Active-site Arg → Lys Substitutions Alter Reaction and Substrate Specificity of Aspartate Aminotransferase

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Arg^{386} and Arg^{292} of aspartate aminotransferase bind the α and the distal carboxylic group, respectively, of dicarboxylic substrates. Their substitution with lysine residues markedly decreased aminotransferase activity. The cat values with L-aspartate and 2-oxoglutarate as substrates under steady-state conditions at 25 °C were 0.5, 2.0, and 0.03 s^{-1} for the R292K, R386K, and R292/KR386K mutations, respectively, cat of the wild-type enzyme being 220 s^{-1}. Longer dicarboxylic substrates did not compensate for the shorter side chain of the lysine residues. Consistent with the different roles of Arg^{292} and Arg^{386} in substrate binding, the effects of their substitution on the activity toward long chain monocarboxylic (norleucine/2-oxocaproic acid) and aromatic substrates diverged. Whereas the R292K mutation did not impair the aminotransferase activity toward these substrates, the effect of the R386K substitution was similar to that on the activity toward dicarboxylic substrates. All three mutant enzymes catalyzed as side reactions the β-decarboxylation of L-aspartate and the racemization of amino acids at faster rates than the wild-type enzyme. The changes in reaction specificity were most pronounced in aspartate aminotransferase R292K, which decarboxylated L-aspartate to L-alanine 15 times faster (cat = 0.002 s^{-1}) than the wild-type enzyme. The rates of racemization of L-aspartate, L-glutamate, and L-alanine were 3, 5, and 2 times, respectively, faster than with the wild-type enzyme. Thus, Arg → Lys substitutions in the active site of aspartate aminotransferase decrease aminotransferase activity but increase other pyridoxal 5'-phosphate-dependent catalytic activities. Apparently, the reaction specificity of pyridoxal 5'-phosphate-dependent enzymes is only achieved by accelerating the specific reaction but also by preventing potential side reactions of the coenzyme substrate adduct.

The pyridoxal 5'-phosphate (PLP)1-dependent enzymes that catalyze transformations of amino acids (for a recent review, see Ref. 1) constitute a few families of evolutionarily related enzymes (2). The member enzymes of such a family use the same protein scaffold to catalyze quite diverse reactions. Apparently, subtle structural differences underlie their catalytic specificity.

Aspartate aminotransferase (AspAT) is probably the most extensively studied PLP-containing enzyme. It catalyzes the reversible transamination of the dicarboxylic L-amino acids, aspartate and glutamate, and the corresponding 2-oxo acids, oxalacetate and 2-oxoglutarate. During the catalytic cycle, the cofactor shuttles between the PLP and the pyridoxamine 5'-phosphate (PMP) forms. High resolution x-ray crystallographic analyses (3–6) in conjunction with site-directed mutagenesis studies (7–14) have elucidated the role of several active-site residues.

The specificity for dicarboxylic amino acids appears to be based mainly on two active-site arginine residues (Fig. 1). Arg^{386} of the small domain binds the α-carboxylic group of the substrate, and Arg^{292} of the large domain of the adjacent subunit interacts with the distal carboxylic group. The spatial orientation of these key residues is determined by steric constraints and polar interactions. The van der Waals contacts of the guanidino nitrogens of Arg^{386} with the side chain carbonyl of Asn^{134} and the aromatic ring of Phe^{390} effectively delimit the conformational space available to Arg^{386}. The guanidino nitrogens of Arg^{292} are within hydrogen bonding distance from the carboxylic group of Asp^{15}, the side chain amide of Asn^{142}, and the hydroxy group of Ser^{296} of the adjacent subunit. The side chain of Arg^{292} is thus maintained in an extended configuration, which favors interaction with the distal carboxylic group of the incoming substrate (15, 12). Arg^{386} is invariant in all known aminotransferase sequences. Arg^{292} is conserved in most AspAT sequences, and other aminotransferases have variable residues at position 292 (16).

