Stability of Quaternary Structure and Mode of Dissociation of Fructosediphosphate Aldolase Isoenzymes†

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ABSTRACT: Using a highly sensitive "subunit exchange" assay, we have studied the relative strengths of interactions between different subunit types (A and C) of fructosediphosphate aldolase and have determined the mode of dissociation of aldolase tetramers in vitro. Interactions between C subunits within C4 tetramers were found to be considerably more resistant to disruption than were interactions between A subunits in A4 tetramers with regard to increasing concentrations of H^+, OH^-, or urea. Slight dissociation of A4 was also observed in 1.2 M magnesium chloride. These observations suggest that the quaternary structure of aldolase C4 is inherently more stable than that of aldolase A4. Also, the symmetrical heterotetramer A2C2 was found to be more resistant to urea-mediated dissociation than was the aldolase A4 homotetramer; this observation suggests that, even when in heteromeric combination, C subunits have a stabilizing influence on the quaternary structure of aldolase tetramers. In no case did we find evidence for a stable dimeric intermediate in the dissociation of aldolase tetramers to monomers. These observations are considered in terms of the tetrahedral arrangement of subunits in the aldolase tetramer. The general applicability of the subunit exchange assay described here for studying the subunit structure and mode of dissociation of oligomeric enzymes is discussed.

Most intracellular enzymes are oligomeric molecules. The highly specific interactions which occur between subunits within the oligomer may be necessary for the biological function of these proteins. These highly specific interactions are also necessary to ensure that newly synthesized monomeric protein subunits, upon release from the ribosome, can selectively seek one another out during biogenesis of the oligomeric species.

We have been using the isoenzymes of fructosediphosphate aldolase as model systems to investigate the synthesis, assembly, and intracellular degradation of oligomeric enzymes. These aldolases are tetrameric molecules (Kawahara & Tanford, 1966; Penhoet et al., 1967), and in vertebrate organisms, three homologous aldolase structural genes are known to code for three different subunit types of the enzyme (Penhoet et al., 1969; Lebherz & Rutter, 1969). These subunits, termed A, B, and C, can interact with each other to form heterotetramers in vivo and in vitro (Penhoet et al., 1967; Penhoet & Rutter, 1971; Lebherz & Rutter, 1969; Lebherz, 1975a,b). Using a highly sensitive "subunit exchange" assay, we previously gave evidence that aldolase tetramers do not dissociate under conditions which do not result in extensive denaturation of these proteins (Lebherz, 1972). Additionally, we gave evidence (Lebherz, 1975c) that subunit exchange between aldolase tetramers probably does not occur in vivo. These observations lead us to suggest that, in vivo, aldolase tetramers are constructed only during the initial assembly of newly synthesized subunits and, therefore, that all four subunits of a given aldolase tetramer may be considered to be degraded in unison (Lebherz, 1975c; Lebherz & Shackelford, 1979).

In our original subunit exchange assay, a mixture of two different aldolase homotetramers, with different electrophoretic mobilities, or a single defined heterotetramer, was treated under selected conditions. After neutralization, detection of any "new" isoenzymes in the samples would have provided unequivocal evidence that dissociation of the tetrameric species had occurred (Lebherz, 1972). It should be noted that observations obtained in subunit exchange experiments may be less prone to misinterpretation than those obtained in ultracentrifugation experiments; unlike ultracentrifugation methods, accurate determinations of solvent densities, partial specific volumes, and frictional coefficients of proteins, and/or other physicochemical parameters, need not be considered. However, our original subunit exchange assay had two inherent major drawbacks. (1) The assay could detect dissociation of homotetramers only if both isoenzymes in the incubation mixture were dissociated since the detection of dissociation depended on the formation of new isoenzymes from the original ones. (2) Because subunit exchange is a rapid, time-dependent process, our original assay could not follow the mode of tetramer dissociation nor could it be used to follow the kinetics of tetramer dissociation and/or re-formation. We have now modified our original assay so that these drawbacks no longer exist.

