Regulation of Glutamine Synthetase. XII. Electron Microscopy of the Enzyme from *Escherichia coli**

R. C. Valentine, B. M. Shapiro, and E. R. Stadtman

ABSTRACT: Electron microscopic examination of the glutamine synthetase purified from *Escherichia coli* shows that the enzyme molecule is a symmetrical aggregate of twelve identical subunits arranged in two hexagonal layers. The dimensions of the molecule estimated directly from electron micrographs are in good agreement with hydrodynamic measurements of the size of glutamine synthetase. No differences in the subunit structures of the previously described taut, relaxed, and tightened enzyme forms could be detected by electron microscopy. However, it was established that the tightened form, produced when the relaxed enzyme is exposed to divalent cations, aggregates in such a way that its double-hexagon molecules interact face to face to produce long hexagonal tubes. This type of aggregate accounts for the lowered solubility of the tightened form. Time-sequence studies showed that these linear aggregates undergo subsequent lateral associations to produce larger cylindrical bundles which finally precipitate from solution as a paracrystalline "wheat-sheaf" structure. Electron microscopic examination of the relaxed enzyme at various times after its exposure to 1 M urea (pH 8.0) shows that the dissociation of subunits involves the intermediary formation of a heterogeneous population of molecular subaggregates. Dissociation does not seem to follow any rigid pattern but the tendency for breaks to occur between subunits in the hexagonal rings appears to be greater than for breaks to occur in the bonds that join the two hexagonal rings together. The individual subunit, once dissociated, seems to change shape, as judged by the failure to observe the well-defined ellipsoidal structures characteristic of the subunit in the aggregated state. Addition of Mn^{2+} to the fully dissociated enzyme results in the reconstitution of double-hexagon structures that are similar to, but less well defined than, those of the native enzyme. The molecular architecture of *E. coli* glutamine synthetase is not altered by either substrates or inhibitors of the enzyme, as judged by electron microscopy.

Glutamine synthetase (L-glutamate–ammonia ligase (ADP),[1] EC 6.3.1.2) as purified from *Escherichia coli* (Woolfolk *et al.*, 1966) is subject to cumulative feedback inhibition by a number of the products of nitrogen metabolism for which glutamine serves as the nitrogen donor (Woolfolk and Stadtman, 1967a). The enzyme, obtained as a crystalline protein ($s^0_{20,w}$ = 20.3 S; M_w = 592,000; Shapiro and Ginsburg, 1968), is homogeneous in the ultracentrifuge and by disc gel electrophoresis (Woolfolk *et al.*, 1966). In 4 M guanidine hydrochloride, the enzyme is disaggregated to subunits of M_w = 48,500 (Woolfolk *et al.*, 1966). The initial ultracentrifuge data (Woolfolk *et al.*, 1966) suggested that the enzyme contained 12–14 subunits, but the analytical results were not of sufficient precision to discriminate between these values. Tryptic peptide maps indicate that these subunits are identical (Shapiro and Stadtman, 1967; Woolfolk *et al.*, 1966). Other studies (Shapiro and Stadtman, 1967) show that, as normally isolated, glutamine synthetase exists in a "taut" state. In this form it contains up to 40 equiv of bound Mn^{2+} and is resistant to dissociation and inactivation by mild protein denaturants or by treatment with mercurials. However, following the removal of divalent cations, it changes to a "relaxed" form, that is highly susceptible to dissociation either by 1 M urea, by increasing the pH to 8.0 (Woolfolk and Stadtman, 1967b; Shapiro and Ginsburg, 1968), or by organic mercurials (Shapiro and Stadtman, 1967). Addition of either Mn^{2+}, Mg^{2+}, or Ca^{2+} converts the relaxed enzyme into a "tightened" state that is generally indistinguishable from the taut form (Shapiro and Ginsburg, 1968; Kingdon *et al.*, 1968) except that it is considerably less soluble in dilute buffer solution. Complete dissociation of the relaxed enzyme to subunits is achieved by treatment with 1 M urea at alklaine pH, and this is partially reversed by the addition of excess Mn^{2+}, which results in reaggregation of the subunits and a transient restoration of catalytic activity (Woolfolk and Stadtman, 1967b).

The present investigation was undertaken to determine if changes associated with the interconversions of the taut, relaxed, and tightened forms of glutamine synthetase are discernible under the electron microscope. It will be shown that the relaxed and taut forms of the enzyme are indistinguisable microscopically; both are composed of twelve subunits, symmetrically arranged in two hexagonal layers. Differences in the solubility of taut and tightened enzyme forms are due to the capacity of the latter to undergo face-to-face as-

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[1] Abbreviations used are as listed in *Biochemistry 5*, 1445 (1966).

