Reaction Intermediate Structures of 1-Aminocyclopropane-1-carboxylate Deaminase

INSIGHT INTO PLP-DEPENDENT CYCLOPROPANE RING-OPENING REACTION*

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The pyridoxal 5′-phosphate-dependent enzymes have been evolved to catalyze diverse substrates and to cause the reaction to vary. 1-Aminocyclopropane-1-carboxylate deaminase catalyzes the cyclopropane ring-opening reaction followed by deamination specifically. Since it was discovered in 1978, the enzyme has been widely investigated from the mechanistic and physiological viewpoints because the substrate is a precursor of the plant hormone ethylene and the enzymatic reaction includes a cyclopropane ring-opening. We have previously reported the crystal structure of the native enzyme. Here we report the crystal structures of the two reaction intermediates created by the mutagenesis complexed with the substrate. The substrate was validated in the active site of two forms: 1) covalent-bonded external aldimine with the coenzyme in the K51T form and 2) the non-covalent interaction around the coenzyme in the Y295F form. The orientations of the substrate in both structures were quite different form each other. In concert with other site-specific mutation experiments, this experiment revealed the ingenious and unique strategies that are used to achieve the specific activity. The substrate incorporated into the active site is reactivated by a two-phenol charge relay system to lead to the formation of a Schiff base with the coenzyme. The catalytic Lys residue may play a novel role to abstract the methylene proton from the substrate in cooperation with other factors, the carboxylate group of the substrate and the electron-adjusting apparatuse of the coenzyme.

The α-amino acid with a cyclopropane ring, 1-aminocyclopropane-1-carboxylate (ACC), is known to be a key intermediate in the biosynthesis of the plant hormone ethylene (1), which is responsible for the initiation of fruit-ripening and for regulating many other plant developmental processes. In higher plants, the pathway of ethylene biosynthesis (Fig. 1) is initiated with the conversion of the methionine to S-adenosylmethionine (2) by methionine adenosyltransferase followed by the conversion of S-adenosylmethionine to ACC by ACC synthase, pyridoxal 5′-phosphate (PLP)-dependent enzyme. ACC is oxidized by mononuclear iron dependent-ACC oxidase (3) to ethylene in the final step.

In a soil bacterium, Pseudomonas sp. strain ACP, ACC was also converted to α-ketobutyrate and ammonia by a bacterial enzyme when ACC was the sole nitrogen source for the growth of this bacterium (4, 5). This degradation is catalyzed by another PLP-dependent enzyme, ACC deaminase (ACCD, EC 4.1.99.4), which has a special ability to break the cyclopropane ring preceding deamination (Fig. 2). The possibility of symbiosis between the plants and the soil bacterium has also been mentioned (6, 7). The introduction of ACCD in higher plants by gene technology reduced the production level of ethylene and delayed the ripening progression of fruits (8, 9). This enzyme has been isolated from a few strains of Pseudomonas species (5, 8, 10, 11), yeast Hansenula saturnus (12), and fungus Penicilium citrinum (13). Furthermore, several open reading frames in fully decoded organisms are annotated as ACCD homologues by BLAST (14) with significant sequence identity.

The PLP enzymes catalyze a wide variety of reactions that act upon the metabolism of amino acids. These include transamination, racemization, deamination, decarboxylation, and the elimination or replacement of the β- and γ-substituent groups besides oligosaccharide or polysaccharide phosphorylation. According to Jansonius (15) or Structural Classification of Proteins (SCOP) database (16), the three-dimensional structures of the PLP enzyme are classified into five folding types. The structure of ACCD from yeast H. saturnus (yACCD) (17) belongs to the tryptophan synthase β-subunit (TRPSβ) family (fold type II), which includes five other proteins whose three-dimensional structures have been analyzed to date: TRPSβ (18), O-acetylserine sulphydrylase (cysteine synthase) (19), threonine deaminase (20), threonine synthase (21), and cystathionine β-synthase (22). The cyclopropane ring-opening reaction of ACCD is regarded as a special case of γ-elimination (4, 23) (Fig. 2), but ACCD also catalyzes β-serine deamination, which involves β-elimination. This seems to be consistent with the perception that all of the members of the TRPSβ family catalyze the β-replacement/elimination of the substrate amino acid.