In an attempt to explore the mechanisms responsible for the reaction specificity of AspAT, we re-engineered the substrate-binding site of the enzyme by substituting the substrate-binding Arg^{292} and Arg^{386} with lysine residues. This conservative substitution was expected not to abolish the catalytic apparatus of the enzyme, but to alter the electron repartition and certain bond angles in the coenzyme-substrate adduct, both important determinants of catalytic specificity.

EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis and Purification of Wild-type and Mutant AspATs—Oligonucleotide-directed mutagenesis of the wild-type aspC gene of Escherichia coli inserted into the BS M13 vector (17) was performed with the mutagenesis kit of Bio-Rad (18) and the oligonucleotides GC CAC ATT TAG TTT ACC AGA AGC and GA GTA GTC AGC TTT AAT CGC CGC T for the R386K and the R292K mutation, respectively. The mutations were confirmed by determination of the nucleotide sequences. The mutated DNAs were expressed in the AspAT-deficient E. coli strain TY103 (19) with the expression vector pK- DHE19 (20).

Wild-type and mutant enzymes were purified as described previously.
The values of enzymes, the absorption maximum shifted from 360 to 430 nm. The ensuing pH values were measured, and spectra were recorded from pH study; coenzyme-binding Lys258 (titrated by the repeated addition of 3–5 mM or 150 mM L-aspartate for wild-type enzyme or mutant AspATs, incubated in 50 mM 4-methylmorpholine, pH 7.5, containing 9 μM (subunit concentration) AspAT and the substrate. The half-reaction from amino acid to oxo acid was followed by measuring the increase in absorbance at 360 nm and the increase in absorbance at 530 nm due to the conversion of enzyme-bound PLP to PMP; the reverse half-reaction was followed in an analogous manner. The PMP form of the enzymes was prepared by incubation of the PLP form with 1-mm PMP and 5 mM cysteine sulfinate for 30 min at 25 °C in the dark followed by Sephadex G-25 chromatography. The reactions were followed with a Beckman 7400 DU spectrophotometer. With rapidly reacting substrates, a stopped-flow apparatus (a pkb-Spectra Kinetic Monochromator 05-109 from Applied Photophysics) with a cuvette of 1-cm path length and a dead time of 2 ms was used. In all cases, the reaction progress curves fitted to single exponential equations with the pseudo-first order rate constant kobs. The values for kcat and Km were obtained from the kobs values at varying substrate concentrations by Lineweaver-Burk analysis using GRAFIT software (Erithacus Software). The calculated values of kcat/Km for competitive inhibitors and pKcat of internal aldimine of all mutant enzymes. The kcat values of the overall steady-state transamination reactions catalyzed by the mutant enzymes were decreased commensurately with the decrease in rate of the half-reactions (Table II).

The pH rate profiles for AspAT R386K and AspAT R292K/R386K did not significantly differ from that of wild-type enzyme (Fig. 2). In contrast, the pH optimum of AspAT R292K was considerably narrower than that of the wild-type enzyme. The decrease in activity at higher pH might be due to the deproteinization of the newly introduced Lys residue. The affinity of AspAT R292K for aspartate decreased only little with pH (Km for Asp = 14 mM at pH 7.5, 18 mM at pH 9.0, and 36 mM at pH 10.0), indicating that kcat rather than Km is affected at higher pH.

All three mutant enzymes were analyzed for newly generated catalytic activities (Table II). AspAT R292K racemizes L-aspartate, L-glutamate, and L-alanine 3, 5, and 2 times faster, respectively, than the wild-type enzyme. The same mutant enzyme catalyzes the β-decarboxylation of L-aspartate to L-alanine with a kcat of 0.002 s−1, i.e. 15 times faster than the wild-type enzyme. The kcat of the wild-type enzyme of β-decarboxylation and transamination is 0.004 and 5.9 × 10−7 for AspAT R292K and the wild-type enzyme, respectively. Both AspAT R386K and R292K/R386K exhibited a 3 times higher β-decarboxylase activity and a 2–3-fold higher alanine racemase activity than the wild-type enzyme. All three mutant enzymes showed about the same serine dehydrogenase activity as the wild-type enzyme.

