Paracatalytic Inactivation of L-2-Haloacid Dehalogenase from *Pseudomonas* sp. YL by Hydroxylamine

EVIDENCE FOR THE FORMATION OF AN ESTER INTERMEDIATE

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Ji-Quan Liu, Tatsuo Kurihara, Masaru Miyagi, Nobuyoshi Esaki, and Kenji Soda

From the Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, and the Biotechnology Research Laboratories, Takara Shuzo Co., Ltd., Otsu, Shiga 520-21, Japan

Asp\(^{10}\) of L-2-haloacid dehalogenase from *Pseudomonas* sp. YL was proposed to act as a nucleophile to attack the \(\alpha\)-carbon of L-2-haloalcanoic acids to form an ester intermediate, which is hydrolyzed by nucleophilic attack of a water molecule on the carbonyl carbon (Liu, J.-Q., Kurihara, T., Miyagi, M., Esaki, N., and Soda, K. (1995) *J. Biol. Chem.* 270, 18309–18312). We have found that the enzyme is paracatalytically inactivated by hydroxylamine in the presence of the substrates monochloroacetate and L-2-chloropropionate. Ion spray mass spectrometry demonstrated that the molecular mass of the enzyme inactivated by hydroxylamine during the dechlorination of monochloroacetate is about 74 Da greater than that of the native enzyme. To determine the increase of the molecular mass more precisely, we digested the inactivated enzyme with lysyl endopeptidase and measured the molecular masses of the peptide fragments. The molecular mass of the hexapeptide Gly\(^6\)-Lys\(^11\) was shown to increase by 73 Da. Tandem mass spectrometric analysis of this peptide revealed that the increase is due to a modification of Asp\(^{10}\). When the enzyme was paracatalytically inactivated by hydroxylamine during the dechlorination of L-2-chloropropionate, the molecular mass of the hexapeptide was 87 Da higher. Hydroxylamine is proposed to attack the carbonyl carbon of the ester intermediate and form a stable aspartate \(\beta\)-hydroxamate carboxyalkyl ester residue in the inactivated enzyme.

Paracatalytic enzyme modification is a catalysis-linked and substrate-dependent enzyme modification (1). It involves a direct chemical reaction between an enzyme-activated substrate and an extrinsic reagent. The catalytic effect of an enzyme can increase the reactivity of a substrate with extrinsic reagents that are not constituents of the normal enzyme-substrate system. The reactive intermediates formed may thus react with extrinsic reagents to branch off from the normal catalytic pathway. Consequently, the enzyme active site may be specifically and irreversibly modified.

L-2-Haloacid dehalogenase (EC 3.8.1.2) catalyzes the hydrolytic dehalogenation of L-2-haloalkanoic acids to produce the corresponding L-2-hydroxyalkanoic acids (2–4). Our recent \(^{18}\)O incorporation experiment showed that the reaction of L-2-haloacid dehalogenase from *Pseudomonas* sp. YL (L-DEX YL)\(^1\) proceeds through the mechanism shown in Fig. 1 (5); Asp\(^{10}\) acts as a nucleophile to attack the \(\alpha\)-carbon atom of the substrate, producing an ester intermediate and a halide ion. Subsequently, a water molecule hydrolyzes this intermediate forming L-2-hydroxyalkanoic acid and restoring the side chain carboxylate group of Asp\(^{10}\). The reactions catalyzed by haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 (6, 7), rat liver microsomal epoxide hydrolase (8), and 4-chlorobenzoyl-CoA dehalogenases from *Pseudomonas* sp. strain CBS3 (9) and *Arthrobacter* sp. 4-CB1 (10) also proceed through similar mechanisms, which involve the formation of an enzyme-substrate ester intermediate.

