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To cite this version:
Laurent Maveyraud, Isabelle Saves, Odile Burlet-Schiltz, Peter Swarén, Jean-Michel Masson, et al.. Structural Basis of Extended Spectrum TEM -Lactamases CRYSTALLOGRAPHIC, KINETIC, AND MASS SPECTROMETRIC INVESTIGATIONS OF ENZYME MUTANTS*. Journal of Biological Chemistry, American Society for Biochemistry and Molecular Biology, 1996, 271 (18), pp.10482-10489. 10.1074/jbc.271.18.10482. hal-03004742

HAL Id: hal-03004742
https://hal-cnrs.archives-ouvertes.fr/hal-03004742
Submitted on 20 Nov 2020

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Structural Basis of Extended Spectrum TEM β-Lactamases

CRYSTALLOGRAPHIC, KINETIC, AND MASS SPECTROMETRIC INVESTIGATIONS OF ENZYME MUTANTS*

(Received for publication, July 31, 1995, and in revised form, January 16, 1996)

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The E166Y and the E166Y/R164S TEM-1 β-lactamase mutant enzymes display extended spectrum substrate specificities. Electrospray mass spectrometry demonstrates that, with penicillin G as substrate, the rate-limiting step in catalysis is the hydrolysis of the E166Y acyl-enzyme complex. Comparison of the 1.8-Å resolution x-ray structures of the wild-type and of the E166Y mutant enzymes shows that the binding of cephalosporin substrates is improved, in the mutant enzyme, by the enlargement of the substrate binding site. This enlargement is due to the rigid body displacement of 60 residues driven by the movement of the Ω-loop. These structural observations strongly suggest that the link between the position of the Ω-loop and that of helix H5, plays a central role in the structural events leading to extended spectrum TEM-related enzymes. The increased Ω-loop flexibility caused by the R164S mutation, which is found in several natural mutant TEM enzymes, may lead to similar structural effects. Comparison of the kinetic data of the E166Y, E166Y/R164S, and R164S mutant enzymes supports this hypothesis.

Bacterial resistance to penicillins and cephalosporins represents an increasing risk in the chemotherapy of Gram-negative bacterial infections. This resistance often arises from the emergence and the dissemination of the plasmid-encoded extended spectrum TEM-β-lactamases (EC 3.5.2.6) (Blazquez et al., 1995; Palzkill et al., 1995; Venkatachalam et al., 1994; Viadiu et al., 1995). Most of these proteins are derived from the Escherichia coli TEM enzyme, via the combination of a few point mutations, which have led, so far, to 27 TEM-related enzymes (Morosini et al., 1995). The parent TEM-1 is a very efficient enzyme and hydrolyzes penicillin substrates through an acylation and a deacylation step (Swarent et al., 1995). Extended spectrum TEM-related enzymes hydrolyze third generation cephalosporin substrates. Based on the kinetic data, two common characteristics of these enzymes are (i) a severalfold reduction in the catalytic turnover toward penicillins, and (ii) an increase in the catalytic efficiency against cephalosporin substrates (Raquet et al., 1994; Sowek et al., 1995).

The E166Y mutant displays one of the characteristic features of extended spectrum enzymes: similar activities toward penicillin and cephalosporin substrates (Delaire et al., 1991). The high resolution structure of this mutant shows unexpected structural differences compared with the wild-type enzyme. Comparison of the kinetic data of the E166Y, E166Y/R164S, and R164S (Sowek et al., 1991; Raquet et al., 1994) mutant enzymes suggests that these structural differences may play a key role in extending the substrate specificity of the TEM-related enzymes.

EXPERIMENTAL PROCEDURES

Protein Production—Site-directed mutagenesis, protein expression, and purification was performed as described (Saves et al., 1995b). For site-directed mutagenesis, the following oligonucleotides were used: E166Y, 3'-GTA ACC ATG GCC CTC GAC 5'; and E166Y-R164S, 3'-CAT TGA GCG GAA CTA TCA ACC ATG GGC CTC GAC TTA CTG 5'.