In the present work, we used the modified subunit exchange assay to compare the stabilities of the quaternary structure of the aldolase homotetramers A4 and C4, as well as that of the symmetrical aldolase A2C2 heterotetramer.[1] We also have determined the mode of dissociation of aldolase tetramers in vitro and give evidence that aldolase tetramers dissociate to monomers without passing through a stable dimeric intermediate. We discuss the applicability of this subunit exchange assay for investigating the subunit structure and mode of dissociation of oligomeric enzymes which, in contrast to the aldolase system, do not naturally exist in multiple, electrophoretically distinct forms.

Materials and Methods

Isolation of Aldolases A4 and C4. Rabbit and chicken skeletal muscles contain only the A4 isoenzyme of aldolase (Lebherz & Rutter, 1969), and these molecules were isolated from muscle by "affinity elution" from phosphocellulose columns as previously described (Petell et al., 1981, 1982). The C4 isoenzyme comprises about 80% of the total aldolase

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[1] A4, C4, A2C2, etc. should have been printed A_4, C_4, A_2C_2, etc. throughout.

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population of chicken brain (Lebherz, 1975a), and this aldolase was isolated from frozen brains (purchased from Pelfreeze Co.) by affinity elution from phosphocellulose followed by ion-exchange chromatography as previously described (Lebherz, 1975a). These aldolase preparations were judged to be greater than 97% pure on the basis of electrophoretic analysis in sodium dodecyl sulfate–polyacrylamide slab gels.

Formation and Isolation of Chicken Aldolase A2C2. Chicken aldolase A2C2 was artificially created by reversible acid dissociation of an equimolar mixture of pure chicken aldolases A4 and C4. A solution containing 12.5 mg of each homotetramer in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 1 mM ethylenediaminetetraacetic acid (EDTA), and 50 mM 2-mercaptoethanol, pH 7.3 (50 mL total volume), was adjusted to pH 2.3 at 0 °C by the addition of 3 mL of 1 M citric acid. After the solution was stirred for 10 min in the cold, reassociation of aldolase tetramers was effected by the dropwise addition of the dissociated enzyme to 450 mL of 100 mM Tris-HCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 8.3, with constant stirring at room temperature. Greater than 80% of the initial activity was recovered. After the reaction mixture was stirred for 30 min at room temperature, the sample was concentrated to about 40 mL by ultrafiltration through an Amicon UM 20 membrane. Then the sample was dialyzed against 5 mM Tris-HCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 8, and aldolase A2C2 was resolved from the other isoenzymes by NaCl gradient elution from DEAE-cellulose essentially as previously described (Lebherz, 1975a). Column fractions containing only aldolase A2C2 were identified by cellulose polyacetate strip electrophoresis as described below. These fractions were pooled; the pool was concentrated by ultrafiltration, and the sample was dialyzed against 10 mM Tris-HCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 7.0.

Aldolase Activity Measurements and Protein Determinations. Aldolase activity measurements were performed at 25 °C by using a previously described continuous spectrophotometric procedure (Lebherz & Rutter, 1975). Activities were expressed as micromoles of fructose diphosphate cleaved to triose phosphates per minute, and specific activities were expressed as units per milligram of protein. Protein concentrations of pure enzyme preparations were estimated by A_{280} measurements assuming an extinction coefficient of 0.9 (Baranowski & Neiderland, 1949) for all aldolase isoenzymes.

Electrophoretic Procedures. Electrophoresis of aldolase isoenzymes in 9% polyacrylamide slab gels in the presence of 0.1% sodium dodecyl sulfate was performed by using the gel reagents and buffer system suggested by Laemmli (1970). Afterward, gels were stained for protein with Coomassie blue. Cellulose polyacetate strip electrophoresis followed by staining the strips for aldolase activity was performed as described by Susor et al. (1975). See appropriate figure legends for additional methods or procedures.