Changes in Substrate Specificity—Aliphatic dicarboxylic and monocarboxylic amino and oxo acids of various length as well as aromatic substrates were tested for transamination (Tables I and III). As previously shown for AspAT R386K (7) and now for AspAT R292K and AspAT R292K/R386K, the decrease in both kcat and kcat/Km compared with the wild-type enzyme was more pronounced with C3 dicarboxylic substrates (glutamate and 2-oxoglutarate) than with C4 dicarboxylic substrates (as-

Results

Spectroscopic Properties of Mutant AspATS—Virtually identical absorption and CD spectra in the visible region for wild-type and mutant enzymes, in both their PLP and PMP forms (not shown), indicate that the mutations leave the active-site geometry essentially undisturbed. Spectrophotometric pH titration gave a pKυ value of 6.4 for the internal aldimine of all three mutant enzymes and of 6.3 for the wild-type enzyme. Apparently, the positive electrostatic potential due to Arg292 and Arg368 that is assumed to account for the low pKυ of the internal aldimine (4) is maintained in the Arg → Lys mutant AspATs.

Changes in Reaction Specificity—Replacement of either Arg292 or Arg368 with a lysine residue resulted in a marked decrease in aminotransferase activity toward dicarboxylic substrates. The kcat values of the half-reaction from L-aspartate to oxalacetate were decreased by 2 orders of magnitude in the single mutant enzymes and by 4 orders of magnitude in the double mutant enzyme (Table I). Due to a general increase in Km values by 1–2 orders of magnitude, an even larger decrease in the catalytic efficiency kcat/Km was observed for all mutant enzymes. The kcat values of the overall steady-state transamination reactions catalyzed by the mutant enzymes were decreased commensurately with the decrease in rate of the half-reactions (Table II).
Kinetic parameters for transamination half-reactions of wild-type and mutant AspATs with dicarboxylic, monocarboxylic, and aromatic amino acids

Values were determined under single turnover conditions in 50 mM 4-methylmorpholine, pH 7.5, at 25 °C and 9 μM subunit concentration by Lineweaver-Burk analysis of the rate of the decrease in ΔA_{292} (see "Experimental Procedures"). The concentration ranges of the tested amino acids were 10–200 mM for dicarboxylic amino acids, 2.5–150 mM for l-alanine, 5–90 mM for l-norleucine, 1–30 mM for l-phenylalanine, and 1–5 mM for l-tyrosine.

| Substrates          | AspAT wild type | AspAT R292K | AspAT R386K | AspAT R292K/R386K |
|---------------------|-----------------|-------------|-------------|-------------------|
|                     | k_{cat} \( s^{-1} \) | K_{m} \( \text{mM} \) | k_{cat}/K_{m} \( s^{-1} \) | k_{cat} \( s^{-1} \) | K_{m} \( \text{mM} \) | k_{cat}/K_{m} \( s^{-1} \) | k_{cat} \( s^{-1} \) | K_{m} \( \text{mM} \) | k_{cat}/K_{m} \( s^{-1} \) |
| L-Aspartate         | 530a 4^b 1.3 × 10^6 | 326 | 4.5b 14 | 9.6b 72 | 133 | 0.055 300 | 0.16 |
| L-Glutamate         | 670c 377 1.8 × 10^4 | 177 | 14c 1.4 | 300 | 4.6 | 0.003 293 | 0.01 |
| L-2-Aminoadipate    | 3.4^c 118 28 | 0.16b 126 1.3 | 4.2 | 0.003 | 5 | 0.001 | 0.01 |
| l-Alanine           | 0.07 0.018 0.51 | 0.005 129 0.04 | 0.002 152 | 0.13 | 152 0.04 | 0.03 400 | 0.01 |
| l-Norleucine        | 0.06 0.018 0.67 | 0.14^c 12 | 11.7 | 0.002 98 | 0.02 | 0.003 304 | 0.01 |
| l-Phenylalanine     | c 150b | c 83b | c 0.06 | c 0.04 |
| l-Tyrosine          | c 420b | c 250b | c 16 | c 7 |

a Ref. 7.
b Determined by stopped-flow technique.
c Saturation was not apparent within the concentration range tested. k_{cat}/K_{m} values were obtained by linear least squares regression of the data.