Determination of Kinetic Parameters of Substrate Hydrolysis and Determination of Acylation and Deacylation Rate Constants by Electrospray Mass Spectrometry (ESMS)†—The Michaelis-Menten kinetic parameters and the elementary rate constants by ESMS were determined as described previously (Saves et al., 1995a, 1995b).

Crystal Preparation—A solution of the E166Y mutant enzyme (14.5 mg/ml) in 45 mM potassium phosphate buffer (pH 7.8), containing 7.5% (v/v) saturated ammonium sulfate solution, was equilibrated, at 6 °C, against 100 mM potassium phosphate buffer (pH 7.8), containing 42%
(v/v) saturated ammonium sulfate solution and 4% (v/v) acetone. After equilibration, the supersaturated medium was seeded with a TEM-1 wild-type macrocrystal, and the ammonium sulfate was slowly increased to 47%. At this stage, the acetone was removed by slow evaporation. This procedure was repeated three times using, at each step, a newly formed E166Y crystal for seeding. Typical crystal size was 300 × 300 × 600 μm³. E166Y crystals are isomorphous to wild-type TEM-1 crystals (space group P2₁2₁₂₁), with cell parameters a = 43.1 Å, b = 64.7, c = 91.5 Å, and α = 90°, β = 90°, γ = 120°. X-ray Data Collection and Processing—Data were collected from a single crystal on a Siemens/Nicolet area detector, mounted on a four-circle goniostat. Cu-Kα X-rays were generated with a Rigaku rotating anode, operating at 40 kV and 80 mA. The crystal-to-detector distance was 12 cm, and the swing angle was 20°. The crystal was cooled at −4 °C during data collection. Data frames of 0.2° oscillation range with exposure time of 60 s were collected. Data were processed with the Xegen program (Howard et al., 1987) (Table I).

Results—All calculations concerning structure factors and electron density maps were done using the CCP4 programs package (Collaborative Computational Project, 1994). Electron density maps and protein structures were displayed on an Evans & Sutherland ESV3/30–33 graphic system, using the XPLOR package (Jones, 1988). Initial model corrections were manually applied according to (3Fo − 2Fc) and (2Fo − Fc) difference Fourier maps computed between 8.0- and 3.0-Å resolution, using calculated structure factors from the refined wild-type structure. The resolution was extended to 2.0 Å in four steps of both simulated annealing with the X-PLOR 2.1 package (Brünger, 1989) (from 300 to 300 K in 2.7 ps, time step of 0.025 ps) and manual corrections. Individual temperature factors were kept to 10 Å² in the first two refinement steps and were individually refined at 2.3- and 2.0-Å resolution. Solvent molecules were included as neutral oxygen atoms when they appeared as an electron density peak four standard deviations above the mean value in a (Fo − Fc) difference Fourier map, and at a reasonable distance from polar atoms. A tetrahedral arrangement of four positive electron density peaks was interpreted as a sulfate ion, provided by the crystallization medium, which was also found in the wild-type enzyme structure (Jelsch et al., 1993). Further refinement included cycles of energy minimization, individual temperature factor refinement, solvent occupancy refinement with PROLSQ (Hendrickson and Konnert, 1980), and model inspection in (2Fo − Fc) and (Fo − Fc) electron density maps. For the comparison of the E166Y mutant and the wild-type enzyme structures, the Cα positions of residues from strands S1 (residues 43–50), S2 (residues 56–60), and S5 (residues 259–266) of the main β-sheet were chosen to determine the superposition parameters. These strands are identical in both structures and are far from the mutation site. Rotation and translation parameters were then applied to all atoms of the E166Y mutant protein. The root mean square differences are 0.13 Å for main-chain atoms (when the 85–142 and 165–170 regions are excluded from the calculation) and 0.31 and 0.48 Å for the 85–142 and 165–170 main-chain atoms, respectively. Positional differences given in the text for whole side chains and residues are root mean square differences.

