Data are presented, based upon subunit complementation experiments, that suggest that Escherichia coli adenylosuccinate synthetase contains two shared active sites between its dimeric interface. This conclusion was alluded to by use of mutant forms of adenylosuccinate synthetase previously prepared by site-directed mutagenesis. The experiments indicate that, although the R143L and D13A mutants have low or no activity independently, when they are mixed, a significant amount of activity was obtained. These results indicate that the subunits exchange with each other to form heterodimers with a single viable wild-type active site. The $k_{cat}$ value for the active hybrid active site in the R143L-D13A heterodimer is virtually identical to that observed with the wild-type enzyme, and the other kinetic parameters are very similar to those found for the wild-type enzyme.

An analysis of the restoration of the activity in the presence of substrates suggests that GTP and IMP stabilize the dimeric structure of the protein. A comparison of the restoration of the activity using different combinations of mutants provides evidence indicating that some of the GTP binding elements, including the P-loop, in the protein are important for subunit integrity. Also, for the first time, a comprehensive analysis of subunit complementation is performed for the two inactive mutants (R143L and D13A) where the dissociation constants for the R143L-D13A heterodimer and the D13A homodimer were determined to be 21 and 2.9 μM, respectively. A concentration dependence of the specific activity of the wild-type protein in this study shows that the $K_d$ for dimer dissociation is approximately 1 μM.

For the majority of enzymes, the amino acid residues constituting an active site are contained within a single polypeptide chain. It is also possible, however, for the active sites to be located at the interfaces between polypeptide chains in oligomeric enzymes and for catalytic activity to be dependent upon the joint participation of amino acid residues from adjacent chains. This type of active site has been recognized for a few enzymes by crystallographic studies (1–3). An alternative approach aimed at determining whether the catalytic activity of an enzyme requires the joint participation of amino acid residues from adjoining chains involves subunit complementation of inactive mutants or enzymes inactivated by chemicals (4–6). However, due to formation of the kinetically inert oligomers in these studies, either in vivo or in vitro complementation using chaotropic reagents is employed where the latter methods require a partial or complete protein unfolding-refolding process.

AMPPhorese (1) (see Ref. 7 for a review) catalyzes a reversible reaction utilizing GTP, IMP, and aspartate in the presence of Mg$^{2+}$ to form adenylosuccinate, GDP, and inorganic phosphate (P). This is the first reaction committed to the formation of AMP from IMP, using GTP as a specific energy source, and it is believed to play an important role in the de novo pathway of purine nucleotide biosynthesis. It has been recognized (8) that the primary sequences of AMPPhorases contain the GTP-binding consensus sequences commonly identified in the GTPhase superfamily (9). These include the (N/T)XKD box and the P-loop sequence, GXXXXGK. Alignment of the known amino acid sequences of AMPPhorases reveals that the GTP-binding sequences are well conserved and, presumably, their functions may be similar to those found in the GTPhase superfamily. Indeed, Asp$^{143}$ of Escherichia coli AMPPhrase within the consensus box, (N/T)XKD, was reported to be responsible for GTP specificity of the protein (10), and mutations in the P-loop region caused drastic decreases in enzyme activity (11). X-ray diffraction studies (12) of crystalline AMPPhrase from E. coli indicated that the GTP binding domain of the protein, including the P-loop, is superimposable on that of the human p21$^{ras}$ protein. The function of the P-loop in AMPPhrase is similar to that of the GTPhase superfamily in that it interacts with the γ-phosphoryl group of GTP; however, in AMPPhrase it has additional roles relative to those of typical G-proteins; e.g. it is involved in conformational changes associated with IMP and GTP binding (13).

Chemical modification studies of E. coli AMPPhrase followed by site-directed mutagenesis suggested that the Lys$^{140}$ and Arg$^{147}$ residues may contribute to catalysis (14, 15). However, an x-ray crystallographic study of the enzyme (16) indicates that the inactivation caused by the chemical modifications at the Lys$^{140}$ and Arg$^{147}$ residues might be due to disruption of the dimeric structure of the protein by the modification, since those two residues are involved in two salt bridges between the subunits. Likewise, the subunit integrity seems to be essential for the catalytic activity of the AMPPhrase. Arg$^{143}$ deserves special attention because it is the only residue projecting into the active site of the other symmetry related subunit where it is ligated to the 5′-phosphoryl group of IMP (16, 17). Therefore, it would be reasonable to assume that this residue is important for maintaining the protein as a dimer, a condition that may be essential for catalysis. However, although a mutation at this residue (R143L) does result in drastic decreases in the binding affinities of IMP and GTP to the protein, no significant change in activity was observed (17). These results raise the following questions. Is the dimeric structure really necessary for AMPHPLC, high pressure liquid chromatography.
Sase activity, and how stable are its dimeric structures compared with the wild-type and other mutant forms of AMPSase?

