The specifically mutated enzyme thiol β-lactamase has been expressed in *Escherichia coli* by means of the trp promoter and purified to homogeneity. The gene for this enzyme results from a single base change N140 A → T in the gene of pBR322 RTEM β-lactamase (EC 3.5.2.6, penicillinase, penicillin amido-β-lactamhydro-lase) which alters the codon for the active site Ser 70 to that for Cys. Precursor thiol β-lactamase is processed to give the same NH2-terminal sequence as that for wild type enzyme. In contrast to the wild type enzyme, thiol β-lactamase contains one free titratable thiol group/molecule. Thiol β-lactamase catalyzes the hydrolysis of β-lactams with a substrate specificity that is distinct from that of wild type enzyme. For benzylpenicillin and ampicillin, the $K_m$ values are similar to wild type values although the $k_{cat}$ values are 1–2% that of wild type enzyme. For the cephalosporin nitrocefin, the $K_m$ is greater than 10-fold that of the wild type and the $k_{cat}$ is at least as large as the $k_{cat}$ for the wild type enzyme. Thiol β-lactamase is different from wild type β-lactamase in that it is not competitively inhibited by boric acid although a small degree of noncompetitive inhibition does occur. Whereas the circular dichroism spectra of both enzymes are nearly identical, thiol β-lactamase at 40 °C is 3-fold more resistant to trypsin than is the wild type enzyme.

The primary sequences of proteins such as hormones and enzymes can be specifically altered by using recombinant DNA methods either to modify existing DNA sequences or to synthesize entirely new sequences. By making predetermined changes in the primary sequence of a protein, the structure-function relationships of any residue can be directly addressed. Within the previous year, several examples illustrating this approach have appeared in the literature. Through specific amino acid substitutions, different studies have investigated the relationship of the sequence of signal peptides to protein secretion (1–3), the role of tyrosine phosphorylation in protein secretion (1–3), the role of tyrosine phosphorylation in the relationship of the sequence of signal peptides to substrate specificity (1–3), and the function of a conserved cysteine in tyrosyl-tRNA synthetase (6). During this period, we reported the construction of thiol β-lactamase, the beginning of our investigation into the role of the specific chemical groups that occur at the active site of hydrolytic enzymes (7).

β-Lactamases hydrolyze the β-lactam amide bond of antibiotics such as penicillins and cephalosporins and are the most common reason for bacterial resistance to these drugs (8). Understanding the mechanism of these enzymes is pertinent to the design of pharmacologically important inhibitors (9). Of the three classes of β-lactamases (A, B, and C), A and C are serine hydrolytic enzymes (10). Recent studies, mainly utilizing poor substrates, have shown that for these enzymes, a serine residue (Ser 70 for Class A and Ser 80 for Class C) acts as an active site nucleophile with the formation of an acyl-enzyme intermediate (11–15). As a result of this intermediate, product partitioning occurs for the Class C enzymes when alcohols are present (15). For one Class A enzyme, RTEM β-lactamase, the actual acyl-enzyme intermediate was spectrophotometrically observed during the slow hydrolysis of the inhibitor cefoxitin (11). These serine β-lactamases together with their homologous β-alanylcarboxypeptidases do not appear to possess a conserved histidine residue and thus they form a family of serine hydrolytic enzymes distinct from proteases like subtilisin (16).

While both serine and cysteine residues can be effective nucleophiles (both serine and cysteine proteases occur in nature), the active site hydroxyl and sulfhydryl groups do have unique chemical and physical properties. The replacement of an active site serine by a cysteine residue, a net substitution of an –OH group by an –SH group, probes the mechanism of catalysis by perturbing in a precise and significant manner the chemical environment of the active site. Chemical methods were previously used to replace the –OH group of subtilisin’s active site serine by –SH (17, 18). We substituted the Ser 70 of pBR322 RTEM β-lactamase (EC 3.5.2.6, (penicillinase, penicillin amido-β-lactamhydro-lase) with Cys by recombinant DNA methods. Specifically, by making a single base mutation in the gene for pBR322 β-lactamase, we changed the codon for Ser 70 AGC to TGC (Cys). The new enzyme, thiol β-lactamase, was active in vivo, and its in vitro β-lactamase activity was sensitive to mercuric ion. We report here the purification of this enzyme and describe some of its enzymatic and physical chemical properties.

