An Aminoacyl-tRNA Synthetase Complex in *Escherichia coli*

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Aminoacyl-tRNA synthetases from several strains of *Escherichia coli* are shown to elute as a high-molecular-weight complex on 6% agarose columns (Bio-Gel A-5M). In contrast, very little synthetase activity was observed in such complexes on Sephadex G-200 columns, suggesting that these enzymes may interact with or are dissociated during chromatography on dextran. The size of the complex observed on Bio-Gel A-5M was influenced by the method of cell breakage and the salt concentrations present in buffers. The largest complexes (>1,000,000 daltons) were seen with cells broken with a freeze press, whereas with sonicated preparations the average size of the complex was about 400,000 daltons. Extraction of synthetases at 0.15 M NaCl, to mimic physiological salt concentrations, also resulted in high-molecular-weight complexes, as demonstrated by both agarose gel filtration and ultracentrifugation analysis. Evidence is presented that dissociation of some synthetases does occur in the presence of higher salt levels (0.4 M NaCl). Partial purification of the synthetase complex on DEAE-Sephacel was accomplished with only minor dissociation of individual synthetases. These data suggest that a complex(es) of aminoacyl-tRNA synthetases does exist in bacterial cells, just as in eucaryotes, and that the complex may have escaped earlier detection due to its fragility during isolation.

Aminoacyl-tRNA synthetases have been shown to exist as high-molecular-weight complexes in mammalian cells (2, 3, 6, 8, 12, 17, 19, 20). The size of these complexes is somewhat variable, ranging from several hundred thousand to two million daltons (2, 5, 16). This variation in size is apparently the result of the fragility of the complexes during isolation. Whereas the physiological significance of the occurrence of these complexes is not fully understood, it has been recently shown that specific synthetases of a rat liver core complex are phosphorylated in vitro, with decreases in their activities (16). Hence, phosphorylation may be involved in the regulation of synthetase activity in mammalian cells. The occurrence of synthetase complexes in lower eucaryotes, such as yeast, has not yet been demonstrated. Indeed, on the basis of sucrose density centrifugation analyses, yeast aminoacyl-tRNA synthetase activity was not seen in high-molecular-weight complexes (4).

It has been generally thought that aminoacyl-tRNA synthetases are not associated in complexes in bacterial cells, since individual enzymes from such sources are readily purified free of other enzymes (4, 18). Direct evidence against a complex was obtained by Nass and Stöffler, who found that nine aminoacyl-tRNA synthetases present in postribosomal supernatants eluted from Sephadex G-200 at positions consistent with their known molecular weights (15). However, as pointed out by Schimmel and Söll, the bacterial synthetase complexes may be fragile enough to escape detection (18). We present evidence here that synthetase complexes may exist in *Escherichia coli*, and that the size of this complex is dependent on the method of cell disruption and the condition of subsequent chromatographic analysis.

MATERIALS AND METHODS

E. coli Q13 and MRE600 were grown in the M9 medium of Anderson (1); the medium for strain Q13 was supplemented with methionine and tyrosine at 0.13 and 0.11 mM, respectively. *E. coli* C6 (a *relA*⁻ Met⁻ Cys⁻ strain) was grown in the same medium, but supplemented with methionine and cysteine at 0.16 and 0.13 mM, respectively (9, 11). In some of the experiments, strain MRE600 cells were purchased as

late-log-phase cells from Grain Processing, Muscatine, Iowa. Axenically grown cells of *Dictyostelium discoideum* Ax-2 were provided by John Ellingson. Cell extracts were prepared in three different ways.

(i) **Sonication.** Cell pellets were suspended in 5 volumes of 0.05 M Tris hydrochloride (pH 7.4) containing 10 mM MgCl₂, 1 mM dithiothreitol, and 10% (vol/vol) glycerol (buffer A). The suspension was sonicated at 4°C using a microtip at a setting of 4 on a Heat-Systems Ultrasonics model 375-W for 2 min at 50% duty.