RESULTS

Structure of the E166Y Mutant—The final R-factor for all reflections between 8- and 1.8-Å resolution was 0.163. The refined structure contained 194 water molecules and one sulfate ion, and the root mean square atomic positional error was estimated to be 0.14 Å from a vs plot (Read, 1986). The deviations from ideal geometry at the end of the refinement are given in Table I. As in the wild-type protein structure, Met⁹⁹ and Leu²²⁰ are the only residues found in high energy conformations on a Ramachandran plot.

The overall structure of the E166Y β-lactamase is very similar to the 1.8-Å wild-type enzyme structure (Jelsch et al., 1993). However, the E166Y mutation leads to significant structural differences in two areas: the region 165–170 (WYPELN), which is part of the ß-loop, and residues 85–142, which move as a rigid body. This region contains helices H3 (109–111), H4 (119–128), and H5 (132–142) and two coil regions (86–108 and 112–118). It is part of the helical domain (residues 62–212) of class A β-lactamases (secondary structure assignment from Jelsch et al. (1993)).

The WYPELN Region—The movement of residues 165–170 (Fig. 1A) is directly related to the point mutation at position 166 and is restricted to these residues by the salt bridges Arg¹⁶⁴-Glu¹⁷¹ and Arg¹⁶⁴-Asp¹⁷². In order to accommodate the bulkier phenol group, 166 C-α moves by 0.7 Å from its position in the wild-type structure, away from the protein core. Tyr¹⁶⁶ OH is now at hydrogen bond distance (2.9 Å) from Ser⁷⁰ O- and Lys⁷³ N-, compared with the 4.2 Å distance between Glu¹⁶⁶ Oe-1 and Ser⁷⁰ O- in the wild-type enzyme. This movement drives the displacement of Trp¹⁶⁵ C-α and Pro¹⁶⁷ C-α, found at 0.3 and 1.0 Å, respectively, from their positions in TEM-1. Movement of the Trp¹⁶⁵ main-chain atoms, without significant modifications of its (φ,ψ) angles, forces the rotation of the indol ring that occupies the same steric volume. The C-α atoms of Glu¹⁶⁶ and Leu¹⁶⁹ move 0.5 and 0.2 Å, respectively, from their positions in the wild-type structure, but their side-chain conformations are little affected. More important changes occur for Asn¹⁷⁰, whose C-α is displaced by 0.5 Å and its side chain by 1 Å. There is no hydrogen bond exchanged between this side-chain amide group and Tyr¹⁶⁶ OH, in contrast to the important interaction, formed in the wild-type structure, with the Glu¹⁶⁶ carboxylate group. These structural modifications induce minimal effects on the main-chain atoms of Ser⁷⁰ and Lys⁷³, which are shifted by 0.15 Å. However, Ser⁷⁰ O- and Lys⁷³ N- move by 0.3 and 0.7 Å, respectively, and the amino group is now at hydrogen bond distance to Tyr¹⁶⁶ OH (3.0 Å) (Fig. 1A).