**EXPERIMENTAL PROCEDURES**

*Strains and Plasmids—* *Escherichia coli* K12 strain HB101 (19) and plasmids pBR322 (20) and pIS2 (7) have been described. Plasmid pBR322 contains the gene for wild type RTEM1 β-lactamase as its ampicillin-resistance marker. Plasmid pIS2 differs from pBR322 by...

*The amino acid and nucleotide sequence numbering is that of Sutcliff (Sutcliff, J. G. (1978) *Proc. Natl. Acad. Sci. U. S. A.* 75, 3737–3741).*
the single base substitution N→T
Expression of Thiol β-Lactamase—Plasmid pIS2 which encodes thiol β-lactamase differs from pBR322 in the one base substitution N^410 A → T which alters codon AGC for Ser 70 of β-lactamase to that for Cys (TGC). Whereas E. coli cells hosting pBR322 are resistant to concentrations of ampicillin greater than 1 g/liter, the resistance given by pIS2 is limited to 10 mg/liter. Antibody precipitation experiments using rabbit anti-β-lactamase serum reveal that levels of thiol β-lactamase expression given by pIS2 are similar to that of wild type enzyme by pBR322. To increase expression, we utilized the plasmid pOTBL in which a partial TspI restriction fragment containing the trp promoter oriented toward the thiol β-lactamase gene has been inserted into the CiaI site of pIS2 (21). The trp promoter was induced by incubating cells in minimal medium. After 4 h, the total cellular protein had increased 6-fold, while the cell density had only doubled. At this point, the protein band corresponding to thiol β-lactamase represented greater than 25% of the total cell protein, an approximate 108-fold enhancement (see Fig. 1). A much lower increase of 15-fold was observed for thiol p-lactamase (22) was found to be less satisfactory for thiol β-lactamase, with reduction in yield occurring during the ion exchange chromatography step. Consequently, an alternate and more rapid purification procedure was developed for purifying thiol β-lactamase. Approximately 30% of the enzyme could be extracted from cells by washing with TE buffer. This preparation was then directly applied to a chromatofocusing column (elution at pH 5.8) followed by size exclusion gel permeation chromatography. At this point, the enzyme was pure by SDS-acrylamide electrophoresis (see Fig. 1).

Sequence Characterization—The sequence of the first 10 residues of thiol β-lactamase was determined by the automated Edman method and was found to be identical to that of mature wild type enzyme with histidine as the NH2-terminal amino acid (31). The mature wild type β-lactamase contains 2 cysteine residues at positions 75 and 121 linked in a disulfide bond (32). Pollitt and Zalkin (32) have shown that the reduction of this disulfide under denaturing conditions can be monitored by the difference in electrophoretic mobilities of the oxidized and reduced forms. The identical phenomenon with similar rates of reduction was observed for thiol β-lactamase (see Fig. 2). Titration of native thiol β-lactamase with 4-pyridyldisulfide reveals the presence of one free thiol group/enzyme molecule. The 4-pyridyldisulfide-derivatized enzyme was inactive but could be reactivated with reducing agents, e.g. 2-mercaptoethanol. In a similar manner, thiol β-lactamase was reversibly deactivated with p-chloromercuribenzoate.

CD Spectral Analysis—The CD spectra of thiol β-lactamase at 24 °C were superimposable onto that for wild type enzyme (see Fig. 3). The fractions of α helices, β sheets, and nonrepeating structure in the proteins were estimated from least squares fits of the observed CD spectra from 204 to 243 nm to linear combinations of the reference spectra for secondary structure (33). The values found were for α, 0.40; β, 0.20; and nonrepeating, 0.40.

