Role of dimerization in yeast aspartyl-tRNA synthetase and importance of the class II invariant proline

(aminoacyl-tRNA synthetase/heterodimer)

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ABSTRACT   Cytoplasmic aspartyl-tRNA synthetase (AspRS; EC 6.1.1.12) from yeast is, as are most class II synthetases, an α_2 dimer. The only invariant amino acid in signature motif 1 of this class is Pro-273; this residue is located at the dimer interface. To understand the role of Pro-273 in the conserved dimeric configuration, we tested the effect of a Pro-273 → Gly (P273G) substitution on the catalytic properties of homo- and heterodimeric AspRS. Heterodimers of AspRS were produced in vivo by overexpression of their respective subunit variants from plasmid-encoded genes and purified to homogeneity in one HPLC step. The homodimer containing the P273G shows an 80% inactivation of the enzyme and an affinity decrease for its cognate tRNA^Asp of one order of magnitude. The P273G-mutated subunit recovered wild-type enzymatic properties when associated with a native subunit or a monomer otherwise inactivated having an intact dimeric interface domain. These results, which can be explained by the crystal structure of the native enzyme complexed with its substrates, confirm the structural importance of Pro-273 for dimerization and clearly establish the functional interdependence of the AspRS subunits. More generally, the dimeric conformation may be a structural prerequisite for the activity of mononucleotide binding sites constructed from antiparallel β strands.

In most cases, oligomerization confers peculiar properties on enzymes: it may determine the direction of a reaction (lactate dehydrogenase), increase its velocity (tryptophan synthetase), or regulate the level of activity (aspartate transcarbamoylase). In addition, subunit association may modify enzyme specificity by generating a new activity (lactose synthetase) or may be required for substrate binding (tyrosyl-tRNA synthetase). Even in cases where no apparent properties seem to be linked to oligomerization, it generally stabilizes the enzyme and/or prevents it from protease degradation.

Aminoacyl-tRNA synthetases (aaRSs) constitute an enzyme family that exhibits a wide diversity of oligomeric arrangements; it is noteworthy that most of the dimeric enzymes of this family are members of the class II aaRSs, which are characterized by acylation occurring on the 3′ hydroxyl of the last ribose of the tRNA and by the presence of three conserved consensus motifs (1). Conservation of the dimeric conformation through evolution in all of these structurally and functionally related proteins of class II aaRS attests to the importance of this configuration in enzyme functionality.

To investigate the signification of this homodimeric association we chose, as a model molecule, yeast aspartyl-tRNA synthetase (AspRS; EC 6.1.1.12) (2), a well-studied $\alpha2$ dimeric member (molecular mass = 2 × 63 kDa) of class II

aaRS. Its amino acid sequence (557 residues) has been established using peptide analysis (3) and gene sequencing (4, 5); AspRS was crystallized (6) as was its complex with its cognate tRNA^Asp (7, 8). The high-resolution x-ray structure of the complex is refined (9). Site-directed mutagenesis studies on AspRS (10, 11) are in agreement with the location of essential residues from motifs 2 and 3 in the catalytic site domain. The enzyme binds two molecules of aminoacyl-adenylate and tRNA^Asp (ref. 12; D. Kern, personal communication); this contrasts with tyrosyl-tRNA synthetase from Bacillus stearothermophilus, a dimeric enzyme of class I that interacts with the latter ligands in an anticooperative way (13). The AspRS monomer has not been isolated, whereas fully active protomers have been obtained after proteolytic cleavage of Escherichia coli methionyl-tRNA synthetase (14). Attempts to locate the subunit interface of the AspRS by deletion mutagenesis from its N terminus beyond residue 90 failed because these overproduced deleted proteins remained insoluble in the crude extract in the absence of detergent (11). Kinetic analysis suggested that AspRS subunits are not functionally independent: two affinity constants differing by a factor 10 were obtained for ATP binding, as was a biphasic curve by K_m determination for ATP in the Michaelis–Menten plot representation (15). The importance of the AspRS dimeric association to function was further suggested by the crystal structure of the tRNA^Asp–enzyme complex: it showed that the subunit interface involves two main contact areas, one of them being the active site domain of one subunit that is in contact with the anticodon binding region of the other subunit. These last contacts suggest potential cooperative recognition upon binding of tRNA^Asp.