If such an ester intermediate is accessible to solvent, nucleophiles other than water could also react with this intermediate to modify it. We used hydroxylamine as a nucleophile (11) and found that L-DEX YL is paracatalytically inactivated by hydroxylamine. Tandem MS/MS spectrometric analysis revealed that the active site Asp\(^{10}\) was specifically labeled. Hydroxylamine is thus useful to probe the active site carboxylate group, which constitutes an enzyme-substrate ester intermediate.

**EXPERIMENTAL PROCEDURES**

**Materials**

Leu\(^{11}\), Ser\(^{170}\), and Arg\(^{185}\) were replaced by Lys by site-directed mutagenesis, and the resultant mutant enzyme, L-DEX T15, yields a small peptide fragment containing active site Asp\(^{10}\) of L-DEX YL by lysyl endopeptidase digestion. (5). Catalytic properties of L-DEX T15 such as the specific activity for L-2-chloropropionate and the optimum pH were indistinguishable from those of the wild type enzyme. L-DEX YL and L-DEX T15 were purified from recombinant *Escherichia coli* cells, which overproduce these enzymes (12, 13). Lysyl endopeptidase of *Achromobacter lyticus* M497-1 and TPCK-treated trypsin were bought from Wako Industry Co., Ltd. (Osaka, Japan) and Worthington Biochemical Corp. (Freehold, NJ), respectively. L-2-Chloropropionate and monochloroacetate were purchased from Sigma (St. Louis, MO) and Nacalai Tesque (Kyoto, Japan), respectively. Hydroxylamine sulfate was obtained from Takara Shuzo Co., Ltd. All other chemicals were of analytical grade.

**Enzyme and Protein Assay**

L-DEX YL and L-DEX T15 were assayed with 25 mM L-2-chloropropionate as a substrate. The chloride ions released were spectrophotometrically determined according to the method of Iwasaki *et al.* (14). In this assay, chloride ions react with Hg(SCN)\(_2\) to form HgCl\(_2\), HgCl\(_2\)\(_2\), and SCN\(^-\), which gives a reddish orange color (Fe(SCN)\(_3\)\(^+\)) with ferric ion

\(^1\) The abbreviations used are: L-DEX YL, L-2-haloacid dehalogenase from *Pseudomonas* sp. YL; MS, mass spectrometry; LC, liquid chromatography; TPCK, N-tosyl-L-phenylalanyl chloromethyl ketone.
in nitric acid solution. One unit of the enzyme was defined as the amount of enzyme which catalyzes the dehalogenation of 1 μmol of L-2-chloropropionate/min. Protein assay was done with a Bio-Rad protein assay kit.

**Inactivation of L-DEX YL and DEX T15 by Hydroxylamine**

For the experiment described in Tables I and II and MS analysis, reactions of L-DEX YL and L-DEX T15 with hydroxylamine were carried out with 1 ml of reaction mixtures containing 1 M Tris-HCl buffer (pH 9.0), 10 μM enzyme, and 1 μM hydroxylamine in the presence or absence of the indicated amount of substrates or monofluoroacetate (a substrate analog).

**Kinetic Analysis of L-DEX YL Inactivation by Hydroxylamine**

To study the relationship between hydroxylamine concentration and the inactivation rate of the enzyme, the reactions were carried out in 1.5 ml of solution containing 0.1 M Tris-HCl buffer (pH 9.5), 9.4 μM L-DEX YL, 100 mM sodium monochloroacetate, and 0–0.5 mM hydroxylamine at 30°C. At intervals, 50 μl of the reaction mixture was taken off, and the residual enzyme activity was measured in 450 μl of assay mixture containing 0.1 M Tris-HCl buffer (pH 9.5) and 25 mM sodium monochloroacetate. After incubation at 30°C for 10 min, the reaction was terminated by the addition of 50 μl of 3 M H2SO4, and the amount of chloride ions was measured by the method described above.