Trypsin Deactivation—While both wild type β-lactamase and thiol β-lactamase are resistant to trypsin at room temperature, at higher temperatures they are cleaved to smaller peptides (31, 34). The initial cleavages can be followed by the loss in β-lactamase activity. At 40 °C under the conditions described (see Fig. 4), the first order rate constants observed for the trypsin deactivations of wild type β-lactamase and thiol β-lactamase were 0.06 and 0.02 min⁻¹, respectively. The product of the wild type gene isolated as a p-chloromercuri-

FIG. 1. Expression and purification of thiol β-lactamase. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was run on whole cell protein from cultures pOTBL/HB101 in which the expression of thiol β-lactamase by the trp promoter was enhanced. The trp promoter was induced by incubating cells at 37 °C in minimal medium. The lanes are, respectively, molecular weight standards, whole cell protein after induction at 0, 1, 2, 3, and 4 h, pure thiol β-lactamase, pure wild type β-lactamase, standards, pure thiol β-lactamase, and standards. The molecular weight standards are phosphorylase b (Mr = 92,500), bovine serum albumin (Mr = 66,200), ovalbumin (Mr = 45,000), carbonic anhydrase (Mr = 31,000), soybean trypsin inhibitor (Mr = 21,500), and lysozyme (Mr = 14,400).

FIG. 2. Electrophoretic monitoring (SDS-10% polyacrylamide gel) of the reduction of the disulfide bonds of wild type β-lactamase and thiol β-lactamase under denaturing conditions. Pure β-lactamase (5 μg) was heated at 95 °C in 30 μl of 50 mM sodium phosphate, pH 6.0, 2% SDS, 4% Ficoll, 40 mM dithiothreitol. Samples were removed at 2-min intervals and cooled to 0 °C.
Thiol β-Lactamase: Purification and Properties

Fig. 3. CD spectra of thiol β-lactamase and wild type pBR322 β-lactamase. CD spectra were recorded on a Jasco 500 spectropolarimeter at 24 °C with protein concentrations of 0.1 mg/ml and a path length of 1 mm for wild-type β-lactamase (Δ) and thiol β-lactamase (○).

FIG. 4. Trypsin inactivation of thiol β-lactamase and wild type β-lactamase. A mixture (16 μg) of thiol β-lactamase and wild type enzyme (100:1) in 70 μl of 20 mM Tris/HCl, pH 7.6, 20 mM NaCl was treated with trypsin (16 μg) in 8 μl of 0.1 M Tris/HCl, pH 8.0. The solution was kept at 40 °C. The progress of the deactivation was monitored by removing 2-μl aliquots and assaying for nitrocefin hydrolysis activity. The amount of wild type enzyme present was determined by measuring the rate of nitrocefin hydrolysis (0.14 mM) after the addition of 2-mercaptoethanol (1 mM) and bovine serum albumin (0.15 mg/ml). A small (5%) correction in the observed rate was made to account for the spontaneous reaction of nitrocefin with the reducing media. Thiol β-lactamase activity was obtained by taking the difference in the observed total and wild type activities. ○, wild type β-lactamase; ○, thiol β-lactamase.

benzilate-resistant revertant of the thiol β-lactamase gene possessed the same trypsin sensitivity as authentic wild type enzyme.