In this study, using analysis of restoration of wild-type AMPSase activity in the absence of any chaotrope from the mixtures of previously generated inactive mutants, we report that the *E. coli* AMPSase has two shared active sites between its two subunits. Also, an unusual dynamic feature of the dimeric protein and its physiological role will be discussed.

**EXPERIMENTAL PROCEDURES**

**Materials—**Wild-type AMPSase was purified from an *E. coli* pur A' strain (H1238), harboring a PMS204 plasmid, by the procedure described previously (18). The mutant AMPSases in this study, previously generated (10), were purified from the H1238 *E. coli* strain containing the modified plasmids. Protein purities were analyzed by using SDS-polyacrylamide gel electrophoresis according to Laemmli (20). The concentration of the purified proteins in solution was determined by using the extinction coefficient for wild-type AMPSase at 280 nm (εmax = 67.85 mm⁻¹ cm⁻¹), where the concentration refers to monomer.

**Subunit Complementation Experiments—**The R143L and other mutants were incubated in 40 mM Hepes buffer (pH 7.7) at room temperature for 1 h before mixing. After the mutants were mixed, the activity was determined at different times. The assay solution contained GTP, IMP, Asp, and MgCl₂ at the levels of 150 μM, 150 μM, 5 mM, and 2 mM, respectively, in 40 mM Hepes buffer. In all experiments in this study, the concentrations of the R143L and other mutants were fixed at 0.25 and 2.0 mg/ml, respectively. 3 μl of the mixture were added to the assay solution in which the final concentrations of the R143L and other mutants were 0.75 and 6.1 μg/ml, respectively. Other experimental details are described in the legends to the figures.

**Determination of Kinetic Parameters for the R143L-D13A Heterodimer—**Predicting the activity assay, the R143L protein was incubated with an excess amount of D13A mutant AMPSase (20 equivalents) for 10 h to allow the major part of the R143L to exist as a heterodimer where the final concentration of the R143L mutant in the mixture was held at 0.25 mg/ml. In order to maximize the formation of the heterodimer, the mixture contained 0.5 mM of IMP, which would be negligible in the assay solution after 3 μl of the incubated mixture were added to 1 ml of an assay solution that contained 200 μM GTP, 250 μM IMP, 5 mM Asp, and 2 mM MgCl₂ in 40 mM Hepes buffer, pH 7.7.

**Gel Permeation Chromatography—**A gel-permeation type HPLC column (HYDROPORE-5-SEC from RAININ) was installed in a Waters HPLC system (model 510), which is equipped with a UV detector (Lambda Max model 481 LC spectrophotometer) and a 20-μl injection loop. The flow rate was 0.5 ml/min, and the buffer was 40 mM Hepes at pH 7.3. Each sample at the appropriate concentration was incubated at room temperature for 30 min before injection. The molecular weight standards were blue dextran (2,000,000), alcohol dehydrogenase (150,000), bovine albumin (66,000), and carbonic anhydrase (29,000). When protein samples of low concentrations (0.2 mg/ml) were employed, 0.5 ml of eluant was collected with a fractional collector and incubated with 0.2 mg of the D13A mutant for at least 1 h, after which the activity was determined by using the assay solution described to identify the position of the protein in the eluant.

**Concentration Dependence of the Wild-type AMPSase—**Prior to determination of activity, the wild-type protein was incubated at room temperature at different concentrations in 40 mM Hepes buffer (pH 7.7). 1 μg/ml from each incubated sample was added to the assay solution described under “Subunit Complementation Experiments.” Specific activity refers to the activity per 1 μg/ml.