To examine the effect of substrate concentration on the rate of L-DEX YL inactivation, the reactions were carried out in dialysis bags each of which contained 5 ml of 0.1 M Tris-HCl buffer (pH 9.5), 0.57 μM L-DEX YL, 0.5 M hydroxylamine, and 0.5 mM sodium monochloroacetate. The reactions were initiated with the addition of sodium monochloroacetate, and the dialysis bags were immediately put into 200 ml of 0.1 M Tris-HCl buffer (pH 9.5) containing sodium monochloroacetate whose concentration was the same as that in the dialysis bag, in order to keep the substrate concentration constant during the reaction. The reactions were carried out at 30°C. After 0, 1, 2, 3, and 4 min, 50 μl of the mixture was taken off from the dialysis bag, and the residual enzyme activity was measured in 450 μl of assay mixture containing 0.1 M Tris-HCl buffer (pH 9.5) and 25 mM sodium monochloroacetate. Since the concentration of hydroxylamine carried into this assay mixture was terminated by the addition of 50 μl of 3 M H2SO4, and the residual enzyme activity was measured in 450 μl of assay mixture containing 0.1 M Tris-HCl buffer (pH 9.5) and 25 mM sodium monochloroacetate. Since the concentration of hydroxylamine carried into this assay mixture was terminated by the addition of 50 μl of 3 M H2SO4, and the amount of chloride ions was measured by the method described above.

**Amino Acid Sequencing**

The amino acid sequences of the enzymes and peptides were determined with a fully automated protein sequencer PPSQ-10 (Shimadzu, Kyoto, Japan).

**Determination of Molecular Mass of L-DEX YL Treated with Hydroxylamine**

Molecular masses of the enzyme and its derivatives dissolved in 50% acetonitrile containing 0.05% formic acid were determined by introducing into a PE-Sciex API III mass spectrometer equipped with an ion spray ion source. The quadrupole mass spectrometer was operated in the single quadrupole mode with a PE-Sciex API III mass spectrometer equipped with an ion spray ion source. The quadrupole was scanned from 300 to 2,000 atomic mass units with a step size of 0.25 atomic mass units and a 0.5-ms dwell time/step. Ion spray voltage was set at 5 kV, and the orifice potential was 80 V. The molecular mass of each peptide was calculated with MacSpec software, and the theoretical average molecular masses of the peptides were calculated with MacBioSpec software supplied by Sciex.

**Proteolytic Cleavage of L-DEX YL and L-DEX T15**

**Digestion of Wild Type L-DEX YL with Trypsin**—One ml of 1 M Tris-HCl buffer (pH 9.0) containing 10 μM L-DEX YL, 1 μM hydroxylamine, and 100 mM sodium L-2-chloropropionate was incubated at 45°C for 30 min. The inactivated enzyme was dialyzed against 5,000 volumes of water, denatured with 3 M urea, and then digested with 5 μg of TPCK-treated trypsin in 0.2 M Tris-HCl buffer (pH 8.0) for 12 h. L-DEX YL treated with hydroxylamine in the absence of the substrate was used as a control.

**Digestion of L-DEX T15 with Lysyl Endopeptidase—Ten nmol of L-DEX T15 inactivated by hydroxylamine in the presence of monochloroacetate under the same conditions as those for the wild type L-DEX YL was denatured with 8 M urea and subsequently digested with 80–100 pmol of lysyl endopeptidase at 37°C for 12 h. L-DEX T15 treated with hydroxylamine in the absence of the substrate was used as a control.

**LC/MS Analysis of the Proteolytic Digest**

The proteolytes of the L-DEX YL inactivated in the presence of L-2-chloropropionate was loaded onto a packed capillary perfusion column (Poros II R/H, 320 μm × 10 cm, LC Packings, San Francisco, CA) connected to the mass spectrometer and then eluted with a linear gradient of 0–90% acetonitrile in 0.05% trifluoroacetic acid over 40 min at a flow rate of 10 μl/min. The total ion current chromatogram was recorded in the single quadrupole mode with a PE-Sciex API III mass spectrometer equipped with an ion spray ion source. The quadrupole was scanned from 300 to 2,000 atomic mass units with a step size of 0.25 atomic mass units and a 0.5-ms dwell time/step. Ion spray voltage was set at 5 kV, and the orifice potential was 80 V. The molecular mass of each peptide was calculated with MacSpec software, and the theoretical average molecular masses of the peptides were calculated with MacBioSpec software supplied by Sciex.