Enzymatic Activity—Thiol β-lactamase catalyzes the hydrolysis of representative penicillins and cephalosporins (see Tables I and II). The observed $K_{\text{m}}$ values for benzylpenicillin and ampicillin, 50 and 120 μM, respectively, are similar to those observed for wild type β-lactamase. Thiol β-lactamase $k_{\text{cat}}$ values for these substrates are 1–2% that of the wild type enzyme. In the hydrolysis of nitrocefin (a cephalosporin possessing a 2,4-dinitrophenyl ring in conjugation with the β-lactam amide nitrogen), saturation of thiol β-lactamase was not observed at accessible substrate concentrations (0.1–2.5 mM) (Table II). At the highest substrate concentration (2.5 mM), the rate constant for nitrocefin hydrolysis was 50% the $V_{\text{max}}$ for wild type enzyme although $k_{\text{cat}}/K_{\text{m}}$ was 2% the wild type value. Since the thiol β-lactamase $K_{\text{m}}$ for nitrocefin is greater than 2.5 mM, its extrapolated $V_{\text{max}}$ is greater than that for wild type enzyme. The presence of penicillin or ampicillin at concentrations equivalent to their $K_{\text{m}}$ values reduced thiol β-lactamase rates of hydrolysis of nitrocefin by 50%. Apparently, these substrates interact with the enzyme at the same active site.

$K_{\text{m}}$ and $k_{\text{cat}}$ values observed at different pH values for benzyl penicillin hydrolysis are presented in Table III. Between pH 4.5 and 9.0, the $k_{\text{cat}}$ for thiol β-lactamase is fairly constant as it is for wild type enzyme. The $K_{\text{m}}$ values for both enzymes increase at high pH.

Boric Acid Inhibition—Previous workers (35) have shown that boric acid competitively inhibits serine β-lactamases with a $K_{\text{a}}$ of 1 mM. A small degree of noncompetitive inhibition could not be excluded. We observe that competitive inhibition

| Substrate               | $K_{\text{m}}$ (μM) | $k_{\text{cat}}$ (μM s⁻¹) |
|------------------------|---------------------|--------------------------|
| Benzylpenicillin       | 0.06 (0.05)         | 20 (1700)                |
| Ampicillin             | 0.12 (0.05)         | 50 (2000)                |
| Cephaloridine          | 1.8 (1.0)           | 2 (1700)                 |
| Nitrocefin             | >2.5 (0.11)         | >400 (900)               |

| [Nitrocefin] | $\nu$ (mM s⁻¹/mol enzyme) |
|--------------|---------------------------|
| 10% dimethyl sulfoxide | 50 mM phosphate, pH 7.0  |
| 50 mM formate   | 4.0, 0.3 M NaCl           |
| 50 mM acetate   | 4.5, 0.3 M NaCl           |
| 50 mM acetate   | 5.5, 0.3 M NaCl           |
| 50 mM phosphate | 6.0, 0.3 M NaCl           |
| 50 mM phosphate | 7.0, 0.3 M NaCl           |
| 50 mM phosphate | 0.1 M ampicillin          |

The observed $K_{\text{m}}$ values for benzylpenicillin and ampicillin, 50 and 120 μM, respectively, are similar to those observed for wild type β-lactamase. Thiol β-lactamase $k_{\text{cat}}$ values for these substrates are 1–2% that of the wild type enzyme. In the hydrolysis of nitrocefin (a cephalosporin possessing a 2,4-dinitrophenyl ring in conjugation with the β-lactam amide nitrogen), saturation of thiol β-lactamase was not observed at accessible substrate concentrations (0.1–2.5 mM) (Table II). At the highest substrate concentration (2.5 mM), the rate constant for nitrocefin hydrolysis was 50% the $V_{\text{max}}$ for wild type enzyme although $k_{\text{cat}}/K_{\text{m}}$ was 2% the wild type value. Since the thiol β-lactamase $K_{\text{m}}$ for nitrocefin is greater than 2.5 mM, its extrapolated $V_{\text{max}}$ is greater than that for wild type enzyme. The presence of penicillin or ampicillin at concentrations equivalent to their $K_{\text{m}}$ values reduced thiol β-lactamase rates of hydrolysis of nitrocefin by 50%. Apparently, these substrates interact with the enzyme at the same active site.