A theoretical model of subunit complementation—Assuming that the R143L mutant exists in a monomeric form without other components, which is confirmed by the HPLC experiment in this study, it is desirable to consider the following two equilibria in the mixture of the R143L and D13A mutants:

\[ M_1 + M_2 \rightleftharpoons M_{12} \]

\[ M_1 + M_2 \rightleftharpoons M_{12} \]

where \( M_1 \) and \( M_2 \) represent the concentrations of the R143L mutant and the D13A mutants, respectively, and \( M_{12} \) and \( M_{12} \) represent the concentrations of the heterodimer composed of the R143L and D13A mutants and the homodimeric form of the D13A mutant, respectively. \( K_1 \) and \( K_2 \) correspond to the dissociation constants for \( M_1 M_2 \) and \( M_1 M_1 \), respectively, which are defined as:

\[ K_1 = M_1 M_2 / M_{12} \]

\[ K_2 = M_1 M_2 / M_{12} \]

where \( (M_1)_0 \) and \( (M_2)_0 \) are the total concentrations of the R143L and D13A mutants.

Substituting \( M_2 = M_1 M_2 + 2 \times M_{12} \),

\[ (M_1)_0 = M_1 + M_{12} + 2 \times M_{12}^2/K_2 \]

\[ (M_2)_0 = M_1 + M_{12} + 2 \times M_{12}^2/K_2 \]

Then,

\[ (M_1)_0 = M_1 + M_{12} + 2 \times M_{12}^2/K_2 \]

**(RESULTS)**

**Restoration of the AMPSase Activity in a Mixture of the R143L and D13A Mutants—**Subunit integrity seems to be essential for catalysis based on an x-ray crystallographic study of *E. coli* AMPSase (16), e.g. structural analysis suggests that the loss of activity associated with the K140L and R143L mutants is essentially the result of a change in the subunit structure. Therefore, based upon fitting the data in Fig. 5 to Equation 2, \( K_1 \) and \( K_2 \) values can be determined.

**kcat** of the D13A mutant has been found to be inactive.² Although the kcat value of the R143L mutant is reported to be close to that of wild-type AMPSase, it exhibits an extremely low activity in the standard assay solution used to study the activity of the wild-type enzyme. This activity is typically less than 0.014/s which is approximately 1% of its maximum activity and is due to its high Km values for IMP and GTP (17). However, when it was mixed with eight equivalents of the D13A mutant, as shown in Fig. 1, the activity increased gradually up to 100-times its intrinsic activity. Because the enhanced activity can be observed only in the presence of the D13A mutant, it is most likely associated with formation of heterodimers between the two mutants. This phenomenon has been interpreted as critical evidence that an oligomeric enzyme contains shared active sites (4, 21). Hence, the data shown in Fig. 1 clearly demonstrate that the subunits of the R143L and D13A mutants exchange subunits with each other to form heterodimers that have hybrid active sites. This situation is diagrammed in Fig. 2 based on the 2-fold symmetry proposed from x-ray diffraction studies (12), where the heterodimer contains two different

² C. Kang, N. Sun, R. B. Honzatko, and H. J. Fromm, unpublished observations.
types of active site residues, i.e. the viable Arg^{143}–Asp^{13} residues, which are identical to those of the wild-type enzyme, and the site which contains the inactive Leu^{143}–Ala^{13} residues.

In Fig. 1, the progress curve of the formation of the hybrid dimer apparently follows a single exponential function plus an offset where the offset is related to the initial activity. The half maximum activity was found at 80 min and the maximum activity was 1.37/s. Considering that the D13A mutant does not have any activity, most of the observed activity should come from the R143L subunit in the heterodimer as shown in Fig. 2. Therefore, the activity of the R143L mutant in the heterodimer is similar to that of the wild-type enzyme when the activities are normalized to activity per monomer. This result indicates that both active sites in dimeric wild-type AMPSase are equally active. At the initial point (less than 1 min), the observed activity (0.12/s, based upon the amount of R143L mutant) is much greater than the combined activity of each mutant, which indicates that the heterodimer is formed from the preexisting monomers at a fast rate compared with the overall rate of the activation. Therefore, the rate-determining step in Fig. 1 seems to be the dissociation of the D13A dimers into the monomeric forms, although the initial offset is associated with the preexisting D13A monomer.

On the other hand, when the mixture was incubated at a lower temperature (4 °C), the reactivation showed a larger initial offset and a smaller but faster activation compared with that of the sample incubated at room temperature (data not shown). These findings indicate that the monomer is relatively dominant and that the dimeric interface becomes unstable and kinetically labile at the lower temperature. These results are consistent with the nature of an oligomeric interface where hydrophobic interactions exist to a significant degree (23).