The active sites of PLP enzymes are conservative in the catalytic lysine residue and phosphate-binding cap (24), but...
their active sites are diverse for recognizing a variety of substrates and promoting the reactions. Most of the PLP enzymes (except in the case of the glycogen phosphorylase family) keep the basic chemical processes of the PLP catalysis: formation of an external aldimine between C4 of PLP and the α-amino group of the substrate, the electron-withdrawing role of the pyridine ring as an electron sink, and the nucleophilic attack on the α-substituent of an α-carbon or α-carboxylate group. In the case of ACCD, the substrate has no α-hydrogen and the carboxyl group is retained in the product, ruling out the above ordinary process. Evidence from kinetic studies and NMR spectroscopy has been accumulated to explain the specificity of the enzyme reaction and to propose hypotheses regarding the cyclopropane ring-opening process.

MATERIALS AND METHODS

Mutagenesis and Enzyme Purification—All of the expression vectors of yACCD mutants were constructed using two-step PCR, and the resulting mutation was confirmed by DNA sequencing using the dRhodamine kit (Applied Biosystems). The cDNA introduced in phagemid pBluescript II KS(+) (Stratagene) was used as the PCR template. The expression vectors pET11d (Stratagene) were transformed into Escherichia coli BL21(DE3) to overexpress the mutated proteins. The mutant proteins were purified using a protocol described previously (12) with slight modification. Among the designed, the solubilized and purified derivatives were K51T, S78A, Y269F, Y295F, and E296Q.

Assay of ACC Deaminase—The activity of ACC deamination was assayed by measurement of α-ketobutyrate formed from 50 mM ACC as

**FIG. 1. Ethylene biosynthesis in higher plants.**

**FIG. 2. The enzymatic reaction catalyzed by ACCD.**
TABLE I

Data statistics

| PDB code         | K51T  | K51T-ACC | Y295F-ACC |
|------------------|-------|----------|-----------|
| Data collection  |       |          |           |
| Wavelength (Å)   | 38.2-2.75 | 38.2-2.20 | 38.2-2.45 |
| Resolution range (Å) | 38.2-2.75 | 38.2-2.20 | 38.2-2.45 |
| Space group      | C222,  | C222,    | C222,     |
| Unit-cell parameters (Å) | a = 65.17, b = 268.26, c = 186.77 | a = 65.25, b = 267.29, c = 187.68 | a = 65.40, b = 269.59, c = 189.91 |
| No. of observations | 241,451 (35,018) | 356,148 (45,758) | 302,793 (41,102) |
| No. of unique reflections | 43,138 (6,182) | 76,220 (10,134) | 60,876 (8,689) |
| Completeness (%) | 99.9 (99.6) | 91.6 (84.1) | 99.4 (98.6) |
|Multiplicity     | 5.6 (5.7) | 4.7 (4.5) | 5.0 (4.7) |
| Averaged I/σ(I) | 11.0 (2.4) | 5.8 (2.2) | 9.8 (2.2) |
| Rfree, Rwork    | 0.073 (0.353) | 0.078 (0.354) | 0.076 (0.368) |

- $R_{\text{merge}} = \sum |m - 1| |\Sigma_{i} I_{h,i} - I_{\text{ave}}|/\Sigma_{i} |I_{h,i}|$, where $I_{h,i}$ is the mean intensities of symmetry-equivalent reflections and $m$ is redundancy.
- $R_{\text{merge}} = \sum |F_{o} - F_{e}|/\Sigma |F_{o}|$, where $F_{o}$ and $F_{e}$ are the observed and calculated structure factor amplitudes, respectively, for the reflection $h$.  
- $R_{\text{free}}$ is equivalent to $R_{\text{merge}}$ for a 8% test set of reflections as the same indexes as the native data not used in the refinement.

The importance of Tyr295 has been proved by Y295F mutation in which the enzyme activity was completely lost. The complex crystal structure of Y295F mutant showed the reason. As described later, the tyrosine residue seems to activate ACC to form the external aldilime.