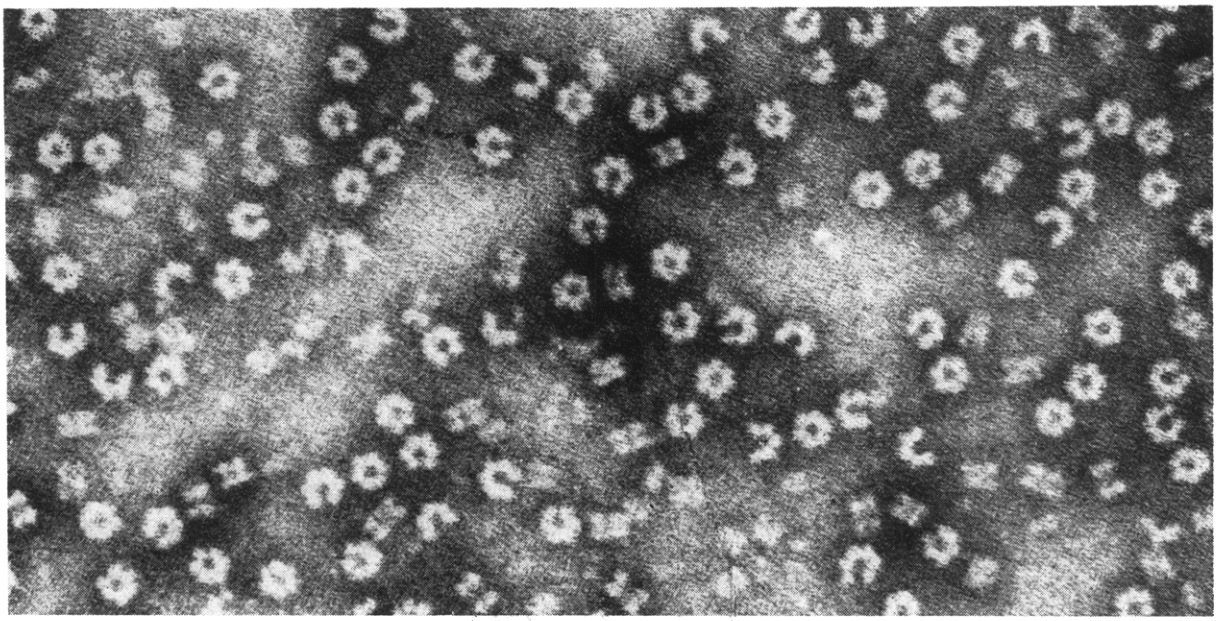

FIGURE 1: An unfixed preparation of glutamine synthetase (200 μg/ml) in 0.01 M imidazole buffer (pH 7.0) with $MnCl_2$ (1 mM). The appearance of the molecules depends on their orientation (see Figure 3) but many of the molecules are broken during the process of negative staining. Magnification × 430,000.

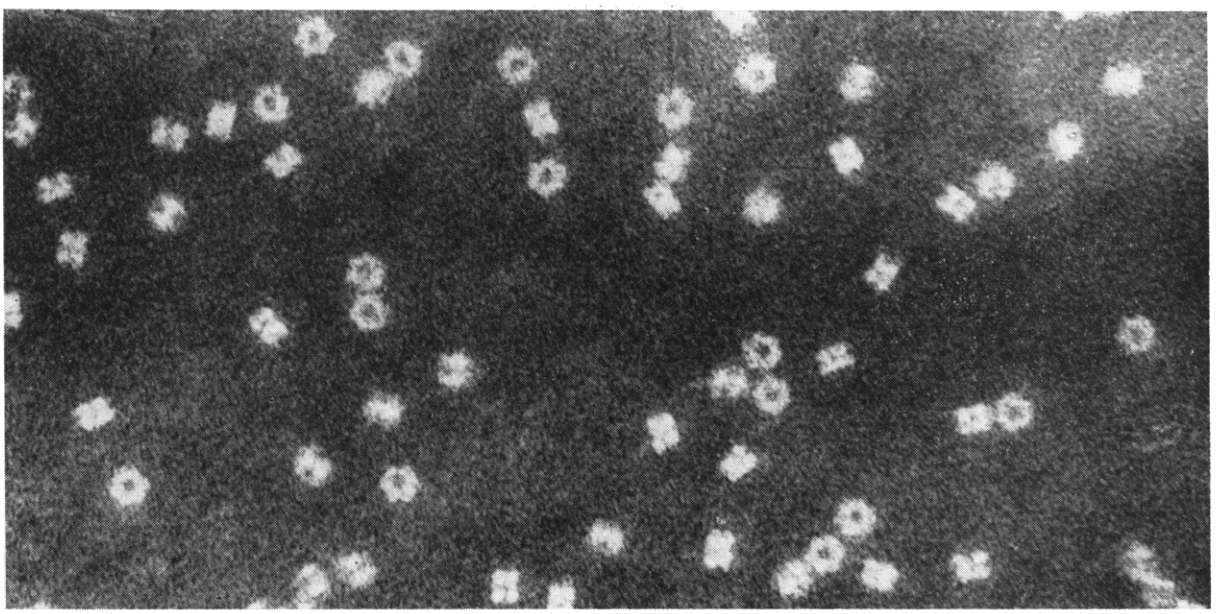

FIGURE 2: The same preparation as shown in Figure 1 after 20-min fixation with glutaraldehyde (0.5%, pH 7.0). There was no detectable difference in the characteristic appearances of the molecules, but as the time of fixation increased the percentage of broken forms decreased until after 20 min broken molecules were rare. Magnification × 422,000.

sociations leading to long hexagonal tubular aggregates which in turn associate to form paracrystalline structures. The urea-induced dissociation of relaxed enzyme to subunits and the reaggregation of the latter in the presence of excess Mn^{2+} were observed. The Mn^{2+}-induced reaggregation of completely dissociated subunits leads to the formation of molecular forms with a double-hexagon structure which are somewhat less well defined than those characteristic of the original associated enzyme. Finally, we were unable to detect any alterations in protein structure which might have been assumed to be associated with the binding of feedback inhibitors and substrates.

Materials and Methods

Sodium silicotungstate and glutaraldehyde of high purity were obtained from Taab Laboratories, Emmer Green, Reading, England. The negative stain was prepared by adding 100 ml of water to 4 g of sodium silicotungstate. This was well shaken and the deposit was allowed to settle for 24 hr. The supernatant solution

was decanted and used without dilution. All other chemicals were of the highest quality obtainable from commercial sources. Glutamine synthetase was obtained in crystalline form from *E. coli* which had been grown as previously described (Woolfolk *et al.*, 1966) in a medium containing glutamate and glycerol as the nitrogen and carbon sources, respectively. The enzyme used for these experiments was dialyzed at 4° against several changes of buffer (10 mM imidazole-chloride–1 mM $MnCl_2$, pH 7.2), and then treated as described below.

Glutamine synthetase (7.3 mg/ml) was diluted with either buffer or some other specified reagent mixture to concentrations of 200–700 μg/ml, and was incubated for appropriate periods of time, as described in the legends to the figures. The treated enzyme preparation was then applied to a carbon film deposited on freshly cleaved mica by floating the film off on the surface of the enzyme solution. The carbon film was transferred to a container of the negative stain (sodium silicotungstate) where it was left floating for several minutes. During this time, diffusion of the inert negative stain into the fluid layer in contact with the film was accompanied by diffusion of the other reagents out of this layer. Loss of enzyme molecules during this procedure is restricted by their adsorption to the film and by their far slower rate of diffusion.

A copper electron microscope grid was placed on the carbon film which was then covered with absorbent paper. When it was wet, the paper was lifted off and the excess liquid was removed by placing it, grid uppermost, on filter paper. As the thin layer of silicotungstate dries, the salt comes out of solution and replicates the still moist molecules in an electron opaque layer. Protein molecules alone scatter an insufficient fraction of the electron beam to form an image. The grids were studied in a Philips EM 200 electron microscope and plates (Ilford N. 60) were exposed to the image at a magnification of 55,000. The magnification was calibrated by the use of the 168-Å spacing of catalase crystals.