In our attempt to assess the significance of the AspRS dimeric association, we focused on Pro-273 in motif 1. As shown in the tertiary structure, this residue is located at the subunit interface area and therefore is predicted to be an excellent structural target for site-directed mutagenesis to determine whether this domain contributes to the active enzyme conformation. Moreover, Pro-273 is the only invariant residue of motif 1, the signature peptide associated with the dimer interface (9) and present in the seven α_2 dimers of class II.

By comparing the catalytic properties of native and Pro-273 → Gly (P273G) homo- or heterodimers of AspRS molecules, we have confirmed the participation of Pro-273 in subunit association and have established the functional importance of dimerization.

MATERIALS AND METHODS

Materials. Yeast tRNA^Asp was a gift from M. Boeglin of our institute (Department of Structural Biology); it was purified

Abbreviations: aaRS, aminoacyl-tRNA synthetase; AspRS, aspartyl-tRNA synthetase.
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according to established procedures (16). L-[^{14}C]Aspartic acid (25 Ci/mol; 1 Ci = 37 GBq) and [^{32}P]PP$_i$ (1–2 cpm/pmol) were purchased from the Commissariat à l'Energie Atomique, Saclay, France and Amersham, respectively. The hydroxyapatite HPLC column (Progel TSK HA-1000; length = 7.5 cm, i.d. = 7.7 mm) was obtained from Supelco.

Strains. *E. coli* TGE900 strain, a gift from M. Courtney from Transgene (Strasbourg, France), has been described (11); it was used for overexpression of plasmid-encoded proteins. *E. coli* strains CJ236 and MV1190 were used for *in vitro* mutagenesis (17).

Plasmid Construction. The plasmid pTG908, provided by M. Courtney from Transgene (11), contains a λ P_L promoter that allows high expression in TGE900 of inserted native or mutated AspRS genes.

The plasmid pTG908-APS contains the 2.3-kb DNA fragment encoding the AspRS gene (11).

pTG908-APS[P273G] contains an AspRS gene mutated at position 273.

pEG91 is a derivative of pTG908 that is ampicillin sensitive and kanamycin resistant. It was constructed starting from pTG908 by inactivation of the ampicillin-resistance gene and insertion, at the *Bgl* II site, of the kanamycin-resistance gene block purchased from Pharmacia (ref. 27489701).

pEG91-APS[ΔN70] and pEG91-APS[ΔN70, RH531] contain AspRS genes deleted from the 70 N-terminal residues (AspRS-ΔN70) and mutated at position 531 (AspRS-ΔN70, RH531).

Oligonucleotide-Directed Site-Specific Mutagenesis. Mutagenic primers were synthesized on a fully automated DNA synthesizer (Applied Biosystems 381A DNA). Mutagenesis of single-stranded DNA from bacteriophage M13mp18-APS was performed according to the method described by Kunkel (18) using the kit from Bio-Rad. Mutated AspRS genes were characterized by dideoxy chain-termination sequencing (19) and were subcloned as a *Sac* I–*Mlu* I DNA fragment into pTG908-APS opened at the same sites.

Cotransformations. Two hundred microliters of competent TGE900 cells (CaCl$_2$ treated) was incubated on ice for 1 hr in the presence of 30 ng of pEG91-APS[ΔN70] and pTG908-APS (native or mutated). Then, the cells were regenerated with 1 ml of LB medium for 1 hr and plated on LB agar containing 100 μg of ampicillin and 70 μg of kanamycin per ml. About 200 cotransformed colonies were obtained after incubation at 30°C for 20 hr.