**Kinetic Characterization of the R143L-D13A Heterodimeric Enzyme**—Table I depicts the kinetic parameters obtained from initial-rate experiments with the wild-type and heterodimeric forms of AMPSase. It is clear from the kinetic data that the heterodimer is as active as the homodimer based upon viable active sites, i.e. two per homodimer and one per heterodimer; however, there is a relatively small increase in the $K_m$ for AMP and a 3-fold increase in the $K_m$ for GTP for heterodimeric AMPSase relative to the wild-type enzyme. This slight impairment of the heterodimer may be due to small conformational alterations in the GTP subsite within the viable active site. It is not clear whether this effect is caused by structural disruptions at the viable active site of the heterodimer that are induced by the R143L-D13A site, or whether it is caused by conformational changes induced by the mutation(s) within the same polypeptide chain. These results suggest that both active sites of dimeric wild-type AMPSase are equally active but not completely independent.

**Subunit Complementation of the R143L Mutant by Other Mutants**—In as much as the subunit complementation is expected to be highly sensitive to the nature of the subunit interface in an oligomeric protein, complementation can be used to investigate the relationship of residues to the subunit interfaces. As demonstrated in Fig. 1, complementation of the R143L mutant by D333E and E14A mutants are different compared with those produced by the H41N and D13A mu-
monomeric forms of AMPSase, whereas the R143L mutant. It indicates that the Arg 143 residue from one subunit forms part of the dimeric interface in AMPSase. On the basis of x-ray crystallographic studies which showed that the Arg143 residue, which interacts with the 5'-phosphoryl group of IMP, is located in the dimeric interface in AMPSase. In the case of the D13A mutant, the activation rate is also fast but with lower maximum activity, which is significantly different from the characteristics associated with the complementation of R143L by D13A. This observation suggests that the E14A mutant has a labile dimeric structure compared with that of the D13A mutant. On the basis of these observations, it can be suggested that the structural elements associated with GTP binding may be important in stabilizing the dimeric structure of wild-type AMPase.

Other combinations of mutant forms of AMPase excluding the R143L mutant, for example, D13A and E14A, did not show any activity enhancement. The subunit complementation can be observed only with mutants where the mutations are located in different subsites within the active site.

Substrate Effects on the Subunit Complementation—Fig. 3 reveals the effects of IMP and GTP on the complementation between the D13A and R143L mutants. The presence of IMP increased the limiting activity although it did not change the reactivation rate. However, GTP causes a 10-fold slower complementation rate compared with that in the absence of GTP as well as an increase in the maximum activity, which is consistent with the suggestion that GTP can stabilize the dimer of the D13A mutant, thermodynamically and kinetically. Although there is no clear evidence for stabilization of the dimer by IMP, presumably, IMP stabilizes the dimer because the Arg residue, which interacts with the 5'-phosphoryl group of IMP, is located in the dimeric interface in AMPase. In addition, these results indicate that IMP and GTP contribute to the stability of the dimeric structure of AMPase.

The R143L Mutant Is Monomeric in the Absence of the D13A Mutant—On the basis of x-ray crystallographic studies which indicate that the Arg143 residue from one subunit forms part of the active site of the other subunit of the dimer, it is likely that the R143L mutant may exhibit unstable subunit integrity, possibly existing as a monomer. An earlier chromatographic study reporting that the wild-type AMPase is a monomer is in contradiction to what was observed with the dimeric crystal. Thus, it is possible that the enzyme may undergo a relatively rapid dissociation and association equilibrium. In order to investigate this possibility, a gel-permeation HPLC experiment was employed. Fig. 4 reveals the results for the oligomeric structures of the wild-type protein at different concentrations and the apparent molecular weights of the R143L mutant in the presence and absence of the D13A mutant. In Fig. 4a, the peaks 1 and 2 correspond to the dimeric and monomeric forms of AMPase, whereas peak 3 represents an unknown impurity because it diminished as the protein concentration was lowered. At high concentrations of the wild-type enzyme (a), most of the protein exists in the dimeric form; however, at low concentrations of AMPase (b), a significant amount is monomer. In panel B, the 17th and 20th tubes from the fraction collector correspond to the positions of peaks 1 and 2 in Fig. 4a, respectively. However, the R143L mutant at the concentration employed in the subunit complementation experiments in this study exists exclusively as the monomer, and, in the presence of the D13A mutant, the majority of the protein is in the dimeric form. These results suggest that dissociation of dimeric wild-type AMPase occurs readily and that the restoration of the activity from the R143L and D13A mixture is due to heterodimer formation between these mutants.