(ii) **Glass bead homogenization.** Cells were suspended in 0.65 volume of buffer A and 3 g of glass beads (100 to 200 µm; Sigma Chemical Co., St. Louis, Mo.) were added per g of cells. The suspension was stirred for 25 min with a Waring blender at low speed (75 to 90 V), keeping the temperature below 10°C. The beads were extracted twice, each time with 2 volumes of buffer A.

(iii) **Freeze press.** Cell pellets were suspended in 2 volumes of buffer A and added to a freeze press (Eaton modification of the Hughes press [7]), which was prechilled to -70°C. The frozen cell suspension was extruded at 8,000 to 12,000 lb/in² with a Carver laboratory press. Three volumes of buffer A was added to the broken cell suspension.

All extracts were clarified by centrifugation at 12,000 × g for 30 min; the resulting supernatant solutions were further centrifuged at 105,000 × g for 90 min. The supernatant solutions were carefully removed from the ribosomal pellets and either used immediately or mixed with glycerol to give 50% (vol/vol) and stored at -20°C. Total synthetase activity was found to be stable to such storage for several months. Protein was measured by the method of Lowry with bovine serum albumin as a standard (13) or by the Bio-Rad method (Bio-Rad Laboratories, Richmond, Calif.) with gamma globulin as a standard. In a typical experiment, the protein concentrations of 105,000 × g supernatant preparations from equal amounts of *E. coli* MRE600 with sonication, glass bead homogenization, and freeze press methods were 13.9, 13.4, and 16.9 mg/ml, respectively.

Chromatography. The 105,000 × g supernatants were chromatographed on Bio-Gel A-5M columns with buffer A as the elution buffer (see legends to figures for exact condi-

tions). The columns were calibrated with dextran blue (2,000,000 daltons), beta-galactosidase (510,000 daltons), pyruvate kinase (237,000 daltons), hemoglobin (64,000 daltons), and lactalbumin (35,000 daltons). Beta-galactosidase was obtained by growing a culture of *E. coli* in M9 medium plus lactose. After sonication and clarification as described above, the extract was chromatographed on Bio-Gel A-5M, and fractions were assayed for beta-galactosidase as described by Miller (14). Purified pyruvate kinase from yeast was the gift of James Blair, and hemoglobin was obtained from rat erythrocytes by hypotonic lysis. Sephadex G-200 chromatography was carried out as previously described (10).

Fractions were assayed for aminoacyl-tRNA synthetase activity by using a previously reported procedure (9). Each assay contained the following components in a final volume of 0.5 ml: 25 mol of Tris hydrochloride (pH 7.3), 5 mol of magnesium acetate, 2 mol of ATP (pH 7), 0.1 to 1.0 μ Ci of a 14 C- or 3 H-labeled amino acid, 0.4 mg of *E. coli* B tRNA, and 0.2 to 0.3 ml of the fraction. After a 30-min incubation at 37°C, 0.1 ml was removed from the reaction mixture, added to 2.4-cm Whatman 3MM disks, and plunged in cold 5% trichloroacetic acid. The disks were washed in trichloroacetic acid, ethanol-ether, and ether and then dried and counted as previously described (9).

Chemicals and radioisotopes. Bio-Gel A-5M was obtained from Bio-Rad Laboratories, Richmond, Calif. Sephadex G-200 and DEAE-Sephacel were products of Pharmacia, Inc., Piscataway, N.J. The 14 C-amino acid mixture was obtained from New England Nuclear Corp., Boston, Mass., and contained 15 purified L-amino acids (lot 1669-065; no cysteine, tryptophan, glutamine, asparagine, or methionine). The specific activity of this amino acid mixture ranged from 113 to 523 mCi/mmol. [14 C]isoleucine (337 mCi/mmol) was also obtained from New England Nuclear, whereas [14 C]serine (10 mCi/mmol), [3 H]glutamic acid (22 Ci/mmol), and [3 H]tyrosine (48 Ci/mmol) were products of Amersham Corp., Arlington Heights, Ill. *E. coli* B tRNA was purchased from Schwarz/Mann, Orangeburg, N.Y. DNase I, from bovine pancreas, was a product of Cooper Biomedical, West Chester, Pa. All other chemicals were of the highest quality commercially available.

