transition states are less polar than the ground states.[15] The acidity of the macrocyclic thiazolium ring is enhanced by the apolar environment provided by the cavity.[14a] In deuterated acetate buffer (pD 4.7), the rate of H/D exchange at C-2 of the thiazolium ring in **1b** is about 2.6 times faster than that measured for **1a**.

The results of control experiments (Table II) show that the high yield obtained in entry a is due to the efficient regeneration of **MeFl** at the anode. Direct oxidation at the anode (entry b) is not an efficient enough process to trap all of the "active aldehyde" intermediates.[16,17]

The full scope of the supramolecular electrochemical process mediated by **1b** and the very useful oxidation of aldehydes to carboxamides are now under investigation.

Acknowledgment. This work was supported by the Office of Naval Research and BASF AG, Ludwigshafen. We thank Dr. Dieter Degner, BASF, for continuing encouragement and support.

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Facilitation of the $\Delta^2 \rightarrow \Delta^1$ Pyrroline Tautomerization of Carbapenem Antibiotics by the Highly Conserved Arginine-244 of Class A β-Lactamases during the Course of Turnover

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Received October 31, 1991

The hydrolytic action of β-lactamases is the primary mechanism of bacterial resistance to β-lactam antibiotics.[1] Within the past several years a variety of new β-lactam drugs have been developed that show resistance to the action of these enzymes.[2] Carbapenems constitute a group of such β-lactamase-resistant molecules, and they possess potent activity against a wide spectrum of bacteria.[3] Studies on the mechanism of action of class A β-lactamases with carbapenems by Knowles and colleagues have indicated a biphasic profile for hydrolysis of carbapenems, with an initial fast phase for substrate turnover leading to a slower one within minutes.[4,5] It was demonstrated that subsequent to active site acylation of a β-lactamase (at Ser-70) by these molecules, the Δ^2-pyrroline analogue **2** may either undergo deacylation or tautomerize to the corresponding Δ^1-derivative (**3**). The ester bond of **3** is kinetically more resistant to hydrolysis because of a less favorable substrate positioning in the active site (Scheme I). We present evidence here that the highly conserved arginine-244[6] is the essential source of proton for the $\Delta^2 \rightarrow \Delta^1$ tautomerization of carbapenem antibiotics, as depicted in Scheme I.

High-resolution crystal structures for two class A β-lactamases from *Staphylococcus aureus* PC1[7] and *Bacillus licheniformis* 749/C[8,9] have been reported recently. The information from crystal structure, in conjunction with kinetic findings from our laboratory, indicated that the substrate carboxylate forms hy-

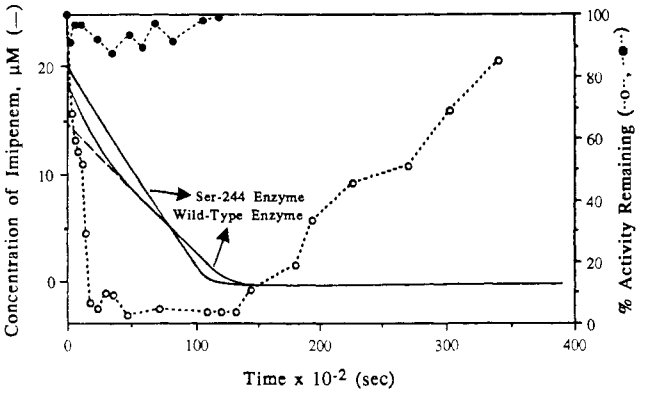


Figure 1. Hydrolysis of imipenem (20 μM) by the wild-type TEM-1 (2 μM) and the Arg-244-Ser mutant (2 μM) β-lactamases, in 0.1 M potassium phosphate buffer, pH 7.0, at room temperature (—). Extrapolation of the linear second phase of hydrolysis by the wild-type enzyme to time zero (---) and inhibition of activity of the wild-type (○) and the Ser-244 mutant (●) TEM-1 β-lactamases (as monitored for the turnover of benzylpenicillin) are indicated.

Scheme I

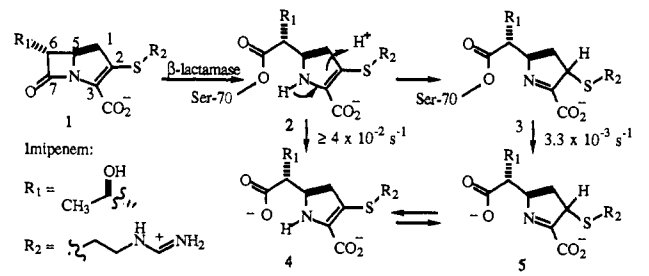
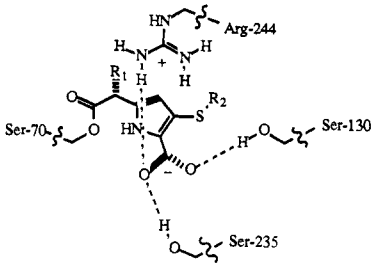