Titation of the Restored Activity of Mixtures of the R143L Mutant with Variable Amounts of the D13A Mutant—One can assume that more complementation activity will be achieved by the greater amount of the D13A mutant used to shift the population of the R143L mutant into the heterodimer. Because dependence of the complementation on the amount of the counterpart mutant will be associated with the nature of the interfaces of the heterodimer and the homodimer in the mixture, the titration curve with a variable amount of the D13A mutant may provide valuable information on the thermodynamics of the dimeric interface in the AMPase. Indeed, the stability constant can be deduced from a theoretical consideration as illustrated under “Experimental Procedures.” In this model, the activity represents that of the equilibrated state in each condition. Fig. 5 shows the dependence of the complementation of the R143L mutant with a variable amount of the D13A mutant. At a fixed concentration of the D13A mutant, as indicated in Fig. 1, the restored activity will converge to a limiting value. The values for each concentration of the D13A mutant were replotted and the results are shown in Fig. 5. Fitting these values to Equation 2 in the model under “Experimental Procedures,” the dissociation constants for the heterodimer formed between the R143L and D13A mutants and the D13A mutant dimer are deduced to be 21 and 2.9 μM, respectively. Presumably, the only major difference between the dimeric interfaces of these dimers is that the D13A homodimeric interface has two Arg residues, whereas the R143L-D13A heterodimeric one has one Arg and one Leu residue. Because replacing an Arg residue with a leucine in the dimeric interface region caused a 7.2-fold decrease in stability, which corresponds to 1.2 kcal/mol, it is possible to conclude that the R143L homodimer is destabilized by 2.4 kcal/mol compared with that of the D13A dimer.

| TABLE I | Comparison of the kinetic parameters for the wild-type and R143L-D13A heterodimeric adenylosuccinate synthetase |
|---------|---------------------------------------------------------------|
|         | $k_{cat}$ | $K_m$, IMP | $K_m$, GTP | $K_m$, IMP |
| Wild type | 1.26 ± 0.15 | 16.0 ± 1.1 | 0.47 ± 0.7 | 225 ± 16 |
| R143L-D13A | 1.35 ± 0.44 | 50.3 ± 7.3 | 20.7 ± 1.7 | 393 ± 37 |
| D13A | 1.04 ± 0.05 | 289 ± 17.4 | 1730 ± 140 | 349 ± 60 |

* From Kang and Fromm (22).
* The $k_{cat}$ value was calculated based on the total concentration of the R143L mutant.
* From Moe et al. (17).
* From C. Kang, N. Sun, R. B. Honzatko, and H. J. Fromm, (unpublished observations).
* ND, the D13A mutant is inactive.

Fig. 3. Substrate effects on the subunit complementation rate. The R143L mutant was incubated with eight equivalents of the D13A mutant in the absence (○), and presence of 150 μM GTP (•), 300 μM of IMP (□), or 150 μM of GTP and 300 μM of IMP (□), respectively.
This study has revealed that catalytically functional dimeric \textit{E. coli} AMPSase has two shared active sites in which both are equally active. It was also demonstrated that the dimeric interfacial of the protein is stabilized by the presence of substrates and that one can determine the dissociation constants of the dimers by using analysis of the subunit complementation data. Previously, the studies regarding subunit structure of AMPSases have reported that it exists presumably as a dimer (7). Also, \textit{E. coli} AMPSase has been crystallized as a dimer in the absence of substrates (12). However, in an early study of \textit{E. coli} AMPSase, the protein has been reported to exist as a monomer (24). This finding was demonstrated by gel-filtration chromatography. These conflicting results raise questions regarding the true nature of the subunit structure and whether the monomer or the dimer is active. The subunit complementation experiments in this study provide strong evidence that \textit{E. coli} AMPSase exists in a relatively rapid monomer-dimer equilibrium and that the dimeric species is active. It is reasonable to assume that the activity of AMPSase would decrease as its concentration is diluted, and a profile of activity \textit{versus} enzyme concentration will be related to dissociation of the dimer. In order to determine the dissociation constant for dimeric wild-type AMPSase, we investigated the concentration dependence of the specific activity of the wild-type protein (data not shown). The results revealed that the specific activity has a sharp transition around 40 \mu g/ml protein which presumably reflects the dissociation of the dimer into the monomers. Also, it was demonstrated that the transition was shifted to a much lower concentration of protein in the presence of IMP or GTP. This finding supports the conclusion from the subunit complementation studies that the substrates stabilize the dimer.
Glu\textsuperscript{14} is believed to be a key element in stabilization of the P-loop in AMPSase because it hydrogen-bonds with a number of other residues (16). On the other hand, the E14A mutant exhibited an alteration in secondary structure, presumably including the P-loop region, whereas the D13A mutant resembles the wild-type in all respects except that it has no activity.\textsuperscript{2}