RESULTS

Aminoacyl-tRNA synthetases in bacteria have been reported to exist as free, monomeric enzymes (15, 18). This is demonstrated in Fig. 1a for Sephadex G-200 chromatography of a $105,000 \times g$ supernatant prepared by sonication of *E. coli* C6. It is clear that the majority of the aminoacyl-tRNA synthetase activity occurs within the resolving portion of the column. This elution pattern agrees well with molecular masses of monomeric synthetases, which range from 63,000 (arginyl-tRNA synthetase) to 237,000 (phenylalanyl-tRNA synthetase) with an average of about 120,000 daltons (18). Indeed, the elution position observed for leucyl-tRNA synthetase in Fig. 1 is consistent with its reported molecular mass of 105,000 daltons. Approximately 5% of the total synthetase activity is observed in the fraction of protein excluded from Sephadex G-200, where one would expect high-molecular-weight complexes to elute. For comparison, Fig. 1b shows the chromatography of a $105,000 \times g$ supernatant preparation from *D. discoideum*, chromatographed on this same column. In this case, much of the synthetase activity is seen in the void volume, indicating the presence of complexes of high molecular weight. We previously reported

similar profiles for supernatants from rat liver (10), showing that eucaryotic synthetases are generally organized in high-molecular-mass complexes, approximately 1,000,000 daltons or larger in size.

Although the results from Sephadex G-200 chromatography suggest that most aminoacyl-tRNA synthetase activity in *E. coli* is not present in high-molecular-weight complexes, a different conclusion is indicated if a similar preparation is chromatographed on 6% agarose. *E. coli* Q13 isoleucyl- and total aminoacyl-tRNA synthetase activities elute together during Bio-Gel A-5M chromatography, with an elution position expected for a protein whose molecular weight is 400,000 (Fig. 2). Similar results were obtained on Sepharose 6B columns with $105,000 \times g$ supernatants from *E. coli* C6 (data not shown). Indeed, enzyme activity was not observed below a molecular weight of 200,000 on agarose columns, whereas little was seen as high as this molecular weight range on Sephadex G-200. These results suggest that synthetase complexes may exist in *E. coli*, although they appear to be smaller in size than complexes in eucaryotes and perhaps are less stable or bind to the matrix during chromatography on dextran columns.

One of the simplest explanations for the appearance of complexes on agarose would be aggregation of synthetases during preparation or as a result of their association with other components of high molecular weight in the extract. Several possibilities were examined. First, to see whether disruption of the cells by sonication could have resulted in the formation of artifactual complexes, we investigated the size of synthetase complexes in *E. coli* MRE600 prepara-

FIG. 1. Sephadex G-200 chromatography of aminoacyl-tRNA synthetase preparations from *E. coli* (a) and *D. discoideum* (b). *E. coli* C6 was harvested at the late-log phase and disrupted by sonication in buffer A (no glycerol). Slime mold cells were broken with a glass-Teflon homogenizer; $105,000 \times g$ supernatants were prepared (see the text), and 5 ml of each (52 mg of protein for *E. coli*; 2.8 mg of protein for *D. discoideum*) was applied to a 2- by 28-cm column of Sephadex G-200. The column was equilibrated and eluted with buffer A (no glycerol) at 0.25 ml/min, and fractions of 3.6 ml were collected. Aminoacylation assays were carried out as described in Materials and Methods. A and B refer to the elution positions of dextran blue (2×10^6 daltons) and hemoglobin (64,000 daltons), respectively. Symbols: (—) absorbance at 280 nm, (○) [3 H]leucine incorporation, (●) 14 C-amino acid mixture incorporation.