Preparation of Native or Mutated Homo- and Heterodimeric AspRS. TGE900 transformed cells were grown in 250 ml of LB containing 100 μg of ampicillin per ml and, additionally, 70 μg of kanamycin per ml in the case of cotransformed cells. All other steps of the 105,000 × g crude extract preparation were as described (11). Purification of the overexpressed AspRS proteins was performed in one fractionation step by HPLC on a hydroxyapatite column (TSK HA-1000): 4 ml of the 105,000 × g crude extract was loaded, at a flow rate of 0.5 ml/min, on the column equilibrated in 275 mM potassium phosphate, pH 7.5/0.01 mM CaCl$_2$. The column was washed at 1 ml/min with 25 ml of the equilibration buffer and eluted by raising linearly the phosphate concentration to 700 mM (20 ml). The fractions containing the homo- or heterodimers (about 3 ml) were pooled, concentrated on a Millipore PM 30 Centricon filter, and washed twice with 1 ml of 50 mM Tris·HCl, pH 8/1 mM MgCl$_2$/0.1 mM EDTA/5 mM 2-mercaptoethanol/10% glycerol. Purity of the proteins was checked by SDS/PAGE and their concentration was established by absorption measurements and gel scanning.

Enzyme Assays. tRNA^{Asp} aminoacylation and ATP–PP$_i$ exchange activities of homo- and heterodimers as well as K_m values for aspartic acid and ATP were measured as described (11).

The binding capacity of AspRS for [^{32}P]tRNA^{Asp} was determined by the nitrocellulose filtration method (20): the binding mix (100 μl) was 3 nM in [^{32}P]tRNA^{Asp} (about 3 × 10^5 cpm/pmol)/100 mM Hepes, pH 7.5/10 mM MgCl$_2$/30 mM KCl and various enzyme concentrations extending from 10^{-8} to 10^{-6} M. The filters (pore size = 0.45 μm, diameter = 25 mm) were washed twice with 1.5 ml of 50 mM potassium phosphate, pH 5.5/50 mM MgCl$_2$, dried, and assayed for radioactivity.

tRNA^{Asp} was labeled on its 5' dephosphorylated end according to ref. 11 and purified from excess [γ-^{32}P]ATP by 2-fold ethanol precipitation in the presence of 1 M ammonium acetate.

RESULTS AND DISCUSSION

Production of AspRS Heterodimers. The heterodimer approach has already been successfully applied in studies of tyrosyl-tRNA synthetase from *B. stearothermophilus* to demonstrate that tRNA^{Tyr} interacts with both subunits of this enzyme (13, 21). In these studies, two alternate methods were developed to produce active heterodimers: (*i*) charged groups engineered at the subunit interface of homodimeric variants allowed pH-governed dissociation and reassociation into heterodimers (21) and (*ii*) a mix of homodimeric variants were first unfolded in 8 M urea and then reassociated into the different homo- and heterodimeric combinations by electrophoresis on native polyacrylamide gels (13). Although both methods were efficient in the case of tyrosyl-tRNA synthetase they may not be uniformly applicable.

First, treatment by urea prior to heterodimer formation results in a significant or even complete irreversible loss of activity for most enzymes. Thus the production level is lowered to the same extent. Second, mutations introduced at the subunit interface to obtain a pH-controlled dissociation may also affect enzyme activity. As a consequence, variation of catalytic properties concurrent with heterodimer formation may be difficult to interpret. Furthermore, this approach requires the existence of an appropriate residue at the enzyme interface.