As shown in this study, the E14A mutant seems to favor the monomeric form of AMPSase compared with the D13A mutant, presumably due to its altered secondary structure. Previously, it had been reported that one of the mutants generated in the P-loop region of the AMPSase (G15V) showed remarkably changed hydrophobicity as revealed by the fact that the mutant bound tightly to a phenyl Sepharose column compared with the wild-type protein (11). The tight binding to the phenyl Sepharose column was also observed for a mutant lacking a salt bridge in the dimeric interfaccial region (15). Thus, the enhanced hydrophobicity found in the G15V mutant, a P-loop mutant, may be associated with reduced stability of its dimer.

Although the E14A mutant did not show an enhanced hydrophobicity in the phenyl Sepharose chromatography experiments, these results suggest that structural alterations in the P-loop region of the AMPSase may cause destabilization of its dimeric structure. However, it is not clear whether the lower activity found with the E14A-R143L heterodimer when compared with that of the D13A-R143L heterodimer, is caused by the lower concentration of the formed heterodimer or by a lower activity of the heterodimer. Nevertheless, the P-loop seems to play an important role in maintaining the dimeric structure of AMPSase.

It is noteworthy that the K140L AMPSase mutant exhibited restored activity during incubation with the D13A mutant (data not shown). Because the Lys\textsuperscript{140} residue is also located in the dimeric interface of AMPSase, it is reasonable to assume that the K140L mutant exists in a monomeric form, and, in the presence of the D13A mutant, part of the protein sample will be in an active dimeric form. The analysis of the subunit complementation used to determine the dissociation constants for the D13A-R143L and D13A dimers in this study can be used to study the roles of this residue in the AMPSase dimer.

In summary, the dimeric structure of the E. coli AMPSase is readily dissociated; however, it can be stabilized by the presence of GTP and IMP. By a comprehensive analysis of the subunit complementation of the two inactive mutants (R143L and D13A), the dissociation constants for the R143L-D13A heterodimer and the D13A homodimer are determined to be 21 and 2.9 \( \mu \text{M} \), respectively. In a comparison of subunit complementation of E14A with R143L relative to that of D13A with R143L, it is suggested that the AMPSase P-loop is responsible for stabilization of its dimeric structure, which is an essential requirement for catalysis.

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Monomeric Adenylosuccinate Synthetase Is Inactive

Fig. 5. The limiting restored activity versus the concentration of D13A mutant AMPSase. The solid line was drawn from fitting the data to Equation 2 under “Experimental Procedures.”

It is noteworthy that there is a significant difference between the effects of IMP and GTP on subunit complementation of AMPSase, i.e. although both nucleotides stabilize the dimer, GTP delays dimer dissociation. This result implies that there may be two different conformations for the dimer. Support for this suggestion comes from earlier studies (14, 16). Previously, it was found that the inactivation of E. coli AMPSase by pyridoxal 5'-phosphate is correlated with modification of Lys\textsuperscript{140} and the inactivation could be completely precluded by the presence of GTP, but not by IMP (14). It was subsequently determined that Lys\textsuperscript{140} is located at the subunit interface region and forms a salt bridge with the Asp\textsuperscript{231} residue from the juxtaposed subunit (16). Taken together, the differences between GTP and IMP in this study suggest that the salt bridge between Lys\textsuperscript{140} and Asp\textsuperscript{231} is formed in the presence of GTP but not IMP and thus represents a major difference between the dimers formed in the presence of the nucleotides. It is of interest to note that the K140L AMPSase mutant (14) exhibited restored activity when incubated with the D13A mutant (data not shown). Thus, it is reasonable to assume that, in the presence of the D13A mutant, part of the protein will be in the active dimeric form. Therefore, the analysis of subunit complementation used to characterize the D14A-R143L and D13A dimers in this study may be applied to study the differences between dimers in the presence of GTP and IMP and the role of salt bridges in the wild-type AMPSase dimer.
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