**Mass Spectrometric Analysis of the Peptide Containing Asp**

For the analysis of the peptide containing Asp, the proteolyses of L-DEX T15 incubated with hydroxylamine in the presence or absence of monochloroacetate were applied to a C18 column (Poreasil 5 μm C18 120 Å, 4.6 × 150 mm; Millipore, Tokyo, Japan) and eluted with 0.05% trifluoroacetic acid for 5 min followed by a linear gradient of 0–80% acetonitrile in 0.05% trifluoroacetic acid over 60 min at a flow rate of 1.0 ml/min. The elution was monitored at 215 nm with a UV detector, and the fractions were collected and injected into a PE-Sciex API III mass spectrometer in the single quadrupole mode under the same conditions as described above.

**Tandem MS/MS Analysis of the Peptide Containing Asp**

The MS/MS daughter ion spectra were obtained in the triple-quadrupole daughter scan mode by selectively introducing the peptides containing Asp from (m/z 723.8, 708.7, or 650.5) from Q1 into the collision cell (Q2) and observing the daughter ions in Q3. Q1 was locked on m/z 723.8, 708.7, or 650.5. Q3 was scanned from 50 to just above the molecular mass of the peptide. A step size was 0.1, and the dwell time was 1 ms/step. Ion spray voltage was set at 5 kV, and the orifice potential was 100 V. Collision energy was 30 eV. The resolutions of Q1 and Q3 were approximately 500 and 1,500, respectively. The collision gas was argon, and the gas thickness was 2.9 × 10^(-11) molecules/cm².

**RESULTS**

**Inactivation of L-DEX YL by Hydroxylamine**—We found that treatment of L-DEX YL with hydroxylamine in the presence of monochloroacetate or L-2-chloropropionate led to an inactivation of the enzyme, whereas the treatment in the absence of the substrate or in the presence of monofluoroacetate, a substrate analog, caused no significant alteration in the enzyme activity.
Extensive dialysis of the inactivated enzyme did not result in its reactivation, suggesting a covalent modification of the enzyme. The enzyme was inactivated as the chloride ions were released (data not shown), and the inactivation followed pseudo-first-order kinetics (Fig. 2A). Fig. 2B shows that the rate of inactivation is proportional to the concentration of hydroxylamine. The initial rates of the inactivation were plotted against substrate concentrations, and the data were computer fitted to the Michaelis-Menten equation as shown in Fig. 3. The concentration of monochloroacetate causing a half-maximum rate of inactivation was thus calculated to be about 1.7 mM, which is close to the $K_m$ value for monochloroacetate in the dehalogenation reaction (1.1 mM) (4). These kinetic data show that this inactivation is due to a reaction of hydroxylamine with an enzyme-substrate complex.

**Molecular Mass and NH$_2$-terminal Amino Acid Sequence of the Inactivated L-DEX YL**—We determined by ion spray mass spectrometry molecular masses of L-DEX YL treated with hydroxylamine in the presence or absence of the substrate (Table II). The molecular mass of the native L-DEX YL was 25,863 Da, which indicates that the COOH terminus of this enzyme preparation lacks the last three amino acid residues (25,862.4 Da) (12). The molecular mass of the enzyme treated with hydroxylamine in the absence of the substrate was 25,863 Da, which is closely similar to the above value. In contrast, the molecular masses of the enzymes incubated with hydroxylamine in the presence of each of two kinds of the substrates, L-2-chloropropionate and monochloroacetate, were 25,952 and 25,937 Da, respectively, which are higher by 89 and 74 Da than that of the native protein. These indicate that L-DEX YL was modified by a molecule (or molecules) derived from the substrate (or plus hydroxylamine) to give molecular masses increased by 89 and 74 Da, respectively.