$K_{\text{m}}$ and $k_{\text{cat}}$ values observed at different pH values for benzyl penicillin hydrolysis are presented in Table III. Between pH 4.5 and 9.0, the $k_{\text{cat}}$ for thiol β-lactamase is fairly constant as it is for wild type enzyme. The $K_{\text{m}}$ values for both enzymes increase at high pH.

Boric Acid Inhibition—Previous workers (35) have shown that boric acid competitively inhibits serine β-lactamases with a $K_{\text{a}}$ of 1 mM. A small degree of noncompetitive inhibition could not be excluded. We observe that competitive inhibition
by boric acid applies also to the wild type pBR322 \( \beta \)-lactamase. For both substrates benzylpenicillin and nitrocefin, the same \( K_a \) of 1 mM was measured (pH 7.0) over a range of boric acid (0.25–10 mM) and substrate concentrations ranging from one to two times their \( K_a \) values.

In contrast to the wild type enzyme, thiol \( \beta \)-lactamase was not competitively inhibited by boric acid. A small noncompetitive inhibition was observed at high boric acid concentrations. Boric acid at 10 mM inhibited thiol \( \beta \)-lactamase hydrolysis of benzylpenicillin by 12–16% for substrate concentrations of 0.05–0.5 mM and inhibited thiol \( \beta \)-lactamase hydrolysis of nitrocefin (0.14 mM) by 15%.

### DISCUSSION

The primary object of the present work was to determine whether the chemical behavior of an enzyme, in this case pBR322 \( \beta \)-lactamase, could be manipulated by specific changes in its primary sequence. Determining the consequences of specific changes sheds light on structure-function relationships which in turn will direct future changes that will produce new proteins with desired properties. We have already described the manner by which the codon for the active site Ser 70 of \( \beta \)-lactamase was changed to that of Cys (7). In the present study, this new protein, thiol \( \beta \)-lactamase, was purified and characterized.

Structurally, thiol \( \beta \)-lactamase is similar to wild type \( \beta \)-lactamase. The disulfide bond (Cys 75—Cys 121) in both enzymes is reduced under denaturing conditions at approximately equivalent rates. At 24 °C, the CD spectra of both enzymes are nearly identical. These spectra reflect a high degree of secondary structure with 40% \( \alpha \) helix and 20% \( \beta \) sheet being predicted by the method of Chen et al. (33). Similar predictions of helical content and CD spectra have been reported for Bacillus cereus and Staphylococcus aureus \( \beta \)-lactamasases (36). A crystal structure remains to be determined for a \( \beta \)-lactamase. In view of the strong homology between known \( \beta \)-lactamasases and D-alanylkcarboxypeptidases, it should be noted that the structure of one serine D-alanylkcarboxypeptidase is known and shows a high percentage of \( \alpha \) helix and \( \beta \) sheet (37).

Although thiol \( \beta \)-lactamase and wild type enzyme appear to have similar structures, they differ in their sensitivity toward trypsin. Denatured wild type enzyme is rapidly and extensively digested by trypsin (31). However, the native enzyme is fairly resistant to trypsin and other proteases and is cleaved only at high (40 °C) temperatures. In contrast, several thermolabile \( \beta \)-lactamasases as well as the precursor form are extremely sensitive to trypsin (0 °C) (34). At 40 °C, thiol \( \beta \)-lactamase is 3-fold more resistant to trypsin than wild type enzyme. This translates to a difference of 0.6 kcal/mol (\( RT \) in 3) between the energies of activation for cleavage of the two enzymes. If we assume that trypsin cleaves only a non-native form of both enzymes and that the rate of this cleavage is proportional to the population of the non-native state, then the Cys to Ser substitution has resulted in a 0.6-kcal/mol stabilization of the native conformation of \( \beta \)-lactamase. The magnitude and direction of this change are consistent with replacing a hydroxyl with a sulfhydryl group in the apolar interior of a protein. Sulfur is larger (van der Waals radii 1.8 Å for O, softer, and more polarizable than oxygen (38). Persh and Dingwall (39) have discussed the difference in hydrophobicity of the \(-SH\) and \(-OH\) groups and the role it might play in the selectivity of RNA synthetases. They calculated a 1.5 kcal/mol of more favorable dispersion energy for the interaction of \(-S\) with a \(-CH_2\) group than for \(-O\).