These structural modifications within the ß-loop affect the solvatation and the hydrogen bond network within the substrate binding site. Four water molecules are lost, and two water molecules and the sulfate ion are shifted compared with the wild-type enzyme (Fig. 1B). The water molecule Wat297,
Fig. 1. A, stereo view of residues 70-73 and 165-170 in the wild-type (thin lines) and E166Y (thick lines) structures. B, stereo view of the substrate binding sites in the wild-type (thin lines) and E166Y (thick lines) structures. The crystallographic water molecules in the TEM-1 \( \beta \)-lactamase are represented by crosses. Those in the E166Y enzyme are represented by dots. The location of the sulfate ion is also represented. C, stereo view of the H5 helix (residues 132-142) and of residues 165-166 of the \( \Omega \)-loop. The hydrogen bonds between the Asn136 side-chain atoms and the main-chain atoms of residue 166, and between Thr140 O-\( \gamma \) and Trp165 N-\( \varepsilon \) are shown by dotted lines. The wild type is in thin lines, and the E166Y enzyme is in thick lines. The shifts in position for 166 C-\( \alpha \), 136 C-\( \alpha \), and 140 O-\( \gamma \) are, respectively, of 0.7, 0.4, and 0.6 \( \AA \).
considered to be the nucleophile group in the deacylation step (Strynadka et al., 1992; Jelsch et al., 1993; Swarén et al., 1995), is shifted by 1.5 Å but maintains hydrogen bonds to Ser70 nitrogen, Ser70 O-γ, Asn70 side-chain amide group, and Wat391. The 1 Å motion of the sulfate ion has several consequences: (i) it leads to the exclusion of Wat323 from the oxyanion hole, and of Wat404, found in the vicinity of Ser130 main-chain oxygen atom in the wild type structure, (ii) one of its oxygen atoms is now at hydrogen bond distance (2.9 Å) from Ala237 nitrogen, and (iii) it provides more space in the vicinity of the Ser130, Ser235, and Arg238 side chains in the E166Y structure, where a new water molecule (Wat518) is found. All other water molecules occupy nearly the same positions in both structures (Fig. 1B). Solvent molecules that are bound to residues of the Ω-loop move accordingly and preserve their interactions, except for the two water molecules (Wat422 and Wat472) that are expelled by the previously described Asn170 side-chain motion. However, the 1 Å displacement of Pro167 generates a cavity that is filled by a new water molecule.

The 85-142 Region—The rigid body motion of the region 85-142 is significant when comparing the wild-type and the E166Y structures, solved and refined at 1.8 Å resolution with R-factors of 0.160 and 0.163, respectively. Residues Asn132, Asn136, and Thr140 located at three consecutive turns of helix H5, are in close contact with residues 165 and 166 and are directly affected by the Ω-loop movement (Fig. 1C). Asn132, whose side chain is at van der Waals distance from the side chain of Glu166 in the wild-type structure, is displaced (main chain, 0.3 Å; side chain, 0.5 Å) as a result of the increased steric volume at position 166. Asn136, located one helix turn away, whose amide side chains form two important hydrogen bonds to the Glu166 main-chain nitrogen and oxygen atoms in the wild-type structure, moves by 0.5 Å in order to preserve these interactions in the E166Y mutant. At the C terminus of helix H5, Thr140 O-γ moves by about 0.6 Å toward Trp165 N-ε, to which it is now hydrogen-bonded (Fig. 1C).

At the edges of this moving domain, residues Arg83 and Thr141, at the C termini of helices H2 and H5, respectively, and Glu89 and Arg93, in the connecting loop between helices H2 and H3, are clustered within a 4 Å radius sphere (Fig. 2). The movement of residue 141 induces the reorientation of the Arg93 side chain and the expulsion of Wat380, which was bridging Arg83 N-π1, Glu99 O-ε1, and Thr141 O-γ in the wild-type structure. In the mutant enzyme, Arg83 N-π1 is now hydrogen-bonded to the Thr141 main-chain oxygen atom.