tions where cells were broken by freeze fracture (Fig. 3a) or by homogenization with glass beads (Fig. 3b). In each case, the synthetase complex is larger than observed with cells broken by sonication. For example, freeze-fractured preparations contain synthetase activity in the molecular weight range of 500,000 to 1,300,000. With preparations from cells broken with glass beads I observed material in this range also; but, in addition, synthetase activity is seen near the 300,000- to 400,000-dalton range. It is tempting to speculate that these smaller complexes in all preparations are derived from the larger complexes such as those seen with preparations from freeze-fractured cells. In short, these data show that synthetase complexes are smallest in preparations derived from sonicated cells, suggesting that sonication itself may disrupt this macromolecular complex.

To see whether the presence of glycerol in buffers could be contributing to the formation of synthetase aggregates, we prepared a $105,000 \times g$ supernatant from *E. coli* MRE600 with the same buffers minus glycerol. This supernatant solution was chromatographed on Bio-Gel A-5M as described in the legend to Fig. 2, but with buffer A minus glycerol. No differences in the elution positions of total aminoacyl- and isoleucyl-tRNA synthetase activities were observed, suggesting that the presence of glycerol did not influence the size of the synthetase complex (data not shown). We also investigated the possibility that the synthetase may be associating with DNA and then coeluting at a higher apparent molecular size. A $105,000 \times g$ supernatant, prepared by sonication, was treated with $10 \mu\text{g}$ of DNase I per ml of supernatant and chromatographed on Bio-Gel A-5M, this time with glycerol in the buffers. The DNase treatment did not change the size of the aminoacyl-tRNA synthetase complex compared with untreated controls (data not shown). Although this experiment does not completely rule out the association of synthetases with DNA, since the

FIG. 2. Bio-Gel A-5M chromatography of a $105,000 \times g$ supernatant obtained after sonication of *E. coli* Q13. A 19.9-mg sample of S105 protein was applied to a 1.5- by 80-cm column of Bio-Gel A-5M at a flow rate of 6.5 ml/h. The column was equilibrated and eluted with buffer A, and 3-ml fractions were collected. Aminoacylation assays were carried out by adding 0.3 ml of each fraction to the standard assay mixture and using $1 \mu\text{Ci}$ of ^{14}C -amino acid mixture and $5 \mu\text{Ci}$ of ^3H -isoleucine in each assay. A sample of 0.1 ml was applied to filter disks, and the disks were washed and counted under double-label conditions. Symbols: (—) absorbance at 280 nm, (○) ^{14}C -amino acid incorporation, (●) ^3H -isoleucine incorporation. The letters indicate the elution positions for known molecular weight standards: A, dextran blue (2,000,000); B, beta-galactosidase (510,000); C, pyruvate kinase (237,000); D, hemoglobin (64,000); E, lactalbumin (35,000).

FIG. 3. Bio-Gel A-5M chromatography of $105,000 \times g$ supernatants prepared after freeze press and glass bead disruption of *E. coli* MRE600. Extracts were prepared as described in Materials and Methods and chromatographed on Bio-Gel A-5M as described in the legend to Fig. 2. Panel a shows the results with 173 mg of $105,000 \times g$ supernatant from extracts prepared by the freeze-press procedure, whereas panel b shows an identical run with 76.2 mg of $105,000 \times g$ supernatant prepared by homogenization with glass beads. In both cases, standard aminoacylation assays were carried out with the ^{14}C -amino acid mixture. Symbols: (—) absorbance at 280 nm, (○) ^{14}C -amino acid incorporation. The letters show the elution position of known standards, as listed in the legend to Fig. 2.

enzymes may protect bound DNA from DNase attack, it clearly does not support the hypothesis that synthetases are forming nonspecific complexes with DNA.