In this study we produced heterodimers by co-overexpression in *E. coli* of corresponding native or mutated AspRS monomers. The two plasmids, pTG908 and pEG91, contained ampicillin- and kanamycin-resistance genes, respectively, allowing their selection and maintenance in the same cell. Fig. 1 represents a fractionation pattern on a denaturing poly-
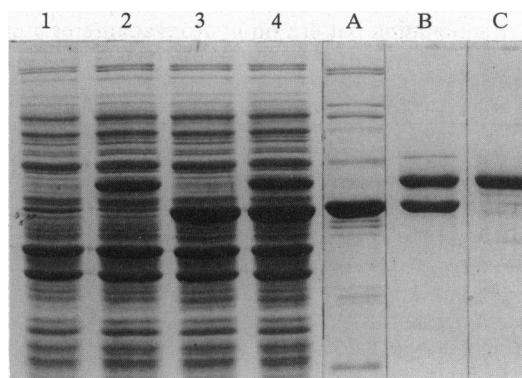

FIG. 1. SDS/PAGE analysis of crude extracts and hydroxyapatite HPLC active eluates. The fractionated crude extracts (30 μg per lane; prepared as described in the text) correspond to wild-type TGE900 cells (lane 1) and TGE900 cells containing pTG908.APS (lane 2), pTG908.APS[ΔN70] (lane 3), and pTG908.APS plus pTG908.APS[ΔN70] (lane 4). In lanes A–C, proteins from the corresponding hydroxyapatite HPLC peaks of activity (Fig. 2) are analyzed.
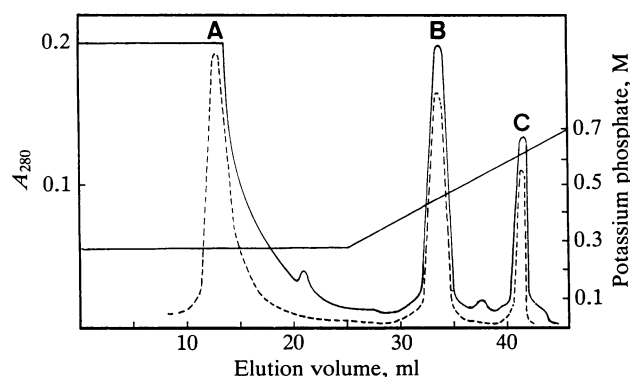
FIG. 2. Purification of the AspRS[ΔN70]-AspRS[P273G] heterodimers by HPLC on a hydroxyapatite column (TSK HA-1000). Four milliliters of a 105,000 × g crude extract corresponding to 250 ml of a culture of cotransformed cells was applied on the column equilibrated with the starting buffer (275 mM potassium phosphate, pH 7.5/0.01 mM CaCl₂) at a flow rate of 0.5 ml/min. The column was eluted at 1 ml/min with the indicated potassium phosphate concentrations. The dashed line corresponds to aspartylation activity. The different peaks of activity correspond to AspRS[ΔN70] (A), AspRS[ΔN70]-AspRS[P273G] (heterodimer) (B), and AspRS (C).

acrylamide gel of a 105,000 × g crude extract (lane 4) from cells cotransformed by pTG908-APS and pEG91-APS[Δ70]; compared to the protein content of nontransformed cells (lane 1), it reveals the presence of the two additional bands corresponding to the migration positions of native and ΔN70-deleted AspRS monomers (lanes 2 and 3, respectively).

Three types of AspRS dimers (one hetero- and two homodimers) are theoretically possible by *in vivo* coexpression depending on the different association possibilities of protomers from two coexpressed AspRS variants. To favor efficient fractionation of these molecules in a further step, they were systematically assembled from a pair of AspRS variants, one of which contained a deletion of its 70 first N-terminal residues. As previously demonstrated (11), this deletion has no effect on the catalytic properties of the enzyme. The resulting hetero- or homodimers contained either one or two or zero 70 N-terminal deleted subunits, respectively, and therefore presented significant differences in their net charge. Indeed, as previously shown (22) the N-terminal domain of AspRS contains a stretch of positively charged residues that confers to the native enzyme, in contrast to its N-terminal deleted variant, increased retention on hydroxyapatite. This is illustrated in Fig. 2, which shows a HPLC fractionation pattern on hydroxyapatite of a crude extract from cells cotransformed by pTG908-APS and