To examine if the active site Asp$^{10}$ was modified at the step of inactivation by hydroxylamine, we carried out amino acid sequencing of the enzyme. The NH$_2$-terminal amino acid sequencing showed that Asp$^{10}$ of the enzyme was modified only in the presence of the substrate (Table II).

**Molecular Masses of the Proteolytic Peptides Derived from L-DEX YL Modified by Hydroxylamine in the Presence of L-2-Chloropropionate**—The enzyme modified with hydroxylamine in the presence of L-2-chloropropionate was digested with TPCK-treated trypsin, and the molecular masses of the resulting peptide fragments were measured by LC/MS. When the spectrometer was in the single quadrupole mode, the total ion current chromatogram displayed several peaks (Fig. 4A). The molecular masses of the peptides for peaks 1 (Fig. 4C) and 3 (Fig. 4B) were 15 and 87 Da higher, respectively, than that for the peak in peak 2 (Fig. 4D). The value of molecular mass of the peak 2 peptide was compatible with that of a peptide corresponding to residues 6–24 derived from the unmodified enzyme. The peptides for peaks 1 and 3 are probably those corresponding to the same region but containing an amino acid residue modified in the hydroxylamine reaction.

**Molecular Masses of the Proteolytic Peptides Derived from L-DEX T15 Modified by Hydroxylamine in the Presence of Monochloroacetate**—We have prepared a mutant enzyme, L-DEX T15, which has been shown to be very convenient for analysis of modification of the active site Asp$^{10}$ because it is possible to obtain several peptides with appropriate sizes which cover the entire enzyme from the NH$_2$ terminus to the COOH terminus after cleavage with lysyl endopeptidase (5). L-DEX T15 incubated with hydroxylamine in the presence or absence of monochloroacetate was digested with lysyl endopeptidase, and the resulting peptide fragments were separated by high performance LC. Amino acid sequencing of the isolated peptides showed that a modified amino acid occurs at the position corresponding to the active site Asp$^{10}$ in the hexapep-
The modified hexapeptide G-I-A-F-X-K (where X is an unknown residue) was subsequently introduced into the ion spray mass spectrometer for detailed analysis. Two major peaks, derived from peptides with molecular masses of 708 and 723 Da, were found (Fig. 5). These values are 58 and 73 Da higher, respectively, than that of the native hexapeptide (650 Da).

These peptide fragments were analyzed in more detail by tandem MS/MS spectrometry. The parent ions of \(m/\text{z} 650.5\), 708.7, and 723.8, corresponding to the native peptide and the two modified peptides, were selected in the first quadrupole and subjected to collision-induced fragmentation in a collision cell in the second quadrupole. The mass spectra of the daughter ions produced are shown in Fig. 6. The peaks at \(m/\text{z} 538.5\), 467.4, and 320.1 from the parent ion of \(m/\text{z} 708.7\) correspond to the peptides A-F-X'-K, F-X'-K, and X'-K, respectively (where X' is an unidentified residue). They are about 58 Da higher than those of the corresponding peaks from the native peptide. However, molecular masses of the remaining portions (K) derived from these three peptides were essentially identical with each other (147.3, 147.3, and 147.1). These show that the active site Asp10 was specifically modified by molecules derived from the substrate and hydroxylamine.