Of particular interest was the difference in enzymatic activity between wild type \( \beta \)-lactamase and thiol \( \beta \)-lactamase. The enzymatic catalyzed hydrolysis of \( \beta \)-lactams is a multistep process in which an acyl-enzyme intermediate forms and then is hydrolyzed. The formation of the acyl-enzyme intermediate is itself a complicated series of chemical steps each of which will be influenced by the \(-OH\) to \(-SH\) substitution. A probable sequence of events involves the attack of the nucleophile \(-OH\) or \(-SH\) (either ionized or with general base catalysis) at the carbonyl carbon to form a tetrahedral intermediate. This tetrahedral intermediate might either be protonated or unprotonated. Protonation would stabilize the intermediate which can in principle break down to give the acyl-enzyme or reactants. In the case of amide substrates, the tetrahedral intermediate must exist long enough for protonation of the incipient amine leaving group to occur (40). Model studies demonstrate that thiols are more effective nucleophiles than alcohols for displacement reactions at carboxyl groups with good leaving groups, e.g. p-nitrophenoxy or imidazolium ions (41, 43). In these studies, the nucleophilic species were the ionized thiolate and alkoxide anions. A large dependence on the leaving group for thiolate nucleophiles might result from competition in the partitioning of the tetrahedral intermediate to product as opposed to reactants (here the thiolate anion being the leaving group). The breakdown of the tetrahedral intermediate to form reactants would occur less rapidly with alkoxides which are poor leaving groups.

In view of these model studies, it is not surprising that thiol-subtilisin hydrolyses activated esters such as p-nitrophenylacetate nearly as rapidly as does subtilisin (17, 18). Furthermore, the rate-determining decacylation for thiol-subtilisin was found to be 10% that for subtilisin which is consistent with the greater stability of thiol esters toward general acid- and base-catalyzed hydrolysis (43). However, thiol-subtilisin lacks activity toward amide substrates although sulfhydryl proteases such as papain and ficin do exist. Previously, this lack of reactivity had been attributed to incorrect orbital orientation (17). Alternatively, in the formation at the acyl-enzyme intermediate, the stabilization of the tetrahedral intermediate and the rapid protonation of the amine leaving group might be more critical for cysteine pro-
teases than for serine proteases.

The situation with β-lactam antibiotics is somewhat intermediate between that of activated esters and amides. The bicyclic ring system found in penicillins and cephalosporins forces the β-lactam amide group to be nonplanar and suppresses the amide resonance -CO-NH- (44). β-Lactam antibiotics are therefore activated amides which are more easily hydrolyzed. An excellent organic model for a β-lactamase is in fact, ionized cysteine which reacts with benzylpenicillin with a rate three times that of hydroxide anion (45).

As this study shows, thiol β-lactamase has appreciable β-lactamase activity. For benzylpenicillin $k_{cat}/K_m$ is $3.5 \times 10^6$ s$^{-1}$ M$^{-1}$ or 1% of the wild type $4 \times 10^6$ s$^{-1}$ M$^{-1}$. This bimolecular constant is $10^6$ greater than that for the reaction of cysteine with benzylpenicillin at pH 7.0 (45). This enhancement in rate over that observed for model systems consisting simply of the chemical catalytic groups results from an unknown combination of factors such as substrate distortion, microenvironment, and orbital orientation which have been proposed to account for enzymatic catalysis (46, 47). The pH dependence for thiol β-lactamase further supports our belief that we are observing a true enzymatic reaction. Whereas in the cysteine hydrolysis of penicillins the ionized thiolate form is the active species, the activity of thiol β-lactamase is actually greater at low pH values. The small differences between the pH profiles for thiol β-lactamase and wild type enzyme at the active site are actually different between the two enzymes.