All of the preparations discussed thus far were prepared in buffer A, a low-salt buffer, which could promote the aggregation of synthetases. To examine this, we used the glass bead homogenization method and buffer A supplemented with 0.15 M NaCl, chromatographing the resulting supernatant on Bio-Gel A-5M equilibrated with the same buffer. The elution profile for total protein (Fig. 4) was altered in buffer A with 0.15 M NaCl, showing less material in high-molecular-weight areas. However, the synthetase activity eluted from 500,000 to 1,300,000 daltons; only the higher-molecular-mass synthetase peaks were observed (compare with glass-bead homogenization, Fig. 3). Hence, extraction and chromatography of the synthetase in the presence of physiological salt levels failed to result in dissociation of the complex.

High-speed sedimentation of the synthetase complex. The eucaryotic synthetase complex can be pelleted by centrifugation of a cell supernatant at $160,000 \times g$ (2, 10). To see whether a similar result could be obtained with bacterial preparations, the $105,000 \times g$ supernatant prepared by glass bead homogenization (Fig. 4) was supplemented with sucrose to 0.25 M and then centrifuged at $160,000 \times g$ for 18 h. Under these conditions, a clear amber pellet was obtained containing 35% of the total protein and 61% of the synthetase activity. If the same postribosomal supernatant was centri-

FIG. 4. Bio-Gel A-5M chromatography of an *E. coli* MRE600 $105,000 \times g$ supernatant extracted with buffer A plus 0.15 M NaCl. Chromatography was as described in the legend to Fig. 2, except that a 2.5- by 57-cm column (282 ml) was employed, 6-ml fractions were collected, and the flow rate was 13 ml/h. Symbols: (—) absorbance at 280 nm, (O) ^{14}C -amino acid incorporation. The letters refer to the elution of previously mentioned molecular weight markers (see legend to Fig. 2).

fuged as above in buffer A with 0.25 M sucrose but no NaCl, 58% of the protein and 66% of the synthetase activity were observed in the pelleted material. Hence, the presence of 0.15 M NaCl did prevent the sedimentation of some protein, possibly by limiting protein aggregation. However, most of the synthetase activity was still seen in the pellet. In these experiments the recovery of both protein and synthetase activities was 90% or more. This centrifugation step resulted in a 2.5- to 3-fold increase in the specific activity of the synthetases and may be a useful step in purification. These results further show that the aminoacyl-tRNA synthetases are organized in a high-molecular-weight complex in *E. coli* and lend strong support to the conclusion drawn from the agarose gel filtration experiments.

Partial purification of the complex. Purification of the synthetase complex has been attempted with ion-exchange chromatography, which resulted in only a partial dissociation to individual enzymes. Chromatography of a $105,000 \times g$ supernatant derived from sonicated *E. coli* Q13 on DEAE-Sephacel (Fig. 5a) shows that isoleucyl-tRNA synthetase activity eluted as a rather sharp peak at 0.13 to 0.2 M NaCl. When these fractions were pooled, concentrated, and applied to Bio-Gel A-5M, three fractions of isoleucyl-tRNA synthetase activity were observed: one in the void volume (15%), a major peak (70%) in position seen previously, and a minor peak (15%) at the position expected for the monomeric enzyme (114,000 daltons; data not shown). The combined DEAE-Sephacel-Bio-Gel A-5M steps resulted in nearly a 40-fold purification of isoleucyl-tRNA synthetase activity, yet most of the activity remained in a high-molecular-weight form. The isoleucyl-tRNA synthetase activity associated with the void volume was not observed when chromatography was carried out in the presence of 0.4 M NaCl (see below), suggesting that slight aggregation of isoleucyl-tRNA synthetase did occur either during ion-exchange chromatography or during the concentration steps. In addition, ion-exchange chromatography resulted in the partial dissociation of isoleucyl-tRNA synthetase from the higher-molecular-weight complex.