RESULTS

K51T-PLP-ACC Complex Structure—The Schiff base covalent bond complex of ACC and PLP with K51T mutant enzyme could be recognized on a 1.5σ-contoured $F_{o} - F_{e}$ omit map (Fig. 3). Compared with the free wild-type enzyme in which the pyridine of PLP ring tilts toward the phenol ring of Tyr295 by 15°, the pyridine ring rotates to be nearly parallel to the phenol ring (Fig. 3). The existence of bulky planar residues such as tryptophan, phenylalanine, tyrosine, and histidine with a nearly parallel position to the pyridine ring has been observed in many PLP enzymes, and they block the movement of PLP so as not to overrotate. However, among the structurally analyzed enzymes of the TRPSβ family, no one has such a tyrosine residue in the active site. The conversion of the internal aldimine to the external aldilime results in a tilt of the pyridine ring by −10°. The angle is almost the same as in the case of TRPSβ-substrate complexes compared with a tilt of −27° in various PLP-dependent amino acid aminotransferases.

The nitrogen atom of the ACC amino group exists with a 3.4-Å distance to the hydroxyl oxygen of Tyr295. The importance of Tyr295 has been proved by Y295F mutation in which the enzymatic activity was completely lost. The complex crystal structure of Y295F mutant showed the reason. As described later, the tyrosine residue seems to activate ACC to form the external aldilime.

Besides the nitrogen atom, around the carboxylate oxygen of ACC, there are a side-chain oxygen atom of Ser79 and a main chain amide nitrogen atom of Asn78 and Gln80 within the hydrogen-bonding distance. Two water molecules (WAT1 and WAT2) were also recognized around the carboxylate of ACC. This entire substrate recognition mechanism was further supported by the fact that the substitution of alanine for Ser79 (S78A) brought the inactivation of the enzyme (data not shown). The water molecules W1 and W2 are hydrogen-bonded to the main-chain carbonyl oxygen of Ile73 and the main-chain amide proton of Gly74, respectively.

Structural analysis has been done with the enzyme-ligand complexes from all fold types of the PLP-dependent enzymes. In the analyzed structures with the exception of those of the TRPSβ family members, the carboxylate groups of the substrate amino acids are recognized by the guanidyl group of the Arg residue. The structure of TRPSβ is analyzed in the presence of substrate/product (31). As shown in Fig. 4, no arginine residue is found near the substrate site of yACCD and TRPSβ.
Fig. 3. Binding of ACC in two mutants. A, the $\sigma_k$-weighted map calculated with omitted PLP and ACC in K51T-ACC-PLP structure. The map was calculated at 2.2 Å contoured at 2.1σ. The protein structures are shown in gray, the PLP are yellow, and ACC are magenta with colored atoms carbon (yellow), nitrogen (blue), oxygen (red), and phosphate (purple). B, the $\sigma_k$-weighted map in Y295F-ACC-PLP structure. ACC was omitted, and the map was calculated at 2.45 Å contoured at 2.1σ. Each molecular is colored as A. C, the detailed interaction in the active site of K51T-ACC-PLP. The protein structures are shown in gray with the exception of Thr51 (green). PLPs are yellow, and ACCs are magenta with colored atoms carbon (yellow), nitrogen (blue), oxygen (red), and phosphate (purple) in each molecule. Two oxygen atoms of water molecules (W1 and W2) are shown with blue spheres. The numbers correspond to interatomic distances in angstroms. D, the detailed interaction in the active site of Y295F-ACC-PLP. Figures are oriented almost the same direction as C. Each molecular is also colored as C.

and groups interacting with the carboxylate of ACC in yACCD are compared with those in TRPSβ. With respect to the carboxylate recognition devices, the basic peptide skeletons that are conserved in both proteins despite the bulkiness of substrate/product are quite different between the two enzymes. Accumulated data show that the architecture used to recognize the substrate/product are quite different between the two enzymes. Are conserved in both proteins despite the bulkiness of sub-structure. The mapping with omitted PLP and ACC in K51T-ACC-PLP structure. The map was calculated at 2.2 Å contoured at 2.1σ. The protein structures are shown in gray, the PLP are yellow, and ACCs are magenta with colored atoms carbon (yellow), nitrogen (blue), oxygen (red), and phosphate (purple). In the K51T-ACC complex (Fig. 5). Nitrogen atoms of the modeled lysine residues can be within the distance of 2.0 Å from the pro-R-methylene carbon of ACC, whereas the corresponding Lys54 residue of PH0054 protein is a distance of 4.7 Å from the pro-R-methylene carbon in the PH0054-ACC complex. No other functional residue of the protein reaches the cyclopropane ring. These figures are important for discussion regarding the catalytic mechanism.

