Two homologous Δ^3-3-ketosteroid isomerases from *C. testosteroni* (TI-WT) and *Pseudomonas putida* biotype B (PI-WT) exhibit different pH activity profiles. TI-WT loses activity below pH 5.0 due to the protonation of the conserved catalytic base, Asp-38, while PI-WT does not. Based on the structural analysis of PI-WT, the critical catalytic base, Asp-38, was found to form a hydrogen bond with the indole ring NH of Trp-116, which is homologously replaced with Phe-116 in TI-WT. To investigate the role of Trp-116, we prepared the F116W mutant of TI-WT (TI-F116W) and W116F mutant of PI-WT (PI-W116F) and compared kinetic parameters of those mutants at different pH levels. PI-W116F exhibited significantly decreased catalytic activity at acidic pH like TI-WT, whereas TI-F116W maintained catalytic activity at acidic pH like PI-WT and increased the *k_\text{cat}/K_m* value by 2.5- to 4.7-fold compared with TI-WT at pH 3.8.

The crystal structure of TI-F116W clearly showed that the indole ring NH of Trp-116 could form a hydrogen bond with the carboxyl oxygen of Asp-38 like that of PI-WT. The present results demonstrate that the activities of both PI-WT and TI-F116W at low pH were maintained by a tryptophan, which was able not only to lower the pK_a value of the catalytic base but also to increase the substrate affinity. This is one example of the strategy nature can adopt to evolve the diversity of the catalytic function in the enzymes. Our results provide insights into deciphering the molecular evolution of the enzyme and creating novel enzymes by protein engineering.

Isozymes carry out the same enzymatic reaction yet exhibit different properties such as substrate affinity, preference of different coenzyme, stability, optimal pH, etc. Even if the sequence differences of those isozymes are evident, the origin of different properties is mostly not clear at the molecular level. Identification of functional divergence on the mechanistic basis is important to understand how the enzyme as a molecular machine can be evolved in the biological system and to rationally modify the enzyme function with desired physical and catalytic properties. In the post-genomic era, protein sequences and high resolution protein structures are being accumulated rapidly, but the remarkable catalytic mechanism of enzymes as well as the catalytic strategies utilized by nature to evolve new catalytic functions remain to be understood at the molecular level. The understanding of the structural basis for different properties of isozymes and their catalytic diversity could provide the insights into not only discovering the properties of the enzymes from their protein sequences or structural data but also creating novel enzymes by protein engineering.

Δ^3-3-Ketosteroid isomerase (KSI, EC 5.3.3.1) is one of the most proficient enzymes catalyzing an allylic isomerization reaction at a rate comparable to the diffusion-controlled limit by an intramolecular transfer of a proton (Scheme 1) (3–8). Although KSIs from two different bacterial sources, *C. testosteroni* and *Pseudomonas putida* biotype B, share only 34% sequence identity, the catalytic residues Tyr-14, Asp-38, and Asp-99 (the residues are numbered according to *C. testosteroni* KSI) are well conserved in the two isomerases (9, 10). Moreover, the overall three-dimensional structures are remarkably similar to each other, indicating that the two KSIs can share the same catalytic mechanism (11–14). In KSIs, Asp-38 acts as the only catalytic base to abstract a proton from the steroid substrate and to transfer the proton to the C-6 position for the allylic rearrangement of Δ^3-3-ketosteroids (3, 14).

KSI from *P. putida* (PI-WT) was reported to exhibit different dependence of *V_\text{max}*, *K_m*, and *k_\text{cat}/K_m* on pH compared with that from *C. testosteroni* (TI-WT) (15). The *V_\text{max}* value of PI-WT was not so sensitive to pH as that of TI-WT, which had been observed before (15, 16). The pH-rate profiles for *k_\text{cat}*, *k_\text{cat}/K_m*, and *k_\text{cat}/K_m* were extensively investigated for TI-WT (17). The pK_a of the catalytically important group on the free enzyme (pK_F) was determined to be 4.57 utilizing a nonsticky substrate, 5,10-estrene-3,17-dione (17). The catalytic activity of TI-WT is supposed to decrease abruptly below pH 5.0 due to the protonation of the catalytic base, Asp-38. The pK_a values of catalytic residues can be affected by the local microenvironment of the active site in the enzyme (18). The environment of Asp-38 in TI-WT is very