DISCUSSION
L-DEXYL is inactivated by hydroxylamine only in the presence of the substrate, whereas a substrate analog that is not subject to dehalogenation does not induce inactivation. The concentration of the substrate causing a half-maximum rate of inactivation is close to its \(K_m\) value in the normal dehalogenation reaction, suggesting that the binding of the substrate to the active site of the enzyme is required for the inactivation by hydroxylamine. Moreover, mass spectrometric analysis of the inactivated enzyme showed that the active site Asp\(^{10}\) of the enzyme is modified with substrate- and hydroxylamine-derived moieties. These results indicate that hydroxylamine inactivates L-DEXYL paracatalytically; the intermediate derived from an enzyme-substrate complex is attacked by an extrinsic reagent.

The molecular mass of L-DEXYL inactivated by hydroxylamine in the presence of monochloroacetate was shown to be about 74 Da higher than that of the native L-DEXYL. The mass spectrometric analysis of the Asp\(^{10}\)-containing peptides derived from the inactivated L-DEXYL showed that there are two different species whose molecular masses are 58 and 73 Da higher than that of the unmodified peptide. Since we could not
detect the modified L-DEX YL whose molecular mass is about 58 Da higher than that of the native L-DEX YL, the peptide species with 58-Da higher mass number probably was formed by degradation of the other peptide species showing 73-Da higher mass number in the course of preparation of the peptide fragments. The increment of the molecular mass of 73 Da, which is more reliable than that of 74 Da observed for the whole modified protein, is postulated to be due to modification of Asp10 leading to the formation of aspartate β-hydroxamate as shown in Fig. 7. The species with 58 Da higher molecular mass would result from hydrolysis of hydroxyimido moiety of the above modified aspartate generating carboxymethylated Asp10.

With L-2-chloropropionate as substrate, the treatment of L-DEX YL with hydroxylamine caused an 87-Da increment in the molecular mass, and a species whose molecular mass is 15 Da higher than that of the native protein was also produced during the preparation of the peptide fragments. These increments in molecular mass can also be explained based on the mechanism shown in Fig. 7. The increment of 87 Da may be due to the conversion of Asp10 to the β-hydroxyimido carboxyethyl ester. The 15 Da higher molecular mass species could be formed by hydrolysis, generating an aspartate β-hydroxamate residue.

Under the normal enzymatic reaction conditions, Asp10 of the enzyme acts as a nucleophile to attack the a-carbon of the substrate leading to the formation of an ester intermediate, which is to be hydrolyzed by nucleophilic attack of a water molecule (5). When hydroxylamine is present, it attacks the carbonyl carbon of the Asp10 ester intermediate as shown in Fig. 7. The nonenzymatic reaction is thought to proceed through pathway 2 in Fig. 7 in preference to pathway 1, resulting in the formation of an aspartate β-hydroxamate residue. However, the mass spectrometric analysis of the modified residue showed that pathway 1 is preferred to pathway 2 in the
case of L-DEX YL modification. The residues facilitating the elimination of the hydroxyl group and suppressing the elimination of the carboxyalkoxyl group are probably present in the active site of the enzyme.

An alternative inactivation mechanism shown in Fig. 8 can also account for the 73- and 87-Da increments in the molecular mass of the enzyme inactivated in the presence of monochloroacetate and L-2-chloropropionate, respectively. Since MS has only identified the atoms incorporated into the inactivated enzyme and not their chemical arrangement, further studies are necessary to determine the exact structure of the modified Asp\(^{10}\) residue.

Hydroxylamine has a much higher nucleophilicity than water and has been used successfully as an acyl group acceptor to trap acylenzyme intermediates of several enzymes, such as chymotrypsin (15), d-alanine carboxypeptidase (16), aliphatic amidase (17), lipoprotein lipase (18), and \(\beta\)-ketoacyl-acyl carrier protein synthetase (19). In the presence of hydroxylamine, these ester intermediates are cleaved by hydroxylamine. Since the acyl moieties of the intermediates in these enzyme reactions are derived from the substrates, the reaction with hydroxylamine leads to the formation of hydroxamate analogs of the normal products. The formation of such hydroxamate analogs is considered evidence for the formation of an ester intermediate in the enzyme reaction. Unlike these enzymes, the active site Asp\(^{10}\) of L-DEX YL was specifically modified by hydroxylamine. This strongly indicates that an ester intermediate, whose acyl moiety is derived from Asp\(^{10}\), is produced during the reaction, which strongly supports the mechanism shown in Fig. 1.