In another experiment, we attempted to dissociate the complex by chromatography of the pooled, concentrated synthetase fractions from the DEAE-Sephacel column on Bio-Gel A-5M, this time equilibrated and eluted with 0.4 M

FIG. 5. (a) DEAE-Sephacel chromatography of a $105,000 \times g$ supernatant from *E. coli* Q13. A sample (6 g) of cells was sonicated, and the supernatant was prepared as described in Materials and Methods. A sample (18 ml) of $105,000 \times g$ supernatant (129 mg of protein) was applied to a 2.5- by 17-cm column of DEAE-Sephacel, previously equilibrated with buffer A. After a wash with 145 ml of buffer A at 1 ml/min, a 400-ml linear gradient from buffer A alone to 0.3 M NaCl in buffer A was applied. Fractions of 5 ml were collected, and conductivity and isoleucine incorporation were measured as described above. (b) Bio-Gel A-5M chromatography of pooled fractions from DEAE-Sephacel. Fractions 75 to 100 were pooled, and 90 ml was concentrated to 11 ml by Amicon ultrafiltration and applied to Bio-Gel A-5M. All conditions were as described in the legend to Fig. 2, except that the column was equilibrated and eluted with buffer A containing 0.4 M NaCl. Isoleucine incorporation was determined as described above by using 0.3 ml of each fraction as a source of enzyme. Symbols: (—) absorbance at 280 nm, (O) $[^3\text{H}]$ isoleucine incorporation.

NaCl in buffer A. The results (Fig. 5) show that total aminoacylation activity is now seen in two main regions, at approximately 300,000 and 100,000 daltons. These peaks probably represent a somewhat smaller multimeric complex and monomeric forms of synthetases. This is further demonstrated by the data shown in Table 1, where individual aminoacyl-tRNA synthetase activities from this same chro-

TABLE 1. Aminoacyl-tRNA synthetase activities in high- and low-molecular-weight fractions from Bio-Gel A-5M

Amino acid	Total incorporation (cpm) ^a	
	High-molecular-weight fractions	Low-molecular-weight fractions
Mixture of 15 amino acids	2,440,000 (71)	999,000 (29)
Isoleucine	88,220 (37)	152,000 (63)
Tyrosine	588,000 (40)	865,000 (60)
Glutamic acid	160,000 (55)	129,000 (45)
Serine	67,300 (48)	72,900 (52)

^a Amino acid acceptances for each of the above amino acids were determined for the high-molecular-weight (fractions 32 through 43) and low-molecular-weight (fractions 49 through 54) fractions shown in Fig. 5. The data are the sum of the incorporation values of these assays of individual fractions, corrected for total volume. The values in parenthesis represent each activity as a percentage of the total measured incorporation.

matogram are measured. With the 15-amino-acid mixture we observed 29% of the activity in the low-molecular-weight fractions and 71% in the complex. The data show that isoleucyl-, tyrosyl-, glutamyl-, and seryl-tRNA synthetases are also distributed in these two fractions. This suggests that these enzymes are partly dissociated from a multimeric complex in the presence of 0.4 M NaCl. The molecular weights of these enzymes have been reported as follows: isoleucyl-tRNA synthetase, 112,000; tyrosyl-tRNA synthetase, 95,000; glutamyl-tRNA synthetase, 102,000 (yeast); and seryl-tRNA synthetase, 103,000. Hence, the elution positions observed near fraction 52 on Bio-Gel A-5M are consistent with their monomeric molecular weights. These results demonstrate that the aminoacyl-tRNA synthetase complex present in *E. coli* is only partially dissociated during ion-exchange chromatography or exposure to high salt during subsequent concentration steps. Thus, it appears that electrostatic forces are important in maintaining the integrity of this complex.

