutations affecting E166 and S130 (the proton abstractor and a residue involved in proton donation) and N132 and K73 (involved in the configuration of the W1 and W2 hydrogen bonding subnetworks) have been examined by optimizing the complexes formed by docking benzylpenicillin, 6-APA and sometimes mecillinam in the mutated-enzyme cavities. The observed changes induced in the hydrogen-bonding network by each of the mutations studied well explain, at least to a first approximation, the effects that these mutations have on the catalytic activity of the β-lactamase.

The present studies also show that, owing to the high density of the hydrogen-bonding network, the enzyme cavity is a structure of high plasticity both structurally and mechanistically. Local modifications that cause the disappearance or weakening of any hydrogen bond may propagate its effects far from the mutated amino acid and modify the entire configuration of the cavity. A cavity, damaged by mutation, may regain functionality upon binding of a properly structured β-lactam compound, either by readopting a hydrogen-bonding configuration comparable with that of the wild-type enzyme or by utilizing an alternate route of proton shuttle from E166 to the nitrogen atom of the β-lactam ring. To all appearances, however, an intact, strongly hydrogen-bonded S70γOH→W1H→Oε1E166 triad is essential to the catalytic mechanism.

Adaptation of the β-lactamases to the environmental conditions is well documented. β-Lactamases show hysteretic kinetics. A unique conformation is induced in the enzymes by each of several closely related β-lactam substrates, and the enzymes can adjust to unfavourable modifications in the substrate (Samuni & Citri, 1979).

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