Y295F-PLP-ACC Complex—The $\sigma_A$-weighted $F_o - F_c$ map showed a distinguishable peak of the ACC molecule in the active site of the Y295F-PLP-ACC complex, and the recognition mechanism of ACC can be understood clearly as follows. The residues and waters, which interact with the carboxylate group of ACC, are in the same positions as those of K51T-PLP-ACC. However, the ACC carboxylate group in the active site cavity moves away from Asn79 and Gln80 residues, and the cyclopropane ring of the ACC faces inversely (Figs. 3 and 4). Furthermore, most importantly, the amino group of the ACC does not form the Schiff base with C4' of PLP.

The Pendulum Movement of the PLP—Fig. 6 is the superposition of the PLP moieties in the six crystals of the yACCD wild type, the ACC complexes (of K51T, Y295F, and PH0054), K51T without ACC structure, and the PH0054 wild type. Because the PLP is located between the large and small domains in each structure and each step of the enzymatic reaction brings a slight change of relative position with respect to domains or residues, the figures were prepared based on the superposition of the large domain main chain. The side chains that are stacked on the pyridine ring of PLP in each structure are omitted for clear visibility.

In the structures of the wild-type yACCD (yellow) and the Y295F-ACC complex (dark gray), the PLPs are bonded with Lys54 amino groups in the upper right area in Fig. 6, whereas in the K51T-ACC (red) and PH0054-ACC (purple) complexes, they are complexed with ACC in the upper left area. The maximum rotation angle of the pyridine ring in yACCD is −10° between the wild type and the K51T-ACC complex, and slight translational movement is observed among all of the structures. The approximate PLP rotation axis corresponds to the line through the nitrogen atom of the pyridine ring and the phosphorus atom of the anchor phosphate. This pyridine nitrogen exists within the hydrogen-bonding distance to the side-chain carboxylate oxygen of Glu296. The existence of the carboxylate group in this position is widely seen in PLP-dependent enzymes with the exception of other members of the TRPSβ family or alanine race-mase from Bacillus stearothermophilus (32).

**DISCUSSION**

The 2.0-Å structure of wild-type yACCD (17) has revealed the detailed structure in the active site. According to the structure, Tyr209, Tyr295, and Glu296 as well as Lys54 (common
features of PLP enzyme) are expected to be important for the enzymatic activity. A previous paper (33) has also noted that the substitution of alanine for Lys51 residue at a PLP binding site of pACCD resulted in a complete loss of ACC-deaminating activity and a characteristic absorption spectrum with maxima at 330 and 405 nm and that an addition of ACC to the K51A mutant enzyme caused a decrease of absorbance at 330 nm and an increase at 425 nm, which shifted the maximum from the 405-nm band. This spectral change indicates the formation of an external aldimine between PLP and ACC in the mutant enzyme. However, because the absorption band around 500 nm was not found, it was concluded that the pACCD-K51A mutant

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**Fig. 4. Comparison of active site from four structures in the presence of substrate (or product) amino acid.** Schematic diagrams of the active site of yACCD-K51T-ACC (A), yACCD-Y295F-ACC (B), FH0064-ACC (C), and TRPS (D) from *Salmonella typhimurium* 8R87T-Trp structures. Residues are enclosed with an oval if their main chain atoms interact with polar atoms (oxygen or nitrogen) of ligands or with a rectangle if their side chains alone contribute to interactions. Corresponding interactions are indicated with dashed lines. The numbers correspond to interatomic distances in angstroms. Each water molecule is labeled with WAT.
one that binds to cofactor PLP by Schiff base. The lysine residue binding to PLP is also so important for catalytic reaction that various mutations have been introduced into many PLP enzymes. The co-crystallization with this mutant and substrate is expected to analyze the reaction intermediate structure. All of PLP enzymes are needed to analyze this external aldimine-type structure to explain substrate specificity or reaction mechanism. In addition, we analyzed the structure, which is prior to external aldimine, in the presence of substrate ACC.