DISCUSSION

We present here the first evidence for the existence of an aminoacyl-tRNA synthetase complex(es) in *E. coli*. We found that only 5% of the total synthetase activity was excluded from Sephadex G-200, indicating that nearly all of these enzymes are monomeric in this bacterium. This is in substantial agreement with previously reported data (15). However, chromatography of an identically prepared $105,000 \times g$ supernatant preparation on 6% agarose (Bio-Gel A-5M) gave evidence of a complex with an average molecular weight of about 400,000. We also showed that aminoacyl-tRNA synthetase activity from a eucaryote, *D. discoideum*, was found in the excluded volume on Sephadex G-200. This is apparently the first observation of a synthetase complex in this organism also. Hence, a large-molecular-weight complex from another organism has the expected behavior on Sephadex, suggesting that the eucaryotic complex is more stable or does not bind to dextran, whereas the synthetase complex of *E. coli* may do so. We also noted that synthetase activity from *E. coli* eluted later than the void volume on Sephadex G-25 during desalting, further suggesting an interaction of the complex with dextran (Harris, unpublished results). It is possible that this interaction with the dextran beads is the reason for the lower molecular size observed for the synthetases on Sephadex G-200 (Fig. 1).

There are two major questions about the demonstration of a synthetase complex in bacteria. Is the complex formed as an artifact during the preparation or chromatography of the extracts, and why has the existence of this complex escaped the attention of previous investigators? To answer these questions, several parameters of the isolation procedure were varied with the following results. First, the apparent size of the synthetase complex was influenced by the method of cell breakage. The freeze-press method of cell breakage gave the highest protein concentrations of all methods tested and also resulted in the least disruption of the synthetase complex. Glass bead homogenization also resulted in preparations with greater molecular weights than those observed with supernatants from sonicated cells. The largest material observed with freeze-press extracts was greater than 1,000,000 daltons, with evidence of multiple peaks of synthetase activity larger than the 400,000-dalton peak seen with extracts prepared by sonication. Since the average synthetase has a mass of 120,000 daltons, the size of a complex with

one of each of the 20 synthetases would be 2,400,000 daltons. Hence, with the best method we tried, the largest synthetase complexes may be half that size. Overall, our results show that the synthetase complex in bacteria is quite labile and is partially dissociated by all methods of cell disruption.

A molecular complex of the size indicated by agarose gel filtration should be sedimented by centrifugation, as was observed with complexes from eucaryotic cells (2, 15). We found this to be the case, since centrifugation of a $105,000 \times g$ supernatant (from cells homogenized with glass beads) at $160,000 \times g$ for 18 h resulted in a pellet containing 35% of the total protein and over 60% of the synthetase activity. This finding strongly supports the conclusions from the gel filtration data that high-molecular-weight complexes of synthetases exist in *E. coli*.

Several potential artifacts were considered. DNase treatment failed to alter the position of elution of aminoacyl-tRNA synthetases on Bio-Gel A-5M. This rules out a non-specific association with DNA as the explanation for the occurrence of the synthetase complex. We found that the elution position of synthetase activity was also unaffected when glycerol was omitted from the extraction and chromatography buffers. In addition, when supernatants were prepared in buffer containing 0.15 M NaCl, to approximate the intracellular salt concentration, we did not observe disaggregation of the synthetases. After elution of the synthetases from DEAE-Sephacel at 0.2 M NaCl only a slight dissociation into monomeric enzymes was observed. However, increasing the monovalent salt concentration to 0.4 M did lead to a partial dissociation of the complex during Bio-Gel A-5M chromatography. Hence, the synthetase complex probably did not form as the result of artifactual electrostatic interactions during extraction. In agreement with results with eucaryotic aminoacyl-tRNA synthetases (18), the *E. coli* complex was partially disrupted by higher salt levels, showing that electrostatic interactions are important for the association of synthetases within the complex. These results further suggest that ammonium sulfate fractionation, a commonly used step in synthetase purifications, would likely cause the dissociation of this complex.

The physiological significance of the finding of a large complex of synthetases in bacteria should be delineated. Since it is likely that all tRNA in bacterial cells is complexed with the synthetases (18), is it possible that this huge complex of synthetases and aminoacyl-tRNA plays a role in protein synthesis or its regulation? Are free and complex forms of the same enzyme different kinetically? What other components are associated with the synthetases in this complex, and what forces are important in their association? The answers to these important questions will require purification and further characterization of this multienzyme complex.

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