pEG91-APS[ΔN70]. Three well-fractionated aspartylation activity peaks were eluted from the column (Fig. 1). Analysis on SDS gels (Fig. 1) shows that peak A corresponds to the AspRS[ΔN70] homodimer that fractionated at the outer side of the bulk protein. Peak B corresponds to the heterodimer and peak C corresponds to AspRS homodimer. These two latter proteins are chromatographically homogenous, as shown in Fig. 1. Thus, this confirms, as predicted, that the elution from the column of the different dimers is according to their content in intact N-terminal sequences. The difference in charge between the homo- and heterodimers is sufficiently important to obtain good resolution of the three AspRS peaks on hydroxyapatite.

Catalytic properties of the three dimers (K_m values for ATP and aspartic acid, K_d values for tRNA^{Asp}, and k_{cat} values in the aminoacylation and ATP–PP$_i$ exchange reactions) were found to be identical to those of native AspRS prepared by classical procedures. This preparation method yielded about 250–500 μg of pure dimeric enzyme per 250 ml of cell culture, a sufficient amount for extensive analysis of catalytic properties. This method was used in this study to produce different types of appropriately designed heterodimers whose catalytic properties were investigated to elucidate the significance of the AspRS dimerization and the role that Pro-273 plays in this association.

Mutagenesis of Pro-273. As it appears from the x-ray crystal structure of AspRS, the invariant Pro-273 in motif 1 is located in the distorted β strand S1 close to the dimer interface. The importance of this amino acid to enzyme function was tested by mutagenesis. To obtain the most easily interpretable result, Pro-273 was replaced by glycine, thus avoiding the formation of new undesirable interactions with other parts of the molecule that might have occurred by substituting hydrophobic, polar, or charged amino acids. As shown in Table 1, the P273G AspRS exhibited exchange and aminoacylation k_{cat} values 6 and 3 times lower, respectively, than those of the native enzyme and had one order of magnitude lower affinity for tRNA^{Asp}. K_m values for ATP or aspartic acid as well as the dimerization capacity remained unaffected (chromatographic controls not shown). These results clearly show that Pro-273 is implicated in AspRS activity and tRNA binding.

Enzymatic Properties of AspRS Heterodimers. Although the above results establish a significant role for Pro-273 in AspRS, they do not address the prediction from the crystallographic structure that Pro-273 participates in dimerization. To address this point, we measured the kinetic behavior of heterodimers containing a P273G subunit associated with a native AspRS protomer previously deleted (for ease in dimer purification) by 70 N-terminal residues. Indeed, assuming that the P273G-induced modifications observed in the case of

Table 1. Kinetic properties of homo- and heterodimeric AspRS molecules

Enzyme variant	Relative specific activity, %		K_m acylation, μM		K_m exchange, μM	K_d tRNA, nM
	Acylation	Exchange	Asp	ATP	Asp	
Homodimer						
AspRS	100	100	400	40	1500	25
AspRS[ΔN70]	100	100	330	32	1000	22
AspRS[P273G]	31	16	400	65	1600	270
AspRS[ΔN70.R531H]	0	0	—	—	—	23
Heterodimer						
AspRS-AspRS[ΔN70]	100	106	330	34	1000	ND
AspRS-AspRS[ΔN70.R531H]	48	55	ND	ND	ND	ND
AspRS[ΔN70]-Asp[P273G]	103	93	450	80	1200	24
AspRS[ΔN70.R531H]-Asp[P273G]	59	52	ND	ND	ND	22

All parameters were determined on electrophoretically homogeneous proteins obtained from overproducing cells after hyroxyapatite HPLC (see text) except AspRS[ΔN70], which was purified according to ref. 23. The ATP–PP$_i$ exchange reaction rates were measured under saturating concentrations of substrates. Acylation rates of tRNA^{Asp} were determined at 0.1 mM aspartic acid. ND, not determined.