Increased rates of side reactions of mutant AspATs

The activities of the enzymes (0.9 mM subunit concentration) toward 20 mM l-aspartate, 8 mM oxalacetate; 20 mM l-glutamate, 20 mM 2-oxoglutarate; 40 mM l-alanine, 10 mM pyruvate; and 20 mM l-serine, 10 mM 2-oxoglutarate were measured in 50 mM 4-methylmorpholine, pH 7.5, at 25 °C. For details, see "Experimental Procedures." No reactions of the substrates were observed in nonenzymic controls with 0.9 mM PLP.

| Substrates | Activities | k_{cat} \( s^{-1} \) | AspAT wild type | AspAT R292K | AspAT R386K | AspAT R292K/R386K |
|------------|------------|-----------------|----------------|-------------|-------------|-------------------|
| L-Aspartate| Transamination | 220 | 0.5 | 2.0 | 0.03 |
|            | β-Decarboxylation | 1.3 × 10^4 | 20 × 10^{-4} | 4 × 10^{-4} | 4 × 10^{-4} |
| L-Glutamate| Racemization | 3 × 10^{-5} | 1 × 10^{-5} | 3.4 × 10^{-5} | 5 × 10^{-5} |
| L-Alanine  | Racemization | 1.5 × 10^{-5} | 8 × 10^{-5} | 1.2 × 10^{-5} | 1.5 × 10^{-5} |
| L-Serine   | Dehydration | 1.5 × 10^{-5} | 3 × 10^{-5} | 5 × 10^{-5} | 5 × 10^{-5} |

a Steady-state assays of overall reaction of transamination (see "Experimental Procedures").

b The increased k_{cat} value of AspAT R292K corresponds to the rate of the steady-state reaction. With some preparations of this mutant enzyme, faster β-decarboxylation was observed in the first minutes of the reaction.

The R386K substitution decreased the catalytic efficiency toward aromatic amino acids by 1–4 orders of magnitude. In contrast, the k_{cat}/K_{m} values of AspAT R292K for aromatic amino acids were only slightly lower than that of the wild-type enzyme and thus higher by 1–2 orders of magnitude than that toward l-aspartate.

Changes in the Binding ofDicarboxylic Inhibitors—The affinity of the mutant enzymes for dicarboxylic substrate analogs of varying length was compared with that of the wild-type enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV). Dicarboxylic acids bind noncovalently to the enzyme (Table IV).

The hydrocarbon chain contributes only insignificantly to the binding of the inhibitors. α-Phthalate behaves like C4 to C6 aliphatic inhibitors. α-Phthalate, however, is the only inhibitor that is bound more tightly by the mutant enzymes than by the wild-type enzyme. α-Phthalate with its fixed conformation may be assumed to interact more strongly with the more flexible lysine residue than with the arginine residue, the side chain of which is fixed by multiple interactions of its guanidinium moiety (see Introduction).

partly and oxalacetate). The k_{cat}/K_{m} value of AspAT R386K but not of AspAT R292K toward C4 dicarboxylic substrates (2-aminoacidipate and 2-oxoadipate) was significantly lower than that toward C5 dicarboxylic substrates. The activity of all mutant enzymes toward alanine and pyruvate was decreased, while k_{cat}/K_{m} of AspAT R292K toward norleucine and 2-oxoacrylic acid was even higher than that of the wild-type enzyme.