Native glutamine synthetase, examined in the electron microscope after negative staining (Figure 1), appeared to consist of a mixture of intact and broken double-hexagon figures (see Results). This molecular heterogeneity was unexpected since the enzyme had been shown to be homogeneous in size by many other physical criteria. Additionally, the extent of heterogeneity differed each time the same enzyme preparation was stained for microscopy. Thus, the disruption of molecular architecture was thought to have been caused by the negative staining technique, perhaps because of the rapid increase in salt concentration which is a concomitant of negative staining with its subsequent drying. For this reason, prior to staining, we attempted to stabilize the enzyme after it had interacted with its effectors. Potassium phosphate (0.1 M, pH 7.3) alone partially protected the enzyme from disaggregation. Much better protection was afforded by fixing the enzyme for 20 min in dilute (0.5%) glutaraldehyde, prepared in 0.1 M potassium phosphate buffer (pH 7.3) prior to staining. After fixation, the molecules could be stained with silicotungstate without breaking; they appeared otherwise identical with the unfixed preparations

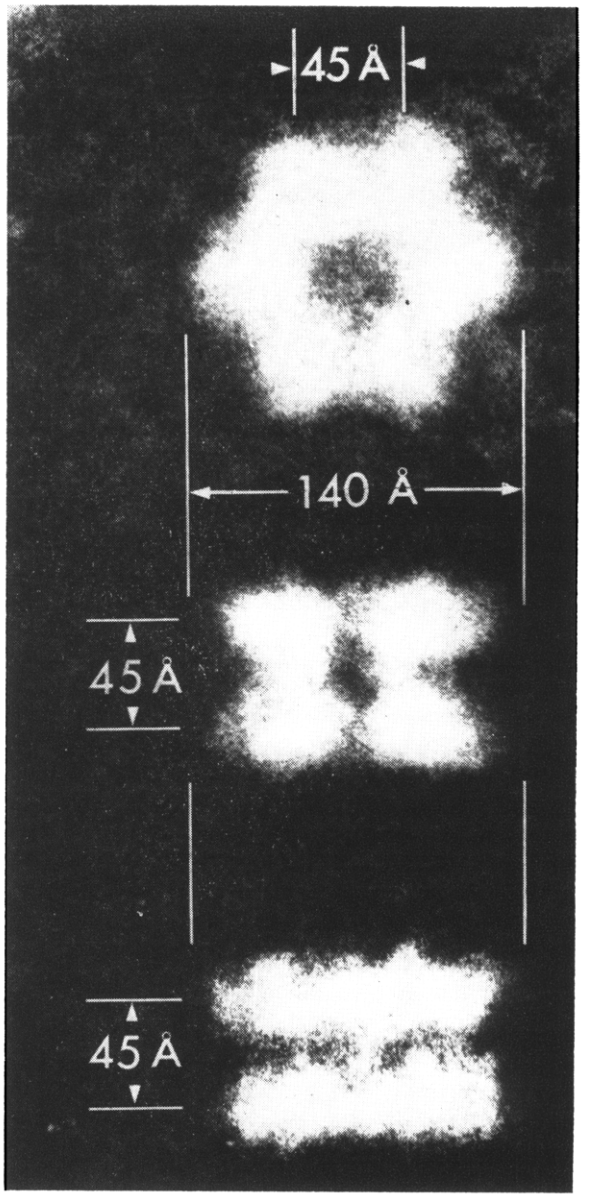

FIGURE 3: A high-magnification picture of five superimposed images of unfixed molecules in the three characteristic orientations. The mean dimensions are indicated. When the molecule rests on a face, the subunits appear as a hexagonal ring (top). Molecules seen on edge show two layers of subunits, either as four spots (center) when viewed exactly down a diameter between subunits, or in general as two lines (bottom). The molecular structure is thus based on twelve identical subunits in two hexagonal layers, the units in one layer being directly above those in the other. Magnification × 3,160,000.

(Figure 2). Relaxed enzyme preparations (see Results) were almost totally destroyed by silicotungstate alone, yet with prior glutaraldehyde fixation they showed a large proportion of intact double-hexagon forms (Figure 4). This finding is in accord with hydrodynamic and light-scattering data (Shapiro and Ginsburg, 1968) which indicated that relaxed and native (taut) enzyme forms have the same size and are quite similar in shape. However, glutaraldehyde-fixed enzyme was catalytically inactive, indicating that the cross-linking reaction

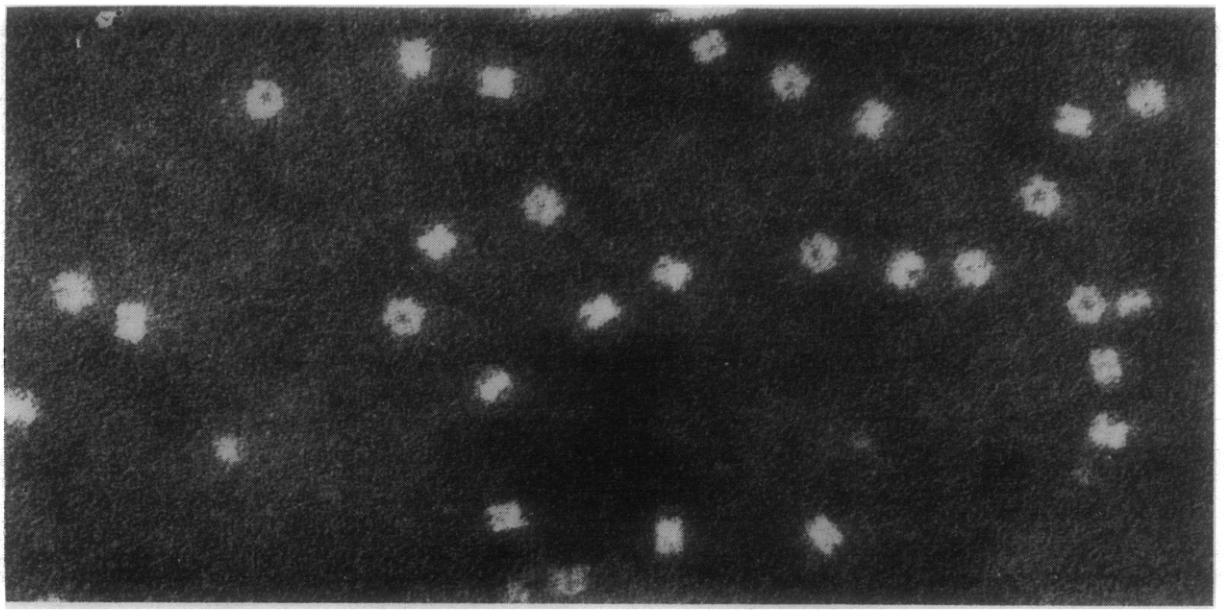

FIGURE 4: Molecules in the "relaxed" state after removal of most of the manganese normally present by incubating the enzyme in 0.01 M EDTA for 3 hr at 0° (0.01 M imidazole buffer, pH 7.2) followed by passage of the enzyme over Sephadex G-25 equilibrated with 0.01 M imidazole buffer (pH 7.0). No consistent change was found in the electron microscope appearance of the fixed molecules. Magnification × 416,000.

may have caused subtle changes in molecular architecture, but the quarternary structural characteristics of the enzyme appear unaltered.