FIG. 3. Overall view of the yeast AspRS-tRNA^{Asp} complex. Pro-273 and ATP for each monomer are shown in dark spheres. Each tRNA molecule is represented in the lighter cpk model.

the AspRS homodimer are due to alterations of the subunit interface, it is likely that a P273G substitution in only one subunit should be sufficient to perturb these interactions, and alteration of one protomer should have an influence on the functional properties of the other intact subunit. Consequently, and in contrast to what would be observed for functionally independent subunits, the activity of such a heterodimer should differ from the sum of its subunit activities as measured in their respective homodimeric context.

Production and purification of heterodimers were performed as described above; purity and identity controls of these molecules by fractionation on denaturing gels are shown in Fig. 1. All kinetic parameter determinations were performed on electrophoretically homogeneous enzymes. Data in Table 1 show that the heterodimer AspRS[P273G]-AspRS[ΔN70] exhibits catalytic properties similar to the native enzyme. Thus, surprisingly, and in contrast to the

prediction that the P273G mutant would affect the native subunit, we found that the catalytic properties of the mutated protomer were restored. A more demonstrative confirmation of this effect was observed with another heterodimer: the P273G protomer was associated with a subunit having a R531H substitution. As shown in Table 1, this mutation, when present in both subunits of AspRS, completely inactivates the enzyme. This drastic effect must be related to the fact that this invariant Arg-531 is located in motif 3, which, from the tertiary structure, appears to be involved in the specific recognition of the ATP molecule (9). In the case of functionally independent subunits, association of P273G and R531H protomers should result in a heterodimer with 8% of the ATP–PP$_i$ exchange activity relative to the wild-type enzyme. In fact, as shown in Table 1, we obtained a heterodimer half as active as native AspRS with a binding constant for tRNA^{Asp} similar to that of the intact dimer. This clearly shows that the P273G subunit completely recovers its activity by interacting with the inactive protomer.

The restoration effect we observed in both heterodimers is triggered at the dimer interface through subunit interactions; this implies that the P273G-induced inactivation is due to alteration of the mutated enzyme interface. Thus, the reactivation effect probably consists, at a molecular level, of the remodeling of the conformation of the P273G-modified interface by interaction with the intact interface domain of the other subunit. The obvious conclusion is that Pro-273 of motif 1 is a structural determinant of the subunit interface domain. Furthermore, it appears that both subunits are functionally interdependent since enzyme properties may be modulated through interface interactions. This implies that the catalytic domain and the tRNA^{Asp} binding site of each subunit have to be structurally connected via subunit interactions. This suggests the existence of allosteric effects upon catalysis or substrate binding; nonequivalent ATP binding sites as well as two different K_m values for this substrate strongly support this hypothesis (15).

Structural Correlations. The critical position of Pro-273 is shown in Fig. 3. This picture highlights AspRS residues in the three motifs characteristic of the seven synthetases of class II, with the location of the strictly conserved proline of motif 1. Not only are the proline residues not too far from each other (15 Å) but also they are in close proximity to motif 2 residues of the other subunit. Pro-273 of one subunit is surrounded by four regions: residues 324–338 and residues 548–557 from the other monomer and residues 324–338 and residues 299–305 from the same monomer (Fig. 4: for clarity, residues in italics are not represented). Key interactions include:

(*i*) E337, invariant in all AspRS and strictly conserved in lysyl-tRNA synthetase, asparaginyl-tRNA synthetase, and prolyl-tRNA synthetase from *E. coli*, binds to K274 and L275
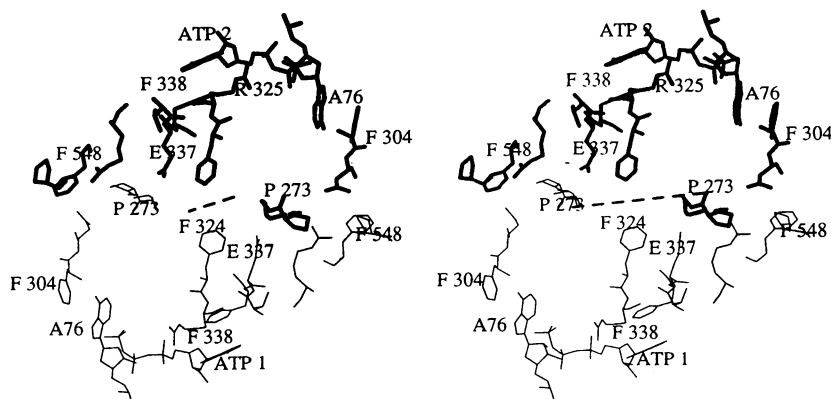