---

Received for publication, March 3, 2003, and in revised form, May 6, 2003 Published, JBC Papers in Press, May 6, 2003, DOI 10.1074/jbc.M302166200

From the Division of Molecular and Life Sciences, the National Research Laboratory of Protein Folding and Engineering, and the National Creative Research Initiative Center for Biomolecular Recognition, Pohang University of Science and Technology, Pohang 790-784, South Korea

Young Sung Yun‡‡, Tae-Hee Lee‡‡, Gyu Hyun Nam‡‡, Do Soo Jang‡‡, Sejeong Shin‡‡, Byung-Ha Oh‡‡, and Kwan Yong Choi‡‡

---

1 The abbreviations used are: KSI, Δ^3-3-ketosteroid isomerase (EC 5.3.3.1); 5-AND, 5-androstene-3,17-dione; 5,10-EST, 5,10-estrene-3,17-dione; CD, circular dichroism; WT, wild-type; MES, 2-(N-morpholino)-ethanesulfonic acid; PI-WT, wild-type KSI from *P. putida* biotype B; PI-W116F, W116F mutant of PI-WT; TI-WT, wild-type KSI from *C. testosteroni*; TI-F116W, F116W mutant of TI-WT; *K_\text{diss}*, ionization constant of the free enzyme; *K_m*, ionization constant of the enzyme-substrate complex.

---

‡‡To whom correspondence should be addressed. Tel.: 82-54-279-2295; Fax: 82-54-279-8290; E-mail: kchoi@postech.ac.kr.
similar to that in PI-WT as judged by crystallographic data of both wild-type enzymes (11, 13, 19). However, there is a significant difference in that the carboxyl oxygen of Asp-38 is hydrogen-bonded to NH on the indole ring of Trp-116 only in PI-WT (Fig. 1B). Trp-116 in PI-WT is homogeneously replaced with Phe-116 in TI-WT (Fig. 1C). The different electrostatic environments around the catalytic bases in TI-WT and PI-WT could affect the pH values of two homologous KSIs.

To investigate the role of Trp-116 in PI-WT, we have prepared the F116W mutant of TI-WT (TI-F116W) and the W116F mutant of PI-WT (PI-W116F), and compared their kinetic parameters at various pHs, respectively. The crystal structure of TI-F116W determined at 2.0-Å resolution exhibited that the indole ring NH of Trp-116 could form a hydrogen bond with the carboxyl oxygen of Asp-38. Our kinetic and structural results demonstrate that different pH activity profiles in the two KSIs originate from a single hydrogen bond between Trp-116 and Asp-38 and that Trp-116 also contributes to the better binding of the hydrophobic steroid substrate to compensate for the lowered basicity of the catalytic residue.

EXPERIMENTAL PROCEDURES

Materials—5-Androstene-3,17-dione (5-AND), and 5,10-estrened-3,17-dione (5,10-EST) were purchased from Steraloids. Chemicals for buffer solutions were from Sigma. Oligonucleotides were from Genotech Inc., Korea. A QuickChange site-directed mutagenesis kit was supplied by Stratagene. pKK223-3 plasmid was from Amersham Biosciences. Superose 12 gel filtration column was obtained from Amersham Biosciences.

Mutagenesis—Phe-116 of TI-WT was replaced with a tryptophan to make TI-F116W. TI-F116W was prepared using the QuikChange site-directed mutagenesis kit (Stratagene) and a thermocycler (MiniCycler, MJ Research). pKSI-TI was used as the DNA template (11). Two primers were designed to introduce the F116W mutation into the KSI gene of C. testosteroni. A forward primer was 5’-AGC ATG CGC GCC TTG TGG GGC GAG AAG AAT ATT-3’ and a reverse primer was 5’-AAT ATT CTG CTC GCC CCA AAA GGC GCG CAT GCT-3’. Underlined nucleotides represent the ones changed by point mutations. Recombinant plasmids were introduced into Escherichia coli XL1-Blue supercompetent cells (Stratagene) and purified by use of QIAprep Spin Miniprep kit (Qiagen). The entire KSI gene was then sequenced to confirm the intended mutation only without the change of other sequences in the KSI gene. The W116F mutation of PI-WT was described previously (20).