Kinetics and Mass Spectrometry—Electrospray mass spectrometry experiments were used to determine the molecular weight of the E166Y mutant protein and of its molecular complexes. This soft ionization technique generates gas phase multicharged ions directly from the solution, and it allows characterization of the covalent intermediates of the reaction (Aplin et al., 1990). The E166Y enzyme readily formed an acyl-enzyme intermediate with penicillin G. This complex (measured molecular mass of 29,318 ± 3 Da) was the only enzyme species that could be detected during the steady state of the reaction. Its formation was extremely fast, and no free mutant enzyme could be detected 10 s after the reaction was started, even at 2.5 °C. The difference between the molecular mass of this complex and that of the free mutant enzyme is in excellent agreement with the molecular mass of penicillin G (334 Da). The acyl-enzyme concentration was equal to the initial E166Y protein concentration, and the determined kcat value at 37 °C (0.36 s−1) is identical to the kcat value measured from steady-state enzyme kinetics. In the mass range of the antibiotic, peaks corresponding to penicillin G and to the penicilloic acid reaction product (18 mass units higher) were followed during the time course of the reaction. After 5 min of reaction time at 37 °C, the only molecular species observed by ESMs were the acyl-enzyme complex (Fig. 3A), unreacted substrate, and the reaction product, penicilloic acid (Fig. 3B). When similar experiments were conducted with cephalorin as substrate, we found that most of the enzyme was present in its free state (data not shown). The acyl-enzyme complex represented less than 10% of the total protein species. Its molecular mass indicated that the only reaction was the β-lactam cycle cleavage upon acyl-enzyme formation and that no elimination of the C-3′ substituent on the dihydrothiazine ring occurred. As previously discussed (Saves et al., 1995a), a precise quantitation cannot be achieved on species amounting to 2-10% of the total ESM signal.

The kinetic data of the E166Y and the E166Y/R164S mutant enzymes are reported in Table III. The single E166Y mutation led to improved Km values for all substrates compared with the wild-type enzyme. However, compared with the E166Y enzyme, the E166Y/R164S double mutant enzyme discriminates between penicillin substrates (decreased Km values) and cephalosporin substrates (increased Km values). When compared with the wild-type enzyme, mutant proteins bearing the E166Y mutation display highly reduced kcat values, although they are higher for cephalosporin than for penicillin substrates. These effects in Km and kcat led to modified substrate spectra in both mutant enzymes, a characteristic of extended spectrum TEM-related enzymes, as exemplified by the R164S TEM-1 mutant (Table III) (Sowek et al., 1991; Raquet et al., 1994). Taken together, these data suggest that the extended substrate spectra of these mutants arise from structural events common to all of these proteins.
DISCUSSION

Effect of the E166Y Mutation on the Enzyme Structure and Consequences for the Binding of Cephalosporin Substrates—The structural modifications observed in the E166Y TEM-1 mutant are of two types. First, the mutation at position 166 induces the optimal short-range effect required to accommodate the larger side chain. Second, it induces an unexpected long range effect, in which a large part of the protein helical domain that bears important residues for catalysis is relocated.

The short range effect on the 165-170 Ω-loop region is restricted by the preceding Arg$^{164}$ and the following Glu$^{171}$. Their side chains are engaged in two salt bridges that are important for the conformation and the stability of the Ω-loop and for the correct location of residue 166 within the active site. The salt bridge between Arg$^{164}$ and Asp$^{179}$ is buried and inaccessible to solvent molecules, which increases the strength of this interaction.

Three hydrogen bonds are exchanged between the Asn$^{136}$


As the movement of residues 85–142 in the E166Y enzyme increases, this is larger than the deacylation rate. Indeed, the determined penicillin G substrate. This indicates that the acylation rate is larger than the thiazolidine ring.

The distance between Ser 130 O- and Thr 140 side-chain and Glu 166 main-chain atoms and between Thr 140 O- and Trp 162 NH. Thus, the movement of the 165–170 region leads to the concerted relocation of Asn 132, Asn 136, and Thr 140 (Fig. 1C). This rigid body motion of the H5 helix drives the displacement of the whole 85–142 region, which represents one-third of the enzyme helical domain (Fig. 4). This movement requires only a few small main-chain dihedral angle rotations and preserves all of the interactions that occur within this protein domain.

The 85–142 region bears residues that delineate one side (residues 104–105, 130, 132) of the substrate binding cavity. The active site bottleneck is found between the hydroxyl groups of Ser 130 and Ser 235, and is precisely the binding site of the thiazolidine ring of penicillins (Fig. 4). The distance between Ser 130 O- and Ser 235 O- is 5.4 Å in TEM-1 (Jelsch et al., 1993). The movement of residues 85–142 in the E166Y enzyme increases this distance to 6.0 Å. As the K_m of cefotaxime, measured by competition procedures, is lowered 1500-fold compared with TEM-1 (Delaire et al., 1991), we suggest that the increased active site aperture favors the better binding of cephalosporins. This is because the dihydrothiazine ring has a larger steric volume than the thiazolidine ring.