FIG. 4. Stereoview of the dimeric interface. Dark and light lines differentiate the monomers.

(main chain group NH) of the other subunit, thus making a direct link between the ATP moiety of one subunit and Pro-273 of the other subunit. The adenine base is stacked over the class II invariant F338.

(*ii*) A second contact of similar nature (across subunit interface) occurs between F324 and L275. A hydrophobic pocket is formed that involves F324, L298, L275, and P322 of both monomers; R325, another class II invariant residue, binds to the α phosphate of the ATP molecule. Any perturbation at this level, such as P273G mutation, would certainly influence the local conformation, affect the position of F324, and in turn alter the correct position of R325.

(*iii*) A third crucial contact between residues of different subunits involves Pro-273 of one subunit and motif 3 residues F548, P549, R550, and P557 of the other. F548 and P549 are strictly conserved within AspRS as well as in lysyl-tRNA synthetase and asparaginyl-tRNA synthetase. An additional intrasubunit interaction occurs with N305, which can also affect the binding of the terminal adenine through the stacking with F304.

The molecular structure of AspRS at the subunit interface, near Pro-273, suggests an easy rationalization of the heterodimer experiments. As shown, Pro-273(a) is anchored to the ATP of the other subunit (b) through the interface interactions: L275(a) with F324(b) and E337(b), K274(a) with E337(b). Two of these residues (L275 and F324) also interact within the hydrophobic pocket; these latter contacts, presumed in the case of the P273G-wt and R531H-P273G heterodimers, probably preserve the intersubunit connections from the disorganizing effect of the P273G mutation. The presence of two mutations in the hydrophobic pocket of the AspRS P273G homodimer may affect its cohesion such that it is no longer stabilized.

Furthermore, the loop between residues F324 and E337 is responsible for the major groove-specific recognition of the tRNA^{Asp} acceptor stem. It is reasonable to assume that a change in position of these residues, as occurs in the AspRS P273G homodimer, induces a conformational modification of the loop that may explain the K_d effect.

Concluding Remarks. The functional importance of dimerization, presently established for AspRS, is probably common to all dimeric aaRSs from class II. Indeed, conservation in most of these enzymes of the dimeric configuration through evolution (7 of 10, with 3 others being tetramers or $\alpha_2\beta_2$) points to its role in catalysis. This prediction is confirmed at least in the case of ProRS from *E. coli* (an enzyme of class II) where dimerization has been shown to be strictly required for activity (24). The question remains: Why do aaRSs of class II require the $\alpha2$ conformation for their activity when class I aaRSs are generally monomers? This structural imperative could be related to the peculiar type of active site conformation, an antiparallel β sheet, characteristic of this class of enzymes. It may be that an isolated subunit of a class II enzyme would not by itself have the capacity to organize spatially, in a functionally active conformation, the different structural elements of its catalytic site. This would require the mutual structuring constraints of the dimeric interaction. Thus, the $\alpha2$ configuration should be considered as an integral structural necessity for a functional antiparallel nucleotide binding site. Confirmation of this hypothesis awaits the discovery of other enzymes containing

this nonconventional ATP binding site. A first supporting example may come from the human asparagine synthetase, which is a homodimer (25). It was recently shown (26) that highly conserved protein motifs characteristic of class II aaRSs align with the functionally important region of *E. coli* asparagine synthetase A, suggesting an antiparallel β sheet conformation for its ATP binding site.