Results

The Structure of Glutamine Synthetase. Figure 1 shows an unfixed preparation of glutamine synthetase in which many hexagonal and tetrad structures are visable. The rather large number of broken rings are apparently caused by the preparative procedure. We interpret the hexagonal and tetrad forms to be two different views of the same molecule and conclude that the complete structure is a dodecahedral aggregate, composed of two hexagons face to face. The glutaraldehyde-fixed enzyme (Figure 2) shows fewer disaggregated molecules and quite clearly illustrates the dihexagonal structure of the glutamine synthetase molecule. By superimposing pictures of five unfixed molecules at higher magnification (Figure 3), we have been able to make measurements of the subunit dimensions both in the end-on view of the molecule, with a hexagonal ring of subunits, and the two side views. These show the two rings of six units either as two lines, or, in the particular case of a view down a diameter passing between subunits, as four ellipsoids. Each of these four spots arises from the superimposition of three subunits. From the spacing between the disks (side view) and the subunits (end view) the height and the width of the subunits were both estimated to be 45 Å. The length of the subunit (*i.e.*, in the radial direction of the ring) is less well defined, but by using the mean maximum diameter of the rings (137 Å) and an approximate value for the diameter of the center hole, the length of the subunit was estimated to be 53 Å. An ellipsoid with these dimensions has a volume of 56,000 Å^3. Assuming a partial specific volume of 0.69 (Shapiro and Ginsburg, 1968), the calculated subunit molecular weight is therefore 48,500, a value identical with that for the subunit determined by sedimentation equilibrium in 4 M guanidine hydrochloride (Woolfolk *et al.*, 1966). Consequently, it appears that the *E. coli* glutamine synthetase is composed of twelve identical subunits of about 50,000 molecular weight, arranged in two hexagonal rings.

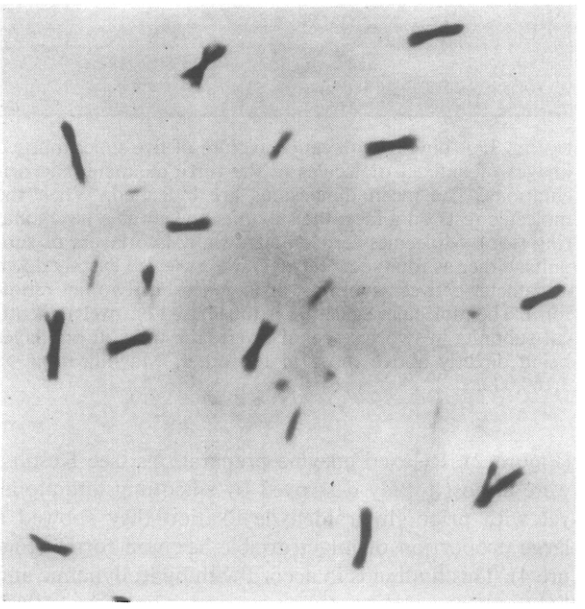

FIGURE 5: A light microscope picture of the crystals of glutamine synthetase 60 min after addition of $MnCl_2$ (5 mM) to a relaxed enzyme preparation (in 0.01 M imidazole). The wheat-sheaf appearance is characteristic. Magnification × 2100.