The reactions catalyzed by haloalkane dehalogenase from X. autotrophicus GJ10 (6, 7), rat liver cytosolic epoxide hydrolase (8), and 4-chlorobenzoyl-CoA dehalogenases from Pseudomonas sp. strain CBS3 (9) and Arthrobacter sp. 4-CB1 (10) are proposed to proceed through the same mechanism that involves enzyme-substrate ester intermediates whose acyl moieties are derived from a carboxylate group of the enzyme. The observation that the enzyme from Arthrobacter sp. 4-CB1 is inactivated by hydroxylamine (10) suggests that hydroxylamine might be used to trap ester intermediates produced in these catalytic reactions and to label the active site residues of these enzymes.

REFERENCES
1. Christen, P. (1977) Methods Enzymol. 46, 48–54
2. Hardman, D. J. (1991) Crit. Rev. Biotechnol. 11, 1–49
3. Janssen, D. B., Pries, F., and van der Ploen, J. R. (1994) Annu. Rev. Microbiol. 48, 163–191
4. Liu, J.-Q., Kurihara, T., Hasan, A. K. M. Q., Nardi-Dei, V., Koshikawa, H., Esaki, N., and Soda, K. (1994) Appl. Environ. Microbiol. 60, 2389–2393
5. Liu, J.-Q., Kurihara, T., Miyagi, M., Esaki, N., and Soda, K. (1995) J. Biol. Chem. 270, 18309–18312
6. Verschuuren, K. H. G., Seljee, F., Rozeboom, H. J., Kalk, H. K., and Dijkstra, B. W. (1993) Nature 365, 695–698
7. Pries, F., Kingma, J., Pentenga, M., van Ponderoyen, G., Jeronimus-Stratingh, C. M., Bruins, A. P., and Janssen, D. B. (1994) Biochemistry 33, 1242–1247
8. Lacroixiere, G. M., and Armstrong, R. N. (1993) J. Am. Chem. Soc. 115, 10466–10467
9. Yang, G., Liang, P.-H., and Dunaway-Mariano, D. (1994) Biochemistry 33, 8527–8531
10. Crooks, G. P., Xu, L., Barkley, R. M., and Copley, S. D. (1995) J. Am. Chem. Soc. 117, 10791–10798
11. Jencks, W. P. (1969) Catalysis in Chemistry and Enzymology, McGraw-Hill Book Co., New York
12. Nardi-Dei, V., Kurihara, T., Okamura, T., Liu, J.-Q., Koshikawa, H., Ozaki, H., Terashima, Y., Esaki, N., and Soda, K. (1994) Appl. Environ. Microbiol. 60, 3375–3380
13. Kurihara, T., Liu, J.-Q., Nardi-Dei, V., Koshikawa, H., Esaki, N., and Soda, K. (1995) J. Biochem. 117, 1317–1322
14. Iwasaki, I., Usumi, S., Hagiho, K., and Ozawa, T. (1956) Bull. Chem. Soc. Jpn. 29, 860–864
15. Caplow, M., and Jencks, W. P. (1984) J. Biol. Chem. 259, 1640–1652
16. Nishino, T., Koizarich, J. W., and Strominger, J. L. (1977) J. Biol. Chem. 252, 2934–2939
17. Woods, M. J., Findlater, J. D., and Orsi, B. A. (1979) Biochim. Biophys. Acta 567, 225–237
18. Burdette, R. A., and Quinn, D. M. (1986) J. Biol. Chem. 261, 12016–12021
19. D'Agnolo, G., Rosenfeld, I. S., and Vagelos, P. R. (1975) J. Biol. Chem. 250, 5283–5288