In the crystal structure of Y295F-PLP-ACC, the internal aldimine between the amino group of Lys\textsuperscript{51} and C4' of PLP is conserved. The aldimine exchange reaction did not occur. This fact suggests that the Tyr\textsuperscript{295} should abstract the proton from the amino group of the substrate ACC to lead to the aldimine exchange from the internal to the external Schiff base of the substrate and the cofactor PLP. As is a common problem with PLP enzymes, the amino group of the substrate has to be deprotonated to perform a nucleophilic attack on the C4' of the internal aldimine. A few strategies were suggested with respect to the activation mechanism of the substrate. They are the effect of the internal aldimine (15), the pH of the solution (37), and the existence of a base to abstract the proton from the substrate (38). The stacking of the tyrosine residues nearly parallel to the pyridine ring was observed in the cystathionine synthase subfamily such as cystathionine \(\beta\)-lyase (38) or cystathionin (39) with its phenol groups directed toward the active pocket. Those stacking phenomena suggested the importance for the enzymatic activity.

However, the \(pK_a\) value of the tyrosine side chain is not sufficient to abstract the proton from the amino group of the ACC at the optimal pH 8.5 for the enzyme reaction. To abstract the proton more effectively, yACCD seems to adopt a further strategy. The presence of the second tyrosine residue (Tyr\textsuperscript{269}) exists back to the Tyr\textsuperscript{295} side chain (Fig. 5). The angle between these two phenol rings is \(-70^\circ\), and the distance between two phenol oxygen atoms is \(-2.6\) Å in the K51T structure (Fig. 5). Substitution of phenylalanine for the Tyr\textsuperscript{269} residue curiously disturbed the ACCD activity, i.e. the purified Y269F mutant enzyme had only <10% of the specific activity of the wild-type enzyme. This shows that the Tyr\textsuperscript{295} may abstract an amino proton from ACC with the help of Tyr\textsuperscript{269}, indicating the existence of a charge relay system between the two phenol hydroxyl groups that can transport a proton. The fact that two tyrosine residues corresponding to Tyr\textsuperscript{269} and Tyr\textsuperscript{295} are conserved in all of the ACCDs, providing putative homologues supports the importance of the charge relay system to activate substrate amino acids regardless of ACC or not. This feature is specific to ACCD, and no structural examples have been discovered so far.

In the TRPS\(\beta\) family, a polar residue such as serine or threonine side chain is always found within hydrogen-bonding distance of the pyridine nitrogen either close to the si face or the re face of the cofactor. The mutation of TRPS\(\beta\) introducing asparate or glutamate residues at the position of this serine residue stabilized the protonated form of pyridine nitrogen of PLP, reducing the \(pK_a\) of the internal aldimine nitrogen and promoting the formation of quinonoid intermediate (40). Consequently, the mutant enzyme of TRPS\(\beta\) with asparate residue interacting with the pyridine nitrogen showed a pH-dependent absorption spectrum and mechanism-based inactivation by L-serine (40). The absorption band shifted from 336 nm at around pH 9 to 416 nm at around pH 6.5, and incubation of the mutant enzyme with L-serine formed a covalent adduct of internal aldimine and aminoacrylate, which was confirmed by spectral data and a compound released from the adduct by alkaline. These characteristics of the mutant TRPS\(\beta\) agree with the published results for pACCD in which pyridine nitrogen inter-
acts with a glutamate residue of the enzyme. The absorption spectrum of pACCD exhibited a spectral change between the 416-nm band at a pH of 7 and the 326-nm band at pH 9 (41), and the covalent adduct of PLP and pyruvate was released by alkaline denaturation of the inactivated pACCD with a weak substrate D-serine or L-chloro-D-alanine (42). In the aminotransferase family, the substitution to neutral residues of these acidic residues retain the degenerated enzymatic activity, whereas the yACCD mutant E296Q does not show ACCD activity at all. It was proposed in the case of aspartate aminotransferase that the charge density on pyridium-nitrogen neighboring Asp is further modulated by hydrogen bonding of other groups, a water molecule and His143-Ser139 (43, 44). However, there is no such charge modulator around Glu 296 in the yACCD active site.