![Fig. 2. pH rate profile of wild-type and mutant AspATs. Activities were measured with the malate dehydrogenase-coupled assay at 25 °C with 20 mM 2-oxoglutarate plus 20 mM l-aspartate or 150 mM l-aspartate for wild-type and mutant AspATs, respectively. The following buffers were used at 50 mM concentration: Mes, pH 5.5–7.0; Hpes, pH 7.0–8.5; Ches, pH 8.5–10. The differences in activity at the overlaps of two buffers were less than 10% of the total activity; the mean values are indicated. a, wild-type AspAT; b, AspAT R386K; c, AspAT R292K; d, AspAT R292K/R386K.](image-url)
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Table III
Kinetic parameters for transamination half-reactions of wild-type and mutant AspATs with dicarboxylic, monocarboxylic, and aromatic 2-oxo acids

Values were determined under single turnover conditions in 50 mM 4-methylmorpholine, pH 7.5, at 25 °C with 9 mM subunit concentration by Lineweaver-Burk analysis of the rate of the increase in A_{420} (see “Experimental Procedures”). The concentration ranges of the tested 2-oxo acids were 0.1–10 mM for oxalacetate; 0.5–20 mM for 2-oxoglutarate and 2-oxoadipate; 0.1–50 mM for pyruvate, 0.1–20 mM for 2-oxoacrylic acid, and 0.1–5 mM for phenylpyruvate.

Table IV
Dissociation equilibrium constants for dicarboxylic competitive inhibitors of wild-type and mutant AspATs

The values were determined by spectrophotometric titration in 10 mM Tris-acetate, pH 8.0, at 25 °C (see “Experimental Procedures”). The enzyme subunit concentration was 9 mM.

Table V

PLP-dependent catalytic activities of AspAT mutants

\(^{a}\) Ref. 7.
\(^{b}\) Determined by stopped-flow technique.
\(^{c}\) Saturation was not apparent within the concentration range tested. \(k_{cat}/K_m\) values were obtained by linear least squares regression of the data.

DISCUSSION

Arg → Lys substitutions are generally considered conservative alterations in protein structure. In AspAT R292K, AspAT R386K, and the double-mutant enzyme, absorption and CD spectra as well as determination of the pKa values of the internal aldime indeed did not reveal changes in the active-site geometry. Nevertheless, both single mutations reduced the catalytic efficiency \((k_{cat}/K_m)\) for transamination of C4 and C5 dicarboxylic substrates by at least 3 orders of magnitude (Table I). Similar results with AspAT R386K have been reported previously (7).2 A decrease by 6 orders of magnitude was brought about by the double mutation. Substitution of Arg\(^{292}\) with aspartate (26), valine, and leucine (27) has been found to have the same effect. Replacement of Arg\(^{386}\) with tyrosine, phenylalanine (8), or alanine (14) reduced the catalytic efficiency by 4 orders of magnitude. Thus, irrespective of whether Arg\(^{292}\) or Arg\(^{386}\) is substituted and irrespective of the nature of the new side chain, the catalytic efficiency is decreased by at least 3 orders of magnitude.

On the basis of chemical modification studies, it was suggested early on that anionic substrates of enzymes are bound by arginine residues (28). In the case of AspAT, determination of the crystal structure of enzyme-substrate analog complexes (3–6) and of enzymic reaction intermediates (29) has confirmed this prediction. The preference for arginine in AspAT and many other enzymes may be explained by the peculiar strength of the guanidinium-carboxylate interaction due to the resonance-stabilized ion pair underlying the two hydrogen bonds that can be formed (30, 31). Moreover, arginine is a poor proton donor because of resonance stabilization and hence would probably not function as a general acid catalyst. Evolutionary selection of arginine thus minimizes nonspecific hydrolysis of substrates (28). The greater number of possible hydrogen bonds not only with carboxylic groups of the substrates but also with other polar active-site residues endows the guanidinium-carboxylate interaction with a more strictly defined geometry than could be achieved with lysine. The crucial role of arginine-carboxylate interactions, both for substrate binding and efficient catalysis, is borne out by our results.