In summary, the enzyme that results from the Ser 70-Cys 70 substitution of pBR322 β-lactamase, thiol β-lactamase, has different physical and enzymatic properties. As such, this enzyme should prove useful in studies of the physiology (secretion and processing), the folding, and the enzymology of β-lactamas.

Acknowledgments—We gratefully acknowledge Milton C. Hillman, Dr. E. Knight, Jr., and Dr. D. C. Blomstrom for assistance in the protein purification, Rusty M. Kutney for obtaining the NH$_2$-terminal amino acid sequence, Theresa P. Chow for providing the antisera, and Dr. R. A. Yates for performing the continuous fermentations. We thank Dr. J. V. Schloss and Dr. V. Chowdhry for helpful discussions and comments on the manuscript and acknowledge the expert technical assistance of Joyce L. Hoopes.

REFERENCES

1. Inouye, S., Soberon, X., Franceschini, T., Nakamura, K., Itakura, K., and Inouye, M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3438-3441
2. Vlassou, G. P., Inouye, S., Ito, H., Itakura, K., and Inouye, M. (1983) J. Biol. Chem. 258, 7141-7148
3. Chabot, A. D., and Gestrens, A. U. (1978) Methods Enzymol. 58, 245-267
4. Fisher, J., and Pratt, R. F. (1980) Biochemistry 19, 3996-4003
5. Fisher, J., Charnas, R. L., Bradley, S. M., and Knowles, J. R. (1981) Biochemistry 20, 2726-2732
6. Koshland, D. E., Jr. (1982) J. Am. Chem. Soc. 104, 5489-5507
7. Ambler, R. P., and Smith, A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 6409-6413
8. Wiler, G., Fersht, A. R., Wilkinson, A. Z., Zoller, M., and Smith, M. (1980) Science 207, 797-802
9. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
10. Polgar, G., Inouye, M., Nakken, K. F., Eldjarn, L., and Pihl, A. (1982) Interferon Res. 1, 297-322
11. Pain, R. H., and Vlurden, R. (1981) Nature (Lond.) 290, 728-731
12. Ambler, R. P., and Smith, A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 321-331
13. Fastrez, J., and Fersht, A. R. (1973) Experiments in Molecular Genetics, p. 3, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
14. Koshland, D. E., Jr. (1982) J. Interferon Res. 1, 297-322
15. Kerber, K., and Tildon, J. W. (1973) in Beta-Lactamases (Hamilton-Miller, J. M. T., and Smith, J. T., eds) p. 151, Academic Press, New York
16. Ambler, R. P., and Smith, A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 7372-7376
17. Pollitt, S., and Zalkin, M. H. (1983) J. Bacteriol. 153, 27-32
18. Chen, Y.-H., Yang, J. T., and Chau, K. H. (1974) Biochemistry 13, 3350-3359
19. Schonaun, G. R., and Bender, M. L. (1967) Biochemistry 6, 610-620
20. Polgar, L., and Bender, M. L. (1976) J. Am. Chem. Soc. 98, 2897-2901
21. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1245-1249
22. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1800-1804
23. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1877-1882
24. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1900-1904
25. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1904-1908
26. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1908-1912
27. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1912-1916
28. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1916-1920
29. Kiener, P. A., and Waley, S. (1974) J. Am. Chem. Soc. 96, 1920-1924
Purification and properties of thiol beta-lactamase. A mutant of pBR322 beta-lactamase in which the active site serine has been replaced with cysteine.
I S Sigal, W F DeGrado, B J Thomas and S R Petteway, Jr

J. Biol. Chem. 1984, 259:5327-5332.