Chart I



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drogen bonds to the side-chain functions of Arg-244, Ser-130, and Ser/Thr-235 upon active site anchoring of the substrate.[10] This mode of active site binding brings one N_η of the Arg-244 side chain from the β-face of an active site-bound carbapenem to approximately 2.5–3 Å from the C_2 of the substrate (Chart I). It appeared to us that the Arg-244 guanidinium moiety could serve—in a truly adventitious manner—as the source of proton for the tautomerization of **2** to yield **3**.[11] To test this concept, we have studied the turnover of imipenem (*N*-formimidoylthienamycin) by the wild-type TEM-1 β-lactamase and the Arg-244-Ser mutant enzyme.[12] The wild-type enzyme hydrolyzes imipenem in the characteristic biphasic manner reported for other carbapenems (Figure 1).[4,5] The more rapid first phase of hydrolysis leads to a slower steady-state phase, which does not end until the substrate is exhausted. Extrapolation of the rate of the linear second phase back to time zero indicated a burst size of 2.5 imipenem molecules hydrolyzed by each enzyme molecule prior to the onset of the slower second phase, as reported for other carbapenems.[4,5] However, the Ser-244 mutant enzyme showed a monophasic hydrolysis of imipenem with a rate close to that of the first phase of hydrolysis by the wild-type enzyme;[13] the transition to the slower second phase was entirely eliminated. This observation suggests that the Ser-244 enzyme catalyzes a steady-state hydrolysis of imipenem without tautomerization of **2** to **3** (i.e., **1** → **2** → **4**). The first-order rate for deacylation of **3** was calculated for the slow phase of imipenem turnover by the wild-type enzyme at 3.3×10^{-3} s^{-1}, according to the method of Glick et al.[14] The corresponding deacylation rates for both the Ser-244 enzyme and the first phase of the wild-type protein were fast and could not be measured accurately. Steady-state kinetic parameters for the turnover of imipenem by the Ser-244 enzyme were evaluated at $K_m = 27$ μM and $k_{cat} = 0.04$ s^{-1}. The value of k_{cat} indicates the lower limit on the rate of deacylation of **2** ($k_{H_2O} \geq k_{cat} = 0.04$ s^{-1}). These findings reveal that the rate of hydrolysis of **2** is at the minimum 12-fold faster than that of **3**. Inhibition of the wild-type β-lactamase as monitored by turnover of benzylpenicillin, which is due to transient active site acylation by imipenem, is rapid and is reversed considerably after all the substrate is consumed (Figure 1). On the other hand, the Ser-244 mutant enzyme was inhibited only marginally (<15%), in part because of rapid hydrolysis of the acyl-enzyme intermediate.

In conclusion, we have shown that Arg-244 of the TEM-1 β-lactamase is responsible for the biphasic turnover profile of imipenem, a representative carbapenem antibiotic, and we were able to estimate the hydrolysis rates of the pyrroline tautomers **2** and **3** from the active site. Furthermore, we have shown that the Ser-244 mutant enzyme is not effectively inhibited by imipenem. The recent report of the occurrence of the plasmid-borne Ser-244 mutant TEM-1 β-lactamase in a clinical isolate might herald an anticipated compromise of the continued effectiveness of imipenem as a backup drug against multiresistant clinical isolates.[15]

Acknowledgment. We thank Dr. James Knox for kindly providing us with the crystal coordinates for the *Bacillus licheniformis* 749/C β-lactamase, and Dr. Elias Manavathu for carrying out the mutagenesis experiments.

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(11) An additional possibility is that a coordinated water molecule to the electrophilic guanidinium moiety of Arg-244, whose pK_a would be lowered by this interaction, is the source of proton. Calculations at ab initio level are being carried out to provide a more quantitative estimate of the actual decrease in pK_a of a water molecule that is hydrogen bonded to a positive ammonium ion center—collaboration with R. Bach of this Department. Preliminary data support the basic idea set forth in this footnote.

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(13) Our measurements of the binding energies of the Arg-244 guanidinium group to the substrate carboxylate (see Chart I) attributed 1.3–2.3 and 0.3–1.0 kcal/mol to the stabilization of both the transition and ground states of penicillins and cephalosporins, respectively (unpublished results). Therefore, the slightly slower rate for hydrolysis of imipenem by the Ser-244 mutant β-lactamase, compared to the early parts of the fast phase of hydrolysis by the wild-type enzyme, is due to the loss of a weak hydrogen bond between imipenem and the mutant protein in the enzyme-substrate complex.

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Switchable Liquid Crystalline Photopolymer Media for Holography

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Received October 8, 1991

Liquid crystalline materials for optical information storage have been sought by several groups in the last few years.[1] The generally high birefringence of liquid crystals offers an attractive means to achieve large refractive index modulations (Δn) through photochemical manipulation of order and/or orientation. In this report, results from a simple approach to orientational control are described, with the introduction of a new type of highly efficient and switchable holographic recording medium. Very few holographic media are known that allow for modulation of the diffracted light intensity (i.e., efficiency).[2]

Photopolymerization of liquid crystalline monomers has been shown in some cases to "lock in" or even increase the mesomorphic order.[3] If the photopolymerization is induced by interfering coherent beams, then a grating will be produced (Figure 1) with the regions of constructive interference becoming locked by polymerization and the regions of destructive interference remaining as fluid monomer. Upon application of an electric field, the monomeric regions should align with the field, increasing Δn to approximately the birefringence of the monomer.[4] The large increase in Δn should produce a corresponding increase in diffraction efficiency.

We have tested this approach with two monomers from the literature: the aromatic diacrylate **1**[5] and the cholesteryl methacrylate **2**.[6] The holographic results were obtained by using either a phase insensitive holography (PIH) or a phase modulated holography (PMH) apparatus similar to those described by Bräuchle and co-workers.[7] These methods use crossed laser beams to create

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