Mechanistic Aspects of the E166Y Mutation—Mass spectrometry measurements showed that during the steady state, the E166Y acyl-enzyme complex is the sole enzyme species with penicillin G as substrate. This indicates that the acylation rate is larger than the deacylation rate. Indeed, the determined k_cat value (0.36 s⁻¹) is 4000-fold smaller than the corresponding wild-type enzyme deacylation rate constant (1500 s⁻¹) (Christensen et al., 1990). Since residue 166 is chemically involved in the deacylation step, a large decrease in k_cat could arise from the different acid-base properties of Tyr compared with Glu as a proton acceptor.

Within experimental errors, k_g is identical to the k_cat value determined from steady-state kinetic measurements, which prevents calculation of k_g. However, examination of the k_cat/K_m values suggests slower acylation rates with the E166Y mutant enzymes compared with the wild-type enzyme. The role of Lys 73 in the acylation reaction was recently described (Swarén et al., 1995). Removal of the negative charge provided by Glu 166 significantly decreases the basicity of the unprotonated Lys 73 in the E166Y Michaelis complex, in line with the decrease of k_cat suggested by the steady-state kinetic data (detailed electrostatic calculations will be presented elsewhere).

ESMS experiments using cephaloridin showed that there is no elimination of the C-3 substituent of the substrate forming the acyl-enzyme complex. Thus, the lower K_m values with the E166Y mutant protein for cephalosporin substrates do not arise from a change in the rate-limiting step resulting from a different kinetic pathway, as was shown to occur with the PC1 enzyme (Faraci and Pratt, 1985, 1986). The rate-limiting step for cephalosporin hydrolysis by the wild-type enzyme is acylation (Saves et al., 1995a). A similar conclusion could be drawn for the E166Y mutant, as ESMS shows that more than 90% of the protein is found as free enzyme during the course of the reaction. However, the detection of a small amount of acyl-enzyme complex would suggest that the k_g and k_cat values are of similar magnitude.
tallographic refinement parameters indicate that the atomic positions in both structures have very little mobility, suggesting that both protein structures are representative of locked conformational states. In both enzymes, the Ω-loop conformation is severely constrained by the identical salt bridge interactions exchanged by Arg₁⁶⁴. The features of the x-ray structures provided the basis for comparing the kinetic data of the three mutant proteins: E₁₆₆Y, E₁₆₆Y/R₁₆₄S, and R₁₆₄S (Sowek et al., 1991; Raquet et al., 1994). They were examined with special emphasis on the Ω-loop conformation and flexibility, assigning a main role to residue 164.

In the TEM-1 enzyme, the R₁₆₄X (except for lysine) mutation, by removing two out of the four salt bridges of the Ω-loop, will release some, but not all, of its conformational constraints. This will likely affect the relative positions of the partners involved in the deacylation step. A slight displacement of the Ω-loop residue Glu₁⁶₆ will decrease the deacylation rate of good TEM-1 substrates. Indeed, this rate is related both to the direction of the electrostatic potential gradient between the Glu₁⁶₆ carboxylate and the ester carbonyl carbon of the acyl-enzyme complex, and to its magnitude, which is very sensitive to atomic positional differences (Swarén et al., 1995). The reduction in $k_{cat}$ is of 10–50-fold in the TEM-related enzymes bearing the Arg₁⁶₄ mutation (R₁₆₄S, R₁₆₄H, R₁₆₄S/E₁₀₄K, and R₁₆₄S/E₂₄₀K) but is only marginally affected in the E₁₀₄K and E₂₄₀K single mutants (Sowek et al., 1991, Raquet et al., 1994, Petit et al., 1995). This proposal is consistent with the situation observed in the PC1 β-lactamase, where a single salt bridge (Arg₁⁶₄→Asp¹⁷⁹) stabilizes the Ω-loop conformation. The substantial disorder of the loop resulting from the D₁⁷₉N mutation (Herzberg et al., 1991), led in that case to a 600-fold decrease in the $k_{cat}$ value for penicillin G.