Relaxed Enzyme. Since the relaxed enzyme (pro-

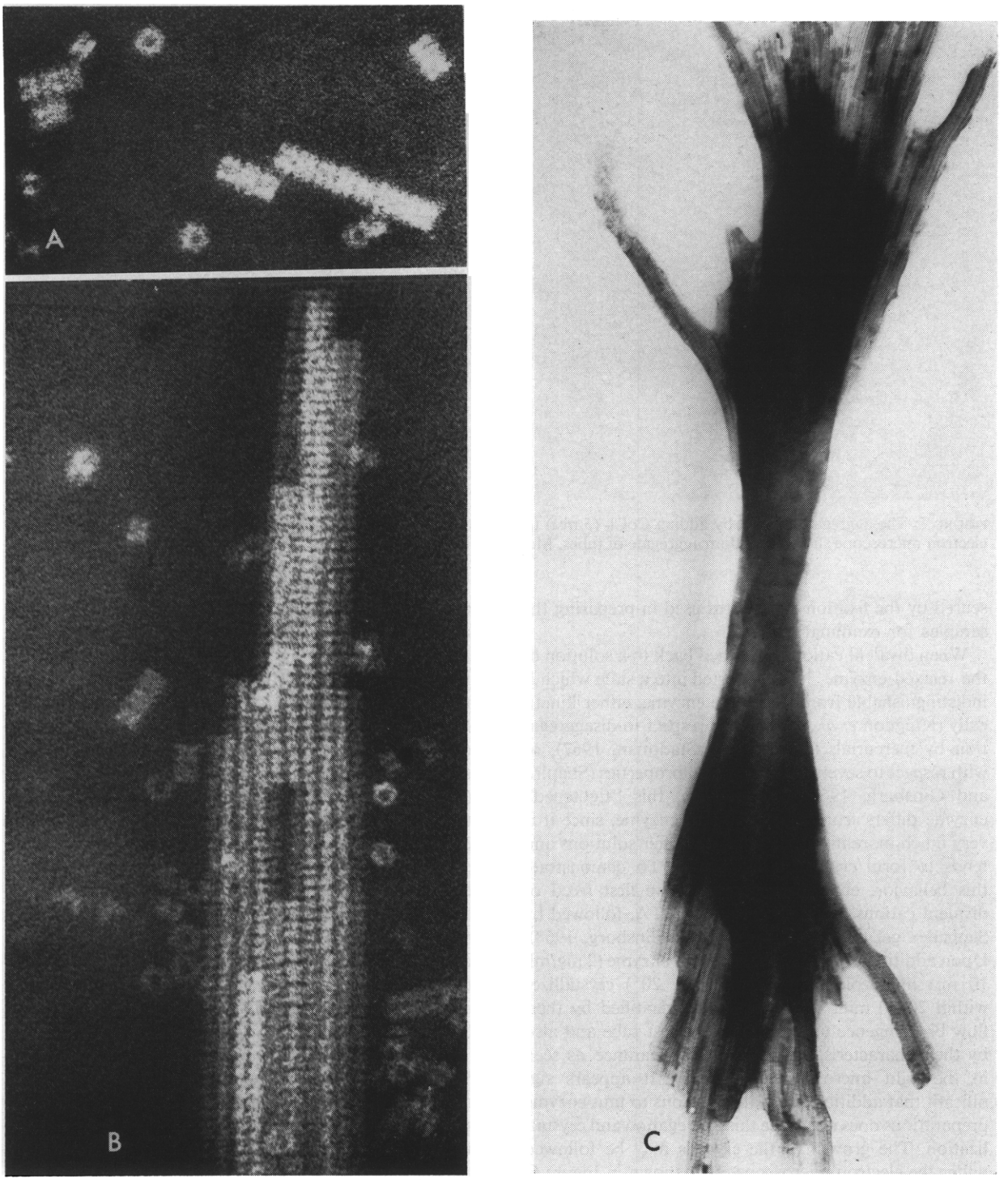

FIGURE 6: Stages in the formation of wheat-sheaf crystals after adding $MnCl_2$ (5 mM) to relaxed enzyme (in 0.01 M imidazole, pH 7.0. (A) After 5 min many of the molecules have stacked face to face to form tubes two to ten molecules long. Magnification × 390,000. (B) After 30 min the tubes have lengthened and aligned themselves side by side in ordered bundles. Magnification × 390,000. (C) The complete crystal (compare with the light microscope picture, Figure 5). The bundles of tubes have aggregated but the electron microscope shows that the wheat sheaf is not a true crystal ordered in three dimensions. Magnification × 71,000.

duced by removal of divalent cations) has the same molecular weight as the native, taut form, but has a lower sedimentation velocity and a higher intrinsic viscosity, (Shapiro and Ginsburg, 1968) it was assumed that it is also a dodecameric aggregate, but is more asymmetric than the native enzyme. Even with precise measurements, no difference in the shapes of relaxed and taut enzyme molecules could be detected (Figure 4). Therefore, the differences in structure between these forms, as evinced by slight differences in their sedimentation and viscosity behavior, are either too subtle to be detected by electron microscopy or they are ob-

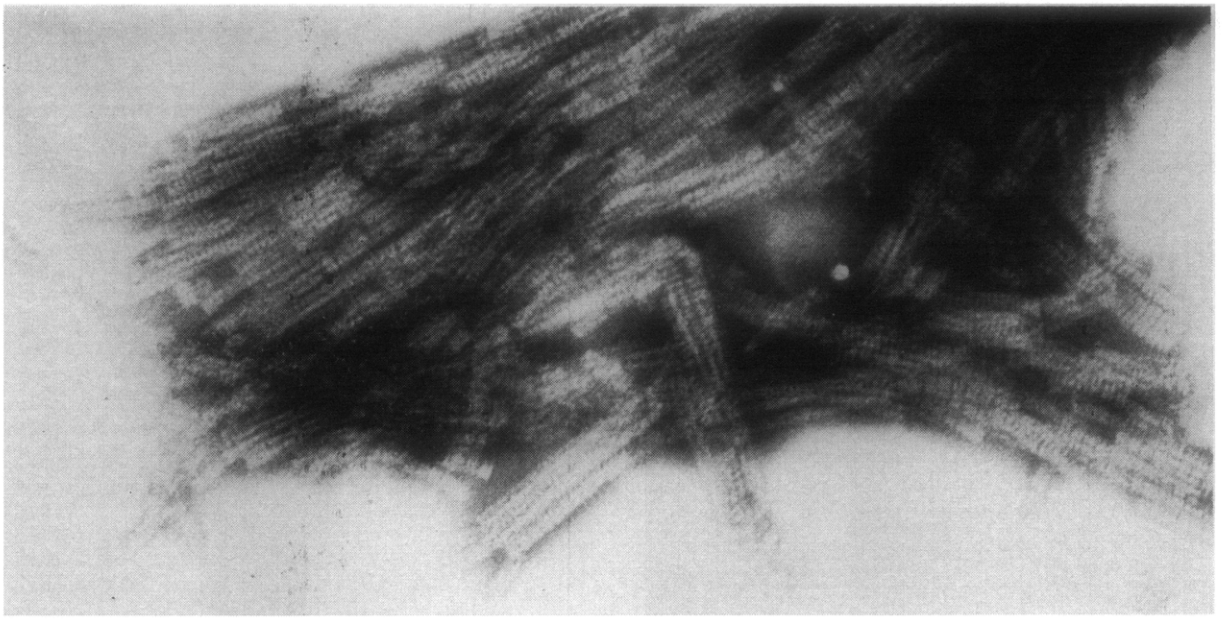

FIGURE 7: The aggregate formed by adding $CoCl_2$ (5 mM) to relaxed enzyme. The wheat-sheaf appearance is not found and the electron microscope shows a random aggregate of tubes. Magnification × 210,000.

scured by the fixation procedures used in preparing the samples for examination.

When divalent cations are added back to a solution of the relaxed enzyme, it is converted into a state which is indistinguishable from the native enzyme, either kinetically (Kingdon *et al.*, 1968), with respect to disaggregation by mercurials (Shapiro and Stadtman, 1967), or with respect to several of its physical properties (Shapiro and Ginsburg, 1968). Nevertheless, this "tightened" enzyme differs from the native taut enzyme, since it is very much more insoluble in dilute buffer solutions and tends to form crystalline aggregates. To demonstrate this behavior, enzyme preparations are first freed of divalent cations by treatment with EDTA, followed by Sephadex gel filtration (Shapiro and Ginsburg, 1968). Upon addition of $MnCl_2$, the resultant enzyme (2 mg/ml, 10 mM imidazole chloride, pH 7.0, 20°) crystallizes within 20–30 min. The crystals are identified by their flow birefringence upon shaking the test tube and also by their characteristic wheat-sheaf appearance as seen in the light microscope (Figure 5). It appears significant that addition of divalent cations to taut enzyme preparations does not cause this aggregation and crystallization. The growth of the crystals may be followed under the electron microscope, as is shown in Figure 6. Figure 6A shows the relaxed enzyme 5 min after the addition of $MnCl_2$ (final concentration 10 mM). Growing aggregates of double hexagons are seen, all lying in side view and forming tubes composed of two to ten glutamine synthetase molecules.