The enzymatic degradation of ACC has long been investigated because of the interest in the cyclopropane ring-opening reaction and in the evolution of PLP enzyme specificity. In particular, the focal point of this investigation is the enzymatic strategy that enables the enzyme to open the cyclopropane ring. Walsh et al. (23) suggest two plausible reaction-mechanisms for the cyclopropane ring-opening reaction based on the experiments of modified substrates and NMR spectroscopy. They are as follows: (a) nucleophilic addition to open the ring followed by -proton abstraction and (b) direct -proton abstraction to initiate cyclopropane cleavage. Of these two possibilities, the idea of nucleophilic addition is better supported by the general chemistry of PLP.

If a step following the nucleophilic addition is blocked by the amino acid replacement, a covalent adduct of the reaction intermediate with a nucleophilic group of the enzyme would be detected by the characteristic absorption band at around 500 nm because of the formation of the quinonoid form. Actually, the absorption band at 510 nm by the quinonoid was observed by the addition of D-alanine to pACCD (45). The preliminarily tested mutant enzymes of the pACCD (33) and the present mutated yACCD losing the ACC-deaminating activity exhibited absorption spectra showing the formation of the external aldimines with ACC, but they did not show any sign of the quinonoid form. Li et al. (46) reported that the mechanism-based inactivation of pACCD by methylene-ACC was consistent with the nucleophilic addition, route A. They identified the turnover product as 2-oxo-3-methyl-3-butenoic acid from a possible substrate (R)-methylene-ACC. This description is not consistent with published results on ACCD, i.e. a good substrate of ACCD is thought to be (S)-form rather than (R)-form to give 2-oxo-3-pentenoic acid. The disagreement remains to be solved.

Our present structure of reaction intermediates highlights the possibility of the route (b) (Fig. 7), because the proposed position of Lys residue in the K51T-ACC complex is near the pro-R-methylene of ACC and there are none of the reactive or nucleophilic residues around the methylene group of ACC in the K51T-ACC-PLP structure. Although experiments with methylene ACC showed that “abstraction of an inert proton from the ring C–H group and such an anion induced ring cleavage is stereochemically unfavored” (46), the function of PLP as an electron sink and the electron-withdrawing carboxylate moiety attached to cyclopropane ring could enable this difficult -proton abstraction to be achieved. The enzymatic inertness of E296Q mutant explains the necessity of a carboxylate oxygen of the Glu residue, forming the electron-withdrawing hydrogen bond with pyridium nitrogen of PLP to induce the electron-deficient state of the ACC cyclopropane ring together with the delocalized system through the electron-withdrawing PLP pyridine ring. As mentioned above, ACCD catalyzes the deprotonation of D-alanine to form the quinonoid intermediate. The nitrogen atom of the proposed Lys residue is impossible to access by the -proton of D-alanine, not that of

**Fig. 7. The putative total mechanism catalyzed by yACCD.**
Deprotonation of D-alanine or D-serine has to be dependent on a group other than Lys^51 residue. One possibility is the phenol group of the Tyr^285 residue existing near the α-proton of D-alanine.

The whole turnover reaction of ACCD is shown in Fig. 7. After the cyclopropane ring is opened, the following steps are generally acceptable. The hydrolysis concomitant with deamination proceeds to the unsaturated aminocrotene to produce the final α-ketobutyrate and ammonia (47). The internal Schiff base between PLP and Lys^51 is formed. As seen in all of the PLP enzymes, the role of the residue Lys^51 is very important and we present the new function of the catalytic lysine, namely the abstraction of methane proton from the cyclopropane ring in combination with ingenious strategies. The action of ACCD brings us to understand the diverse roles of the PLP enzymes in biological transformations.