Wild-type AspAT shows an inverse relationship between \(k_{cat}/K_m\) values and the side chain length of dicarboxylic amino acids (Table I), as has also been shown in another study (32). The side chain of lysine is shorter than that of arginine, the C=–N distance being 5.64 and 6.50 Å, respectively. However, the Arg → Lys substitution cannot be compensated by longer dicarboxylic substrates. Neither of the substituted arginine residues directly participates in the covlancy changes catalyzed by AspAT. Apparently, the loss in enzymic activity is due to modes of binding of the substrates that do not allow the catalytic apparatus to become fully effective. Indeed, all Arg → Lys mutant AspATs show a decrease in catalytic competence with increasing length of the dicarboxylic substrates, although the binding of dicarboxylic reversible inhibitors is independent of their length (Table IV). Arginine residues that are responsible for the formation of catalytically competent enzyme-substrate complexes and cannot be replaced by lysine without substantial loss in catalytic activity have also been found in enzymes other than AspAT (33–37).

Consistent with the different roles of Arg\(^{292}\) and Arg\(^{386}\) in substrate binding, the effects of their substitution on the activ-
ity toward long-chain monocarboxylic (norleucine/2-oxocaprylic acid) and aromatic substrates diverge. Arg292 binds the distal carbanion group of dicarboxylic substrates, and its substitution hardly impairs the catalytic competence toward monocarboxylic and aromatic substrates. In contrast, substitution of Arg292, which binds the proximal carbanion group, decreases the activity toward all types of substrates (Tables I and III).

The introduction of a lysine residue at position 292, which is situated on the re face of the coenzyme-substrate adduct (Fig. 1), increases the rate of racemization of L-aspartate, L-glutamate, and L-alanine 2–3-fold (Table II). A somewhat greater effect was observed when Trp140 on the re face of PLP was replaced by histidine (Table V; Ref. 13). Reprotonation of the intermediate E. coli in the mutant enzymes. 1), increases the rate of racemization of L-aspartate, L-glutamate, and L-alanine 2–3-fold (Table II). A somewhat greater effect was observed when Trp140 on the re face of PLP was replaced by histidine (Table V; Ref. 13). Reprotonation of the intermediate E. coli in the mutant enzymes.

Intermediates in the reactions of aspartate catalyzed by wild-type and mutant AspATs.