The nascent tubes always contain an even number of layers showing that the basic unit is the two-layered molecule. The alternate spaces between the layers of neighboring molecules are distinctly clearer than the spaces between layers belonging to the same molecule. This confirms the dihedral nature of the molecule; *i.e.*, its two component layers join face to face so that top and bottom surfaces are identical. Figure 6B was taken after 30-min aggregation, when the tubes of glutamine synthetase had begun to wrap around one another, forming cylindrical bundles of the linear hexagonal tubes; each of these bundles is 15–20 tubes in diameter. Finally, in Figure 6C is shown an electron micrograph of the completed manganese-induced crystal; this is similar in appearance to the phase-contrast micrograph (Figure 5). It is of some interest that 0.1 M NaCl solutions, as well as solutions of inhibitors and substrates of glutamine synthetase, cause dissolution of these crystal forms. Crystallization of previously relaxed enzyme was also brought about when magnesium or cobalt ions were added to the relaxed enzyme. Mg^{2+} addition led to a paracrystalline array similar to that observed with Mn^{2+}. However, as is shown in Figure 7, the addition of Co^{2+} results in a more random array of tubular aggregates which exhibit flow birefringence, but which do not have the wheat-sheaf form.

Reversible Dissociation of the Enzyme. Woolfolk and Stadtman (1967b) have demonstrated that dissociation of *E. coli* glutamine synthetase to catalytically inactive subunits is effected by treating the enzyme at pH 8.0 with 1.0–1.6 M urea and 0.01 M EDTA. Presumably the enzyme passes through the relaxed state mentioned above, and then breaks down to subunits. With the addition of excess Mn^{2+} and lowering of the pH (7.0–7.7), it was shown that the subunits reassociate to form aggregates similar in size to that of the original enzyme. This reaggregation is accompanied by a transient restoration of catalytic activity. Although these results suggest that dissociation of the subunits is reversible, the fact that the restoration of catalytic activity is only transient indicates that the structure of the reaggregated enzyme molecule is not the same as that of the original undissociated enzyme. This conclusion is supported by direct electron microscopic examination of

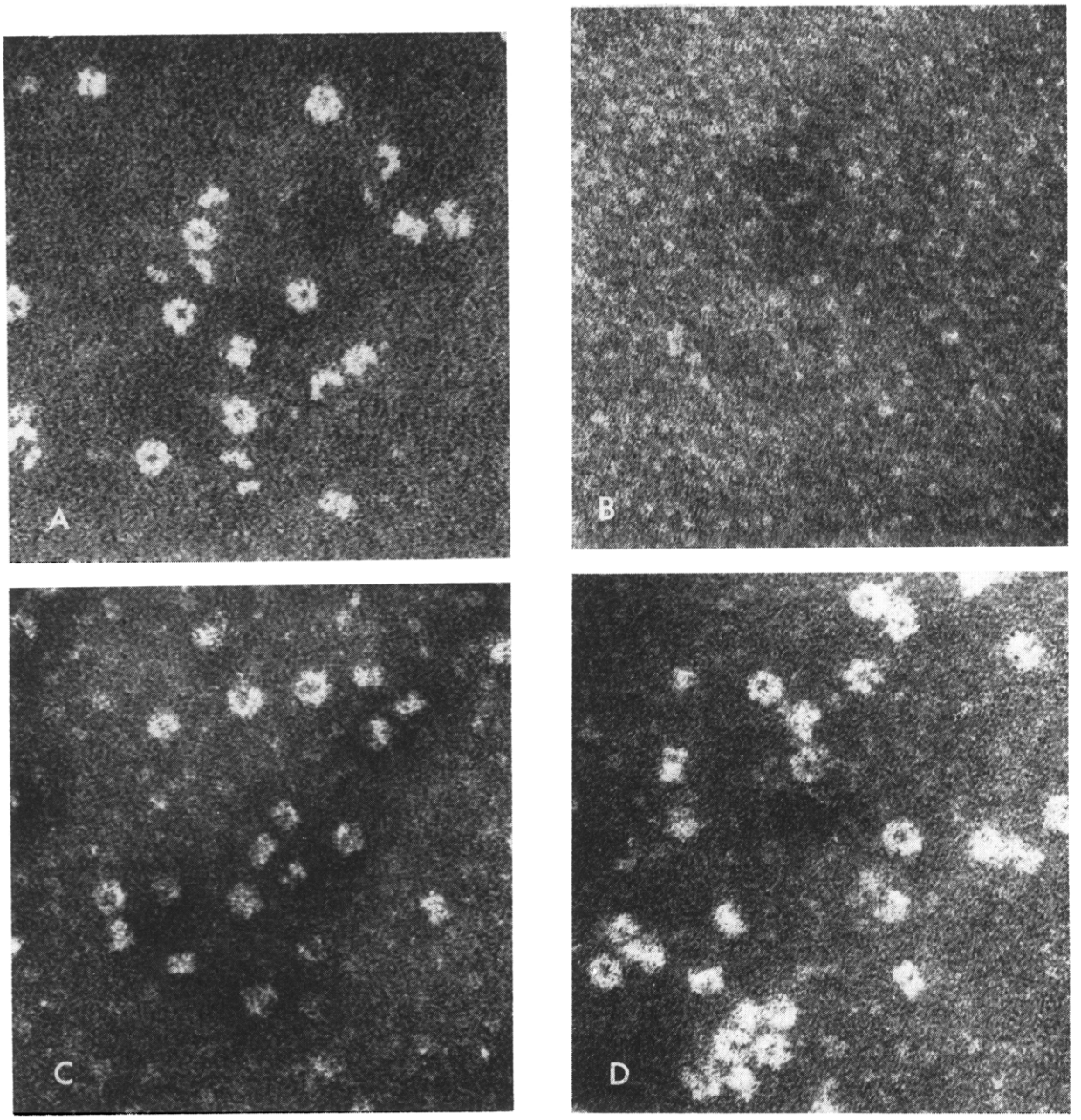

FIGURE 8: The breakdown and reassembly of glutamine synthetase molecules. (A) After 20 min 0.01 M EDTA, 1.0 M urea, and 0.1 M Tris pH 7.90) at 0°. There is a heterogeneous appearance of dissassociating subunits. (B) After 3 hr the molecules have completely dissassociated and only subunits (apparently themselves partly unwound) are seen. (C) The preparation shown in B after 10-min reactivation by enzyme with 5 mM $MnCl_2$ and 0.05 M imidazole (pH 7.0) at 0°. (D) After 30-min reactivation. Each magnification × 410,000.