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REFERENCES
1. Adams, D. O., and Yang, S. F. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 170–174
2. Yu, Y. B., Adams, D. O., and Yang, S. F. (1979) Arch. Biochem. Biophys. 198, 280–286
3. Zhang, Z., Barlow, J. N., Baldwin, J. E., and Schofield, C. J. (1997) Biochemistry 36, 15999–16007
4. Honma, M., and Shimomura, T. (1978) Agric. Biol. Chem. 42, 1825–1831
5. Sheehy, R. E., Honma, M., Yamada, M., Sasaki, T., Martinez, B., and Hiatt, W. R. (1991) J. Bacteriol. 173, 5260–5265
6. Glick, B. R., Jacobson, C. B., Schwarze, M. M. K., and Pasternak, J. J. (1994) Can. J. Microbiol. 40, 911–915
7. Glick, B. R., Penrose, D. M., and Li, J. (1998) J. Theor. Biol. 190, 63–68
8. Klee, H. J., Hayford, M. B., Kretzmer, K. A., Barry, G. F., and Kishore, G. M. (1991) Plant Cell 3, 1187–1193
9. Reed, A. J., Kretzmer, K. A., Naylor, R. M., Finn, R. M., Magin, K. M., and Fuchs, R. L. (1996) J. Agric. Food Chem. 44, 388–394
10. Jacobson, C. B., Pasternak, J. J., and Glick, B. R. (1994) Can. J. Microbiol. 40, 1019–1025
11. Campbell, B. G., and Thomson, J. A. (1996) FEMS Microbiol. Lett. 138, 207–210
12. Minami, R., Uchiyama, K., Murakami, T., Kawasaki, J., Mikami, K., Yamada, T., Yokus, D., Ito, H., Matsu, H., and Honma, M. (1998) J. Biochem. (Tokyo) 123, 1112–1116
13. Jia, Y. J., Kakuta, Y., Sugawara, M., Igarashi, T., Nakagawa, A., Nakagawa, T., and Honma, M. (1999) J. Biochem. (Tokyo) 125, 542–549
14. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
15. Jansonius, J. N. (1998) Curr. Opin. Struct. Biol. 8, 759–769
16. Murzin, A. G., Brenner, S. E., Hubbard, T., and Chothia, C. (1995) J. Mol. Biol. 247, 536–540
17. Yao, M., Ose, T., Sugimoto, H., Horuchi, A., Nakagawa, A., Nakagawa, T., Yokus, D., Murakami, T., Honma, M., and Tanaka, I. (2000) J. Biochem. 127, 34557–34565
18. Hyde, C. C., Ahmed, S. A., Padlan, E. A., Miles, E. W., and Davies, D. R. (1988) J. Biol. Chem. 263, 17875–17871
19. Burkhart, P., Rao, G. S., Huh, H. B., Schnackerz, K. D., Cook, P. F., and Jansonius, J. N. (1998) J. Mol. Biol. 283, 121–133
20. Gallagher, D. T., Gilliland, G. L., Xiao, G., Zondlo, J., Fisher, K. E., Chinchilla, D., and Eisenstein, E. (1998) Structure 6, 465–475
21. Tomazou, K., Curien, G., Dumas, R., and Bien, V. (2001) Protein Sci. 10, 638–648
22. Meier, M., Jonasik, M., Kery, V., Kraus, J. P., and Burkhart, P. (2001) EMBO J. 20, 3910–3916
23. Walsh, C. T., Pascal, J. R., A., Johnston, M., Raine, D., Bir, D., and Honma, M. (1981) Biochemistry 20, 7509–7519
24. Denesuk, A. L., Denesuk, K. A., Kupeda, T., and Johnson, M. S. (2002) J. Mol. Biol. 316, 155–172
25. Weber, I. T., Johnson, L. N., Wilson, K. S., Yeates, D. G., Wild, D. L., and Jenkins, J. A. (1978) Nature 274, 435–437
26. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
27. Burroughs, L. F. (1957) Nature 179, 360–361
28. Collaborative Computational Project N. (1994) Acta Crystallogr. Sec. D 50, 760–763