REFERENCES

1. John, R. A. (1995) Biochim. Biophys. Acta 1248, 81–96
2. Alexander, F. W., Sandmeier, E., Mehta, P. K., and Christen, P. (1994) Eur. J. Biochem. 219, 953–960
3. Kirsch, J. F., Eichele, G., Ford, G. C., Vincent, M. G., Jansonius, J. N., Gehring, H., and Christen, P. (1984) J. Mol. Biol. 174, 497–525
4. Arnone, A., Rogers, P. H., Hyde, C. C., Briley, P. D., Metzler, C. M. and Metzler, D. E. (1985) Transaminases (Christen, P., and Metzler, D. E., eds) pp. 138–155, John Wiley & Sons, Inc., New York
5. Kamitori, S., Okamoto, A., Hirotsu, K., Higuchi, T., Kuramitsu, S., Kagamiyama, H., Matsuura, Y., and Kataube, Y. (1990) J. Biochem. (Tokyo) 108, 175–184
6. Jager, J., Mose, M., Sauder, U., and Jansonius, J. N. (1994) J. Mol. Biol. 239, 285–305
7. Inoue, Y., Kuramitsu, S., Inoue, K., Kagamiyama, H., Hirono, K., Tanase, S., and Morino, Y. (1989) J. Biol. Chem. 264, 9673–9681
8. Danishefsky, A. T., Onuffer, J. J., Petsko, G. A., and Dinge, D. (1991) Biochemistry 30, 1980–1985
9. Taney, M. D., and Kirsch, J. F. (1991) Biochemistry 30, 7456–7461
10. Ziajk, M., Jager, J., Malashchekiev, V. N., Gehring, H., Jaussi, R., Jansonius, J. N., and Christen, P. (1993) Eur. J. Biochem. 211, 475–484
11. Yang, T., Hinosue, Y., Chen, Y. J., Metzler, D. E., Miyahara, I., Hirotsu, K., and Kagamiyama, H. (1995) J. Mol. Biol. 254, 1218–1229
12. Almo, S. C., Smith, D. L., Danishefsky, A. T., and Ringe, D. (1994) Protein Eng. 7, 405–412
13. Varca, R. A., Christen, P., Malashchekiev, V. N., Jansonius, J. N., and Sandmeier, E. (1995) Eur. J. Biochem. 227, 483–487
14. Graber, R., Kasper, P., Malashchekiev, V. N., Sandmeier, E., Berger, P., Gehring, H., Jansonius, J. N., and Christen, P. (1990) Eur. J. Biochem. 232, 686–690
15. Jansonius, J. N., and Vincent, M. G. (1987) Biological Macromolecules and Assemblies (Jurnak, F. A., and McPherson, A., eds) Vol. 3, pp. 157–285, Wiley, New York
16. Mehta, P. K., Hale, T. I., and Christen, P. (1993) Eur. J. Biochem. 214, 549–561
17. Malcolm, H. A., and Kirsch, J. F. (1985) Biochim. Biophys. Res. Commun. 132, 915–921
18. Kunkel, T. A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 488–492
19. Yang, T., Kuramitsu, S., Tanase, S., Morino, Y., Hirotsu, K., and Kagamiyama, H. (1991) J. Biol. Chem. 266, 6079–6085
20. Kamitori, S., Hirotsu, K., Higuchi, T., Kondo, K., Inoue, K., Kuramitsu, S., Kagamiyama, H., Higuchi, Y., Yasuoka, N., Kusunoki, M., and Matsuura, Y. (1997) J. Biochem. (Tokyo) 121, 813–816
21. Kuramitsu, S., Hirono, K., Hayashi, H., Morino, Y., and Kagamiyama, H. (1990) Biochemistry 29, 5469–5476
22. Fonda, M. L., and Johnson, R. J. (1970) J. Biol. Chem. 245, 2709–2716
23. Marley, P. (1984) Carlsberg Res. Commun. 49, 591–596
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24. Kochhar, S., and Christen, P. (1988) *Eur. J. Biochem.* **175**, 433–438
25. Gallagher, T., Snell, E. E., and Hackert, M. L. (1989) *J. Biol. Chem.* **264**, 12737–12743
26. Cronin, C. N., and Kirsch, J. K. (1988) *Biochemistry* **27**, 4572–4579
27. Hayashi, H., Kuramitsu, S., Inoue, Y., Morino, Y., and Kagamiyama, H. (1989) *Biochem. Biophys. Res. Commun.* **159**, 337–342
28. Riordan, J. F., McElvany, K. B., and Borden, C. L., Jr. (1977) *Science* **195**, 884–886
29. Malashkevich, V. N., Toney, D. N., and Jansonius, J. N. (1993) *Biochemistry* **32**, 13451–13462
30. Hwang, J-K., and Warshel, A. (1988) *Nature* **334**, 270–272
31. Mitchell, J. B. O., Thornton, J. M., Singh, J., and Price, S. L. (1992) *J. Mol. Biol.* **226**, 251–262
32. Onuffer, J. J., Ton, B. T., Klement, I., and Kirsch, J. F. (1995) *Protein Sci.* **4**, 1743–1749
33. Hart, W. H., Clarke, A. R., Wigley, D. B., Waldman, A. D. B., Chia, W. N., Barstow, D. A., Atkinson, T., Jones, J. B., and Holbrook, J. J. (1987) *Biochim. Biophys. Acta* **914**, 294–298
34. Yan, H., Shi, Z., and Tsai, M. D. (1990) *Biochemistry* **29**, 6385–6392
35. Engler, D. A., Campion, S. R., Hauser, M. R., Cook, J. S., and Niyogi, S. K. (1992) *J. Biol. Chem.* **267**, 2274–2281
36. Van Berkel, W., Westphal, A., Eschrich, K., Eppink, M., and De Kok, A. (1992) *Eur. J. Biochem.* **210**, 411–419
37. Delle Fratte, S., Iurescia, S., Angelaccio, S., Bossa, F., and Schirch, V. (1994) *Eur. J. Biochem.* **225**, 395–401
38. Kochhar, S., and Christen, P. (1992) *Eur. J. Biochem.* **203**, 563–569