the dissociation–reassociation phenomena. Figure 8A is an electron micrograph taken shortly after disaggregation of the enzyme was initiated by the addition of 1.0 M urea and 0.01 M EDTA. The picture shows a heterogeneous population of molecular subaggregates, along with some intact molecules. The dissociation does not seem to follow any rigid pattern, but there is apparently a tendency for breaks to occur between units in the same layer before breaks in the bonds that join the layers together. Figure 8B shows the same reaction mixture of 3 hr after the addition of the urea–EDTA mixture. Dissociation of the subunits is now complete. No intact structures are apparent. The dissociated subunits appear as ill-defined particles on the granular background. The lack of isolated well-defined ellipsoidal-shaped particles that are characteristic of subunits in the intact molecule suggests that once they are dissociated the subunits may undergo significant changes in shape. Figure 8C is an electron micrograph taken 10 min after the addition of excess Mn^{2+} to the completely dissociated subunits. It is evident that Mn^{2+} causes reaggregation of the subunits to molecular forms that resemble the native undissociated enzyme. However, whereas the double-hexagon structure is clearly

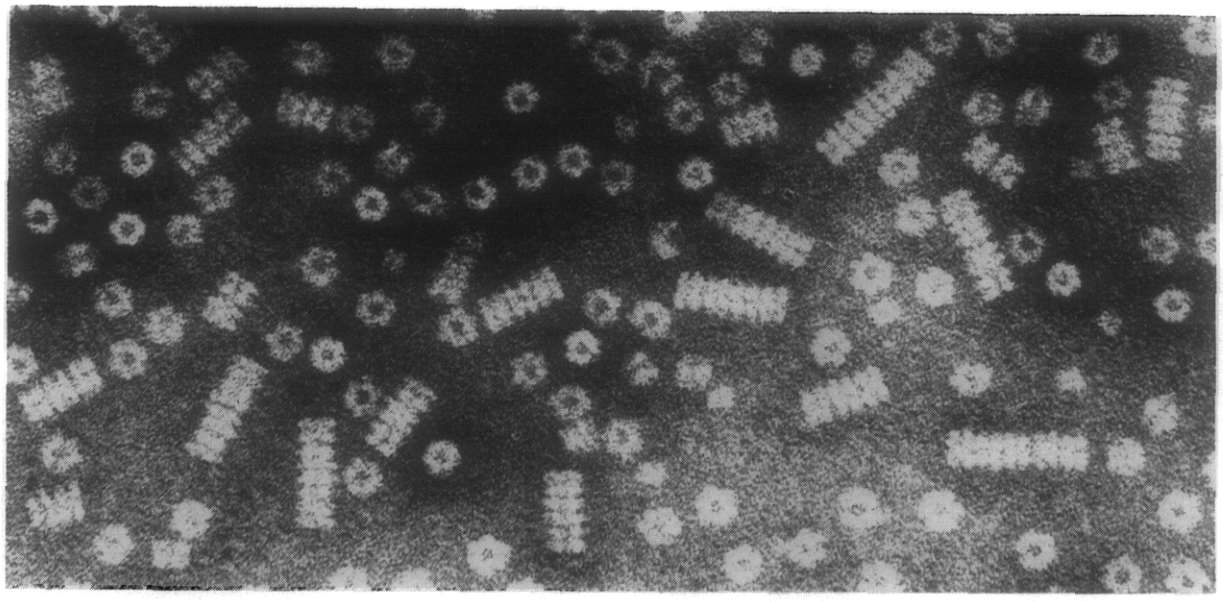

FIGURE 9: Glutamine synthetase molecules containing only 1.2 moles of covalently bound AMP/mole of enzyme. This preparation was treated identically with that examined in Figure 2. The enzyme preparations differed only in their extent of adenylylation (see text).

apparent, the reconstituted aggregates are less well defined than that of the original taut enzyme. The electron micrograph shown in Figure 8C was taken at a time when catalytic activity of the reconstituted enzyme should be maximal (Woolfolk and Stadtman, 1967b). With further incubation under these conditions there is a rapid loss in catalytic activity. This loss in activity appears to be associated with physical instability of the reconstituted enzyme since a picture taken 30 min after the addition of Mn^{2+} (Figure 8D) shows that the enzyme is now more distorted and the molecules have undergone loose aggregation to produce nondescript masses of higher weight.

Mercurial Inactivation of Relaxed Glutamine Synthetase. Previous studies (Shapiro and Stadtman, 1967) have shown that the relaxed form of glutamine synthetase is susceptible to disaggregation by organic mercurials at an alkaline pH. Sucrose density gradient examination of the disaggregated enzyme demonstrated that it existed as a heterogeneous population of protein fragments. Although not illustrated here, electron microscopic examination of organic mercurial disaggregated enzyme also showed the presence of species of intermediate stages of disaggregation between intact dodecamer and monomer, confirming our previous results.

Interaction of Glutamine Synthetase with Its Metabolic Effectors. As noted earlier, glutamine synthetase is subject to feedback inhibition by eight different end products of glutamine metabolism (Woolfolk and Stadtman, 1967a). The binding of these inhibitors must therefore cause conformational changes, albeit perhaps subtle ones, that affect the substrate binding site of the enzyme. These effectors also induce conformational changes in the relaxed enzyme which are manifested by alterations in the reactivity of exposed sulfhydryl groups with organic mercurials (Shapiro and Stadtman, 1967). We therefore investigated the possibility that the conformational changes caused by these effectors involve modifications in the molecular geometry of the enzyme that could be detected by electron microscopy. No changes could be detected, either in the presence of various substrates or in the presence of inhibitors. In the presence of inhibitors a greater number of partially disaggregated molecules were evident, but the number was variable and unrelated to the extent of inhibition. With preparations of enzyme stained in the absence of glutaraldehyde and in the presence of concentrations of inhibitors that cause almost complete inhibition of the enzyme, about 50% of the molecules were broken; the remaining intact molecules were indistinguishable from the molecules shown in Figure 1. These results suggest that the inhibitors may alter the strength of the intersubunit binding mechanisms, allowing the staining procedure to break the molecules more readily; however the direct changes brought by inhibitors are too subtle to be seen under the electron microscope.