The movement of the 85–142 region, in the E₁₆₆Y mutant enzyme structure, explains why binding is improved for cephalosporin substrates compared with the wild-type enzyme. A similar movement of the 85–142 region can be assumed to occur in the E₁₆₆Y/R₁₆₄S mutant protein. Interestingly, the additional R₁₆₄S mutation in the E₁₆₆Y enzyme has no effect with respect to the substrate spectrum ($k_{cat}/K_m$) for cephalexin substrates, whereas the single R₁₆₄S mutation in TEM-1 led to major kinetic differences. This paradox is explained if one assumes that the R₁₆₄S mutation allows structural perturbations similar to those observed, and already achieved, in the E₁₆₆Y protein. It explains why the additional R₁₆₄S mutation in the E₁₆₆Y enzyme is kinetically silent and offers a structural explanation of the consequences of the Arg₁⁶₄ mutation in the wild-type enzyme.

We propose that the conformational constraints of the Ω-loop, partly controlled by residue 164, and the position of the 85–142 region are interdependent in the TEM-1 enzyme. However, in the R₁₆₄X TEM-1 enzymes, this structural link would only be kinetically discernible when large substituents on the substrate molecule reach the Ω-loop residues, as is the case with third generation cephalosporin substrates (Raquet et al., 1994). The release of short contacts, achieved by the Ω-loop movement that drives the 85–142 region, accounts for the kinetic effects that are consistently found in extended spectrum TEM-related enzymes bearing the Arg₁⁶₄ mutation. First, the improvement of the binding of large cephalosporin substrates (i.e. ceftazidime) should increase the catalytic efficiencies for such molecules relative to penicillin substrates. $k_{cat}/K_m$ are, indeed, 1–3 orders of magnitude larger in the R₁₆₄S mutant than in the wild-type enzyme (Table III). Second, the large $k_{cat}/K_m$ differences found in TEM-1 within the cephalexin substrates should level out in the R₁₆₄X mutant enzymes. Indeed, the $k_{cat}/K_m$ of ceftazidime versus ceftazidime is decreased from $3 \times 10^4$ in the TEM-1 enzyme to 33 in the R₁₆₄S mutant enzyme. This property is fulfilled in all TEM mutant proteins bearing the R₁₆₄X mutation (Sowek et al., 1991, Raquet et al., 1994).

Mutations that occur in the vicinity of the Ω-loop residues, such as G₂₃₈S, were shown to drastically reduce the deacylation rate constant (Saves et al., 1995a). Natural mutants in this position also display extended substrate spectra, and work is in progress that will further illustrate the involvement of the Ω-loop region in the molecular evolution of the TEM-1 β-lactamase.

Acknowledgment—We are grateful to Martin Welch for a critical reading of the manuscript.

REFERENCES
Adachi, H., Ohta, T., and Matsuzawa, H. (1991) J. Biol. Chem. 266, 3186–3191.
Aplin, R. T., Baldwin, J. E., Schofield, C. J., and Waley, S. G. (1990) FEBS Lett. 277, 212–214.
Blazquez J., Morosini, M.-I., Negri, M.-C., Gonzalez-Leiza, M., and Baquero, F. (1995) Antimicrob. Agents Chemother. 39, 145–149.
Bruenger, A. T. (1990) X-PLOR Manual, version 2.1, The Howard Hughes Medical Institute.
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J. Biol. Chem. 1996, 271:10482-10489.
doi: 10.1074/jbc.271.18.10482

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