1. Eriani, G., Delarue, M., Poch, O., Gangloff, J. & Moras, D. (1990) *Nature (London)* **347**, 203–206.
2. Lorber, B., Kern, D., Dietrich, A., Gangloff, J., Ebel, J. P. & Giegé, R. (1983) *Biochem. Biophys. Res. Commun.* **117**, 259–267.
3. Amiri, I., Mejdoub, H., Hounwanou, N., Boulanger, Y. & Reinbolt, J. (1985) *Biochimie* **67**, 607–613.
4. Sellami, M., Prévost, G., Bonnet, J., Dirheimer, G. & Gangloff, J. (1985) *Gene* **40**, 349–352.
5. Sellami, M., Chatton, B., Fasiolo, F., Dirheimer, G., Ebel, J.-P. & Gangloff, J. (1986) *Nucleic Acids Res.* **14**, 1657–1666.
6. Dietrich, A., Giege, R., Commarmond, M. B., Thierry, J. C. & Moras, D. (1980) *J. Mol. Biol.* **138**, 129–135.
7. Ruff, M., Cavarelli, J., Mikol, V., Lorber, B., Mitschler, A., Giegé, R., Thierry, J. C. & Moras, D. (1988) *J. Mol. Biol.* **201**, 235–236.
8. Ruff, M., Krishnaswamy, S., Boeglin, M., Poterszman, A., Mitschler, A., Podjarny, A., Rees, B., Thierry, J. C. & Moras, D. (1991) *Science* **252**, 1682–1689.
9. Cavarelli, J., Rees, B., Ruff, M., Thierry, J.-C. & Moras, D. (1993) *Nature (London)* **362**, 181–184.
10. Prévost, G., Eriani, G., Kern, D., Dirheimer, G. & Gangloff, J. (1989) *Eur. J. Biochem.* **180**, 351–358.
11. Eriani, G., Prévost, G., Kern, D., Vincendon, P., Dirheimer, G. & Gangloff, J. (1991) *Eur. J. Biochem.* **200**, 337–347.
12. Giegé, R., Lorber, B., Ebel, J. P., Moras, D., Thierry, J. C., Jacrot, B. & Zaccai, J. (1982) *Biochimie* **64**, 357–362.
13. Carter, P., Bedouelle, H. & Winter, G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1189–1192.
14. Koch, G. L. E. & Burton, J. (1974) *FEBS Lett.* **40**, 180–182.
15. Kern, D., Lorber, B., Boulanger, Y. & Giegé, R. (1985) *Biochemistry* **24**, 1321–1332.
16. Dock, A. C., Lorber, B., Moras, D., Pixa, G., Thierry, J. C. & Giegé, R. (1984) *Biochimie* **66**, 179–201.
17. Kunkel, T. A., Roberts, J. D. & Zabour, R. A. (1987) *Methods Enzymol.* **154**, 367–382.
18. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488–492.
19. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
20. Yarus, M. & Berg, P. (1967) *J. Mol. Biol.* **28**, 479–485.
21. Ward, W., Jones, D. & Fersht, A. (1986) *J. Biol. Chem.* **261**, 9576–9578.
22. Lorber, B., Mejdoub, H., Reinbolt, J., Boulanger, Y. & Giegé, R. (1988) *Eur. J. Biochem.* **174**, 155–161.
23. Vincendon, P. (1991) Ph.D. Thesis (Université Louis Pasteur, Strasbourg, France).
24. Lee, M. L. & Muench, K. H. (1969) *J. Biol. Chem.* **244**, 223–230.
25. Van Heeke, G. & Schuster, S. M. (1989) *J. Biol. Chem.* **264**, 5503–5509.
26. Hinchman, S. K., Henikoff, S. & Schuster, S. M. (1992) *J. Biol. Chem.* **267**, 144–149.