Adenylylated Forms of Glutamine Synthetase. After the experiments reported above were completed, it was found that purified preparations of *E. coli* glutamine synthetase contain different amounts of covalently bound AMP, depending upon the conditions of growth of the organism; moreover the divalent cation specificity and inhibitor response of the enzyme are dramatically modified by the state of adenylylation (Shapiro *et al.*, 1967; Kingdon *et al.*, 1967). The data presented above were obtained with enzyme preparations possessing 9.0 equiv of covalently bound AMP/mole. Figure 9 shows an electron micrograph of another enzyme preparation that contained only 1.2 equiv of AMP/mole. It is evident that the basic molecular architecture of this poorly adenylylated preparation is identical with that of the enzyme with 9.0 equiv of AMP/mole (Figure 2). However, the enzyme with fewer covalently

bound AMP residues (Figure 9) has an increased tendency to form short tubular aggregates analogous to those of the tightened enzyme described above (Figure 6A). Since both enzyme preparations were isolated by identical purification procedures, they should both be in the native, taut state. It thus appears that the covalent attachment of AMP obscures some binding sites which may be active in intermolecular associations. It should be mentioned that the intrinsic viscosity of the poorly adenylylated enzyme was no higher than that of the more fully adenylylated one (Shapiro and Ginsburg, 1968), indicating that these tubular aggregates probably do not exist in solution, but occur as a result of the preparative procedure attendent to microscopy. This is in marked distinction to the divalent cation-induced aggregation of relaxed enzyme (Figures 5–7), which occurs in solution without relation to the microscopic fixation procedure.

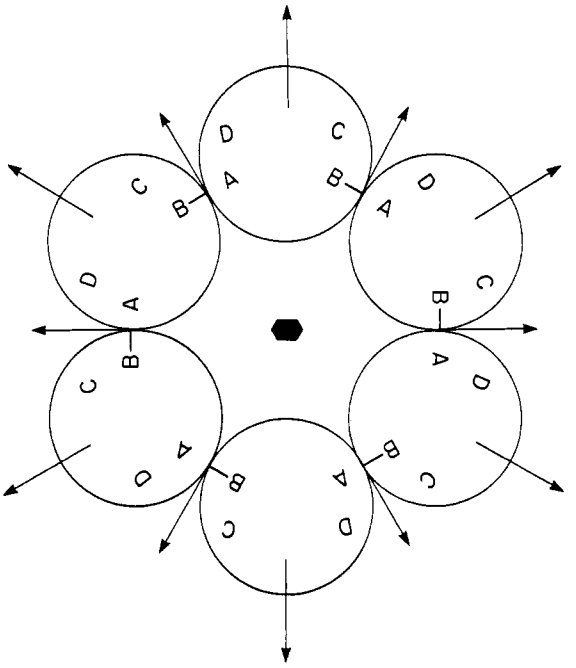

FIGURE 10: Geometry of the association of twelve identical subunits as in *E. coli* glutamine synthetase to form a molecule showing sixfold dihedral symmetry. The binding of the subunits to make a closed ring is dependent upon sites A and B, which participate in heterologous associations; their separation on any one unit is 120°, and consequently the resultant structure is a hexagon. C and D represent the isologous associations. A second hexagon inverted on top of the first will have all its C sites above and binding to D sites on the lower hexagon and similarly D sites bind to C sites; ○ indicates a sixfold axis perpendicular to the page; → indicates the twofold axes in a plane parallel to the page and halfway between the two hexagonal layers.

Discussion

The twelve subunits of the *E. coli* glutamine synthetase are arranged in a double hexagon with one sixfold axis of rotational symmetry and six twofold axes. The diagram of this dihedral type of symmetry in Figure 10 illustrates the fashion in which identical subunits with equivalent packing would be expected to interact. In the terminology defined by Monod *et al.* (1965), each hexagonal ring is formed by heterologous associations between the subunits, and the two hexagons are held together by isologous associations, which prevent the native enzyme from polymerizing into long tubes. Symmetry considerations do not preclude the rotation of one hexagon relative to the other about the sixfold axis, but the pictures suggest that one hexagon in fact lies directly above the other. The fact that manganese and cobalt added to relaxed enzyme can lead to long tubes indicates that in the relaxed enzyme the conformation which confers the symmetry responsible for the formation of a closed system is altered. New potential binding sites form on the outer regions of the double hexagons, and these permit a second type of isologous association between different glutamine synthetase molecules to make linear polymers. All of the polymers observed had even numbers of single hexagons, indicating that the isologous attachments of the two rings composing each molecule had not been altered by relaxation or subsequent "tightening." The aggregate produced when relaxed enzyme is mixed with manganese chloride is markedly less random than that obtained with cobalt chloride, although in both cases the glutamine synthetase molecules polymerize to form hexagonal tubes. This linear association of molecules is similar to that reported to occur with dihydrolipoyltransacetylase (Willms *et al.*, 1967).

The finding of intermediate states of disaggregation of glutamine synthetase when it is treated with organic mercurial at an alkaline pH was in accord with previous chemical data (Shapiro and Stadtman, 1967). Electron microscopic examination of enzyme disaggregated by treatment with EDTA and urea as was previously deduced from hydrodynamic data (Woolfolk and Stadtman, 1967b) clearly illustrated that disaggregation proceeds all the way to subunits. It is further evident that reaggregation and return of activity involves the reformation of the characteristic double-layered hexagon structure. One cannot say from these experiments, however, that the hexagonal molecules are the only active species, for smaller aggregates also present may have catalytic activity. Previous studies showing that the return of activity accompanying reassociation of subunits in a transient phenomenon, and that the subsequent loss is associated with formation of higher molecular weight aggregates (Woolfolk and Stadtman, 1967b) is also supported by direct electron microscopic examination (Figure 8D) which shows that random associations of protein fragments occur after the preliminary formation of the double-hexagon structure.

The fact that no changes in the structure of glutamine synthetase in the presence of any of its effectors, either substrates or inhibitors, could be detected, indicates that the effector-induced conformational changes are too subtle to be detected by the electron microscope. However, an alternate explanation is that the fixing and staining procedures obscure small modifications of molecular architecture.

Acknowledgments

The authors are grateful to Dr. H. S. Kingdon for making the preparation of glutamine synthetase containing 1.2 equiv of covalently bound AMP/mole available to us.

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