Results

Characteristics of the Subunit Exchange Assay. The new feature of our modified subunit exchange assay involves treating a given aldolase homotetramer under a particular condition and then rapidly combining this solution with a solution of fully dissociated subunits of a different type, under conditions which highly favor tetramer formation. The sole purpose of the fully dissociated subunits is to "trap" dissociated species of the isoenzyme in the test solution. Note that if the test condition causes dissociation of aldolase tetramers to monomers, a five-membered set of isoenzymes, in the form of X4, X3Y, X2Y2, XY3, and Y4, would be produced upon neutralization; however, if the test condition causes dissociation of tetramers to dimers instead, then only a three-membered set of isoenzymes, in the form of X4, X2Y2, and Y4, would be produced. Also, the extent of dissociation of tetramers under a particular test condition can be estimated by comparing the amounts of homo- and heterotetramers produced in the subunit exchange experiment with those amounts theoretically expected for the random combination of the two different subunit types into tetramers. Finally, the time course for tetramer dissociation and/or re-formation can be assessed by mixing the two isoenzyme solutions at different times after initiation of dissociation or reassembly of aldolase tetramers. For example, using this approach, we showed that complete assembly of acid-dissociated aldolase subunits into tetramers was completed within 10 min after neutralization (data not shown).

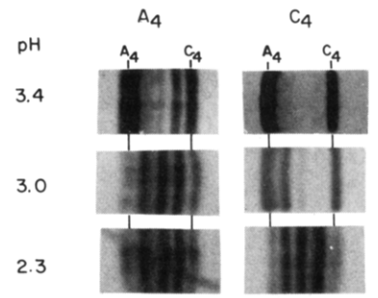

FIGURE 1: Dissociation of aldolases A4 and C4 by H^+. Solutions of chicken aldolases A4 (left panel) and C4 (right panel) at protein concentrations of 0.5 mg/mL in 10 mM Tris-HCl, 1 mM EDTA, and 50 mM 2-mercaptoethanol, pH 7.3, were titrated to the indicated pH with 1 M citric acid at 0 °C in plastic tubes. After 30 min on ice, these solutions were rapidly added to 9 volumes of 100 mM Tris-HCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 8, simultaneously with fully dissociated C or A subunits (previously titrated to pH 2.3) at room temperature. After being stirred for 30 min, these solutions were concentrated about 10-fold by ultrafiltration with Amicon PM 30 membranes. The isoenzymes present in the neutralized solutions were detected by cellulose polyacetate strip electrophoresis followed by staining of the strips for aldolase activity. In all cases, 60% or more of the initial enzymatic activity was recovered.

Stability of Tetrameric Structure and Mode of Dissociation of Aldolases A4 and C4. Using the modified subunit exchange assay described above, we investigated the relative stabilities of the quaternary structure of aldolases A4 and C4 homotetramers as well as the mode of dissociation of these isoenzymes. It is well-known that these aldolase tetramers are fully dissociated to monomers at pH 2 (Penhoet et al., 1967; Lebherz, 1972), and in the first series of experiments, we determined the effect of decreasing pH on the structures of chicken aldolases A4 and C4. Aldolase A4 was incubated in solutions of decreasing pH, and then an equal amount of fully dissociated aldolase C subunits (previously maintained at pH 2.3) was added under conditions which highly favor tetramer formation. As shown in the left side of Figure 1, aldolase A4 was found to be slightly dissociated at pH 3.4 as evidenced by the production of AC3 heterotetramers; extensive dissociation of this isoenzyme was observed at pH 3.0. The fact that AC3 and not A2C2 was the predominant heterotetramer produced at pH 3.4 suggests that an A2 dimer is not an important intermediate in the H^+-mediated conversion of A4 tetramers to monomers; if a stable population of A2 dimers had existed at this pH, a predominant A2C2 species would have been produced. The right side of Figure 1 shows results of a similar experiment conducted with chicken aldolase C4. In contrast to the A4 isoenzyme, no evidence for dissociation of C4 tetramers was