Expression and Purification of KSI Proteins—The protein was overproduced in E. coli BL21(DE3) utilizing the pKK223-3 plasmid containing the wild-type or mutant KSI gene and purified by deoxycholate affinity chromatography and Superose 12 gel filtration chromatography as described previously (21). The purity of the protein was confirmed by a single band on SDS-PAGE. The protein concentration was determined utilizing the difference extinction coefficient between tyrosinate and tyrosine at 295 nm as described previously (22). The accuracy of the protein concentration was confirmed by the quantitative analysis of the band on SDS-PAGE by use of an imaging densitometer (Bio-Rad, GS-700) and a software program provided by its manufacturer (Molecular Analyst/PC).

Steady-state Kinetic Analysis—The catalytic activities of the purified KSIs were determined spectrophotometrically using 5-AND as a sub-

![Figure 1](http://www.jbc.org/)

**FIG. 1.** Three-dimensional structure of KSI. **A**, ribbon diagram of a dimeric KSI from *P. putida* KSI complexed with d-equilenin, an intermediate analogue. Three catalytic residues and d-equilenin are shown in ball-and-stick model representation. **B**, monomeric structure of the dimeric KSI from *P. putida*. **C**, monomeric structure of the dimeric KSI from *C. testosteroni*. The molecular surface of each KSI monomer using the probe of 1.4 Å radius was generated. Positive and negative charged regions are indicated by blue and red, respectively. Asp-38 and Trp-116, or Phe-116 are shown in ribbon diagram of KSI complexed with d-equilenin, an intermediate analogue. Three catalytic residues and d-equilenin are shown in ball-and-stick model representation. The figures were drawn using the programs WebLab Viewer/F, Accelrys.
**Origin of Different pH Activity Profile of KSI**

### Table I

| Enzyme       | $k_{cat}$ | $K_M$ | $k_{cat}/K_M$ | $pK_a$ | $pK_{ES}$ |
|--------------|-----------|-------|---------------|-------|-----------|
| PI-WT        | 21230 ± 610 | 49.9 ± 1.3 | 4.25 x 10^4 | 3.75 ± 0.04 | 4.24 ± 0.15 |
| PI-W116F     | 4780 ± 36 | 149 ± 20 | 3.19 x 10^3 | 5.00 ± 0.20 | 5.25 ± 0.20 |
| TI-WT        | 31683 ± 1200 | 152.8 ± 8.2 | 2.07 x 10^8 | 4.57 ± 0.05 | 4.76 ± 0.04 |
| TI-F116W     | 7155 ± 194 | 48.6 ± 1.4 | 1.47 x 10^9 | 3.52 ± 0.04 | <3.8 |

<sup>a</sup> Data from Kim et al. (20).<br><sup>b</sup> The upper limit was indicated, because $k_{cat}$ did not decrease even at pH 3.8 using the above substrates.

### pH Activity Profiles

All the buffers used to determine the kinetic constants contained 80 mM NaCl to adjust the ionic strength of the solution. The buffer solution contained 20 mM sodium acetate for pH 3.8–4.9, 20 mM sodium MES for pH 5.2–6.3, or 20 mM potassium phosphate for pH 6.6–8.0. The procedures for obtaining kinetic parameters were the same as those for the steady-state kinetic analysis as described above. The stability of the enzyme was confirmed by determining the catalytic activity at pH 7.0 after incubating the enzyme in the respective assay buffer for 2 min. The observed kinetic parameters were fitted to Equations 1 and 2 to obtain the $pK_a$ and $pK_{ES}$ values, respectively, by nonlinear least-squares analysis utilizing a computer program (Abelbeck Software, Kaleidagraph version 3.06).

### Results

#### Kinetic Parameters

The $k_{cat}$ values for PI-WT, TI-WT, and TI-F116W were determined with a nonsticky substrate, 5,10-estrene-3,17-dione. $K_M$ values for PI-W116F were determined with 5-androstene-3,17-dione.

### CD Spectra at Different pHs

To investigate the effect of pH on the conformation of KSI, far-UV CD spectra were obtained in the pH range from 3.8 to 7.2 (Fig. 5). The CD spectra were obtained between 200 and 250 nm to estimate the content of the secondary structure in the protein. TI-WT and TI-F116W absorbance at 222 nm were almost the same as those for PI-WT, consistent with the previous results for two substrates, 5,10-EST and 5-AND (17). The $pK_a$ value of TI-WT was determined with 1.05 pH unit relative to that of TI-WT. The $pK_{ES}$ value of PI-F116W was increased by 2.5-fold and by 4.7-fold for two substrates, 5,10-EST and 5-AND, respectively, with a significant decrease of the $pK_a$ value of TI-WT.

The crystal structure of TI-F116W was determined at 2.0-Å resolution. The statistics of the crystallographic data and refinement are summarized in Table II. No significant structural changes were observed in the mutant KSI compared with TI-WT. The structure of TI-F116W exhibited the $k_{cat}$ and $K_M$ values of PI-F116W and PI-W116F were compared with those of their respective wild-type enzyme (Table I). The $k_{cat}$ values of PI-WT and PI-W116F were lower than those of TI-WT and PI-WT by 4.42- and 4.46-fold, respectively. The $K_M$ values of PI-W116F were higher than that of PI-WT by 2.98-fold, whereas the $K_M$ value of TI-F116W was lower than that of TI-WT by 3.14-fold. TI-F116W exhibited the $k_{cat}/K_M$ value nearly comparable to that of the wild-type, because both $K_M$ and $k_{cat}$ values were decreased simultaneously at pH 7.0.
exhibited similar spectra in the pH range from 3.8 to 7.2, respectively, whereas the spectra of PI-WT and PI-W116F were marginally changed below pH 5.0, indicating that there were no significant structural changes over the pH range from 3.8 to 7.2 in all different forms of KSI.

Native PAGE—The ionization state of the catalytic base in the two KSIs can affect the overall charge of the enzyme, because the catalytic base, Asp-38, is located in the boundary between the cavity of the hydrophobic active site and the solvent (Fig. 1). Mobilities of PI-WT, PI-W116F, TI-WT, and TI-F116W were compared using native PAGE. The ionization state of the catalytic base in the two KSIs can affect the overall charge of the enzyme, because the catalytic base, Asp-38, is located in the boundary between the cavity of the hydrophobic active site and the solvent (Fig. 1).
F116W in the gel electrophoresis were compared at two different pHs, 4.3 and 7.3 (Fig. 6). PI-WT and PI-W116F had the same mobility at pH 7.3, whereas PI-W116F migrated more slowly than PI-WT toward the anode at pH 4.3. TI-WT and TI-F116W also had the same mobility at pH 7.3, whereas TI-WT migrated more slowly than TI-F116W toward the anode at pH 4.3, indicating that TI-WT and PI-W116F have less effective negative charges than TI-F116W and PI-WT, because the carboxylic group of Asp-38 in those KSIs could be protonated at pH 4.3.

**DISCUSSION**

The analyses of pH activity profiles and crystallographic structures at high resolution for the wild-type and mutant forms of two homologous KSIs from two different bacterial sources allowed us to identify the role of Trp-116, including a hydrogen bond formed by the indole ring NH with the catalytic base, Asp-38, in PI-WT. A different environment around Asp-38 was found to be the origin of the different pH activity profiles of the two KSIs. The carboxylate ion could be destabilized in a relatively hydrophobic region of the active sites in TI-WT and PI-W116F, resulting in the increase of the pK_a value.

**TABLE II**

Crystallographic data and refinement statistics for TI-F116W

|                           |       |
|---------------------------|-------|
| Resolution (Å)            | 2.0   |
| R_{sym} (%)               | 6.0   |
| Data completeness, F> 1 σ (%) | 91.8 |
| R_{standard} (%)          | 22.88 |
| R_{free} (%)              | 28.24 |
| No. of refined atoms (atom/water) | 4161/377 |
| Average B factor          | 21.454 |
| r.m.s.d. bond length (Å)  | 0.005970 |
| r.m.s.d. bond angles (deg) | 1.16570 |
| Ramachandran plot (%)     |       |
| Most favored regions      | 90.8  |
| Additional allowed regions| 8.3   |
| Generously allowed regions| 0.9   |
| Disallowed regions        | 0.0   |

*a r.m.s.d., root mean square deviation.*

**FIG. 3.** Stereoview of the catalytic bases of PI-WT (A), TI-WT (B), and TI-F116W (C). Hydrogen bonds between Asp-38 and Trp-116 are represented by dashed lines. The distance between Asp-38 Oδ1 and Trp-116 NE1 in PI-WT was 2.87 Å (A). Phe-116 in TI-WT was located at the corresponding position of Trp-116 in PI-WT (B). The distance between Asp-38 Oδ1 and Trp-116 NE1 in TI-F116W was 2.87 Å (C). The figures were drawn using the programs WebLab ViewerPro; Accelrys. PDB codes are 4TSU and 8CHO for PI-WT and TI-WT, respectively.
of Asp-38. In contrast, the $pK_a$ values of Asp-38 in PI-WT and TI-F116W could be decreased, because the carboxylate ion in Asp-38 was stabilized by a hydrogen bond with Trp-116 (Fig. 7).

Considering the different environment of the critical catalytic base, Asp-38, in two KSIs, the low $pK_a$ value of PI-WT can be attributed to a hydrogen bond between the carboxylate of

![Stereoview of the catalytic base of TI-F116W with 2F$_o$ – F$_c$-simulated annealing omit electron density map contoured at 1.0 $\sigma$. Residues Trp-116 and Asp-38, which were omitted from the model, display clear electron density. A hydrogen bond between Asp-38 and Trp-116 is represented by a dashed line. The figure was drawn by using the program BobScript and rendered using Raster3D.](image)

![Far-UV CD spectra of WT and mutant KSIs at different pH.](image)
Asp-38 and the indole ring NH of Trp-116. The replacement of Trp-116 with a phenylalanine in PI-WT to mimic the active-site environment of TI-WT resulted in a significant decrease of the catalytic activity at low pH, and the W116F mutation increased the pK_a value by 1.25 relative to that of PI-WT. The increase of pK_F in PI-W116F is due to the pK_a increase of Asp-38, because the hydrogen bond between Trp-116 and the carboxylate of Asp-38 could not be formed. The low mobility of PI-W116F on native PAGE is the evidence for the protonated state of Asp-38 at pH 4.3, because the apparent pK_a value of Asp-38 was kinetically estimated to be 5.0 in PI-W116F. The far-UV CD spectra of PI-WT and PI-W116F between 200 and 250 nm were almost indistinguishable at low pH, indicating that the enzymes did not undergo any significant conformational change at low pH. Therefore, the decreased activity of PI-W116F at low pH originates not from any structural change of the protein, but from the protonation of the catalytic base.

TI-F116W was prepared to confirm that a hydrogen bond between Trp-116 and Asp-38 could compensate for the decreased activity of TI-WT at low pH by stabilizing the ionization state of the catalytic base, Asp-38. As expected, TI-F116W exhibited no decrease of catalytic activity at low pH and increase of k_cat/K_m by 4.7- and 2.5-fold compared with TI-WT when 5-AND and 5,10-EST were used as substrates at pH 3.8, respectively. As shown in the crystal structure of TI-F116W, a hydrogen bond can be formed by the indole ring NH of Trp-116 with a carboxyl oxygen of Asp-38 (Fig. 3). The hydrogen bond could cause the pK_a value of Asp-38 to be decreased in TI-F116W. The lower mobility of TI-WT relative to TI-F116W on native PAGE at pH 4.3 was also consistent with the pK_a values estimated kinetically, reflecting the change of ionization state of the critical catalytic base, Asp-38. The far-UV CD spectra of TI-WT and TI-F116W showed almost identical patterns in the pH range from 3.8 to 7.2, indicating that any significant conformational change over the pH range did not take place. Hence, the low activity of TI-WT at low pH is not from the structural change but from the protonation of the catalytic base, Asp-38.

The importance of the indole ring NH of tryptophan was previously suggested in its interaction with other residues in the protein (29, 30). Tryptophan can have characteristics of both hydrophobicity and hydrophilicity due to the amphipathic property, which originates from the indole ring in its side chain (30). The amphipathic characteristic of tryptophan plays two different roles in the catalysis of KSIs. One role of tryptophan is that the bulky hydrophobic indole ring contributes to the better binding of the hydrophobic substrate like steroids. Compared with TI-WT and PI-W116F, PI-WT and TI-F116W have relatively lower K_m values for the hydrophobic steroid substrate (Table I). This notion that Trp-116 contributes to the favorable binding of the steroid is consistent with the previous result in the characterization of PI-W116F (20). The other role of tryptophan is the stabilization of ionized state of the catalytic base residue, Asp-38, at low pH in the hydrophobic active site of KSI, due to a hydrogen bond between Trp-116 and Asp-38. Although the hydrogen bond could decrease the basicity of Asp-38, this adverse effect for catalytic activity could be overcome by the increased binding affinity to the substrate.

The alteration of pH activity profiles by changing the pK_a value of a catalytic base in an enzyme has been observed previously (31–33). The pK_a value of the active site histidine was raised by 0.2 pH unit by acetylation of all surface lysines in trypsin (31). Shifted pH activity profiles and higher catalytic activity were obtained by changing the surface charge of subtilisin using site-directed mutagenesis (32). The pK_a value of His-64 in the active site of subtilisin was lowered by 0.4 unit at low ionic strength in the range of 0.005–0.01 m by the removal of one surface carboxylate (33). The lowering of the pK_a by employing the change of the surface charge is dependent basically on the long range macroscopic electrostatic interaction, which requires a specific condition of low ionic strength to unmask electrostatic interaction (32). During evolution, enzymes may utilize a long-range electrostatic interaction to regulate the pK_a value of the catalytic residue. However, the low ionic condition to unmask the electrostatic interaction may not be allowed in the biological system. In both intracellular and extracellular environments of a typical mammalian cell, the ion concentration can be higher than 0.1 M (34), and total ion concentration of the bacterial cell was reported to be comparable to that of the mammalian cell (35). The direct regulation by use of the neighboring residue could be more effective than the indirect regulation through the long range electrostatic interaction in the aspect that it is relatively independent of ionic strength of the solution. In this study, we demonstrated that the alteration of the pK_a value of the catalytic residue in a mutant with higher activity than the wild-type enzyme at low pH could be achieved not by changing surface charge but by directly changing the microscopic active-site environment of the catalytic residue utilizing a single hydrogen bond.

In summary, based on comparative studies using two homologous KSIs on the role of a tryptophan, we demonstrated that
tryptophan could alter the pH activity profile by both forming the hydrogen bond with the catalytic base and increasing simultaneously the substrate affinity. PI-WT could employ a kind of a protective strategy to stabilize the deprotonation state of a catalytic base, which can play a critical role as a base catalyst at low pH. This strategy is based on the fine regulation of a catalytic base, which can play a critical role as a base catalyst at low pH, independent of external biological buffer during evolution. We assume that this strategy can be found in other enzymes and applied to the alteration of pH activity profile by protein engineering.

Acknowledgment—We thank Yeon-Gil Kim for help in preparing the simulated annealing omit map.

REFERENCES
1. Chen, R. (2001) Trends. Biotechnol. 19, 13–14
2. Gerlt, J. A., and Babbitt, P. C. (2001) Annu. Rev. Biochem. 70, 209–246
3. Pollack, R. M., Thornburg, L. D., Wu, Z. R., and Summers, M. F. (1999) Arch. Biochem. Biophys. 370, 8–15
4. Batsford, F. H., Benson, A. M., Covey, D. F., Robinson, C. H., and Talalay, P. (1976) Adv. Enzyme. Regul. 14, 243–287
5. Schwab, J. M., and Henderson, B. S. (1990) Chem. Rev. 90, 1203–1245
6. Haskinson, D. C., Eames, T. C., and Pollack, R. M. (1991) Biochemistry 30, 6956–6964
7. Haskinson, D. C., Pollack, R. M., and Ambulos, N. P., Jr. (1994) Biochemistry 33, 12172–12183
8. Xue, L., Kulikopoulos, A., Mildvan, A. S., and Talalay, P. (1991) Biochemistry 30, 4891–4897
9. Kim, S. W., Joo, S., Choi, G., Cho, H.-S., Oh, B.-H., and Choi, K. Y. (1997) J. Bacteriol. 179, 7742–7747
10. Kim, S. W., and Choi, K. Y. (1995) J. Bacteriol. 177, 2602–2605
11. Cho, H.-S., Choi, G., Choi, K. Y., and Oh, B.-H. (1998) Biochemistry 37, 8325–8330
12. Wu, Z. R., Ebrahimian, S., Zawrotny, M. E., Thornburg, L. D., Perez-Alvarado, G. C., Brothers, P., Pollack, R. M., and Summers, M. F. (1997) Science 276, 415–418
13. Kim, S. W., Cho, S.-S., Cho, H.-S., Kim, J.-S., Ha, N.-C., Cho, M.-J., Joo, S., Kim, K. K., Choi, K. Y., and Oh, B.-H. (1997) Biochemistry 36, 14030–14036
14. Ha, N.-C., Choi, G., Choi, K. Y., and Oh, B.-H. (2001) Curr. Opin. Struct. Biol. 11, 674–678
15. Smith, S. B., Richards, J. W., and Benisek, W. F. (1980) J. Biol. Chem. 255, 2685–2689
16. Weintraub, H., Alpich, A., and Baulieu, E.-E. (1970) Eur. J. Biochem. 12, 217–221
17. Pollack, R. M., Bantia, S., Bounds, P. L., and Koffman, B. M. (1986) Biochemistry 25, 1905–1911
18. Fersht, A. (1999) Structure and Mechanism in Protein Science, pp. 169–189, W. H. Freeman and Co., New York
19. Cho, H.-S., Ha, N.-C., Choi, G., Kim, H.-J., Lee, D., Oh, K. S., Kim, K. S., Lee, W., Choi, K. Y., and Oh, B.-H. (1999) J. Biol. Chem. 274, 32863–32868
20. Kim, D.-H., Nam, G. H., Jang, D. S., Choi, G., Joo, S., Kim, J.-S., Oh, B.-H., and Choi, K. Y. (1999) Biochemistry 38, 13810–13819
21. Choi, G., Ha, N.-C., Kim, S. W., Kim, D.-H., Park, S., Oh, B.-H., and Choi, K. Y. (2000) Biochemistry 39, 903–909
22. Copeland, R. A. (1993) Methods of Protein Analysis, pp. 51–54, Chapman and Hall, New York
23. Thornburg, L. D., Henot, F., Bash, D. P., Haskinson, D. C., Bartel, S. D., and Pollack, R. M. (1998) Biochemistry 37, 10499–10506
24. Langsetmo, K., Fuchs, J. A., and Woodward, C. (1991) Biochemistry 30, 7603–7609
25. Otwinowski, Z. (1993) in Proceedings of the CCP4 Study Weekend (Sawyer, L. et al., eds) pp 56–62, SERC Daresbury Laboratory, Warrington, UK
26. Brugger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuzezewski, J. Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. 54, 903–921
27. Knowles, J. R. (1976) Crit. Rev. Biochem. 4, 165–173
28. Cleland, W. W. (1977) Adv. Enzymol. Relat. Areas Mol. Biol. 45, 273–387
29. Ippolito, J. A., Alexander, R. S., and Christianson, D. W. (1990) J. Mol. Biol. 216, 457–471
30. Samanta, U., Pal, D., and Chakrabarti, P. (2000) Proteins 38, 288–300
31. Spomer, W. E., and Wootton, J. F. (1971) J Mol. Biol. 69, 215–246
32. Russell, A. J., and Fersht, A. R. (1987) Nature 328, 496–500
33. Russell, A. J., Thomas, P. G., and Fersht, A. R. (1987) J. Mol. Biol. 193, 803–813
34. Albert, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. Molecular Biology of the Cell, pp. 597–549, 3rd Ed., Garland Publishing, Inc., New York
35. Albert, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. Molecular Biology of the Cell, pp 89–90, 3rd Ed., Garland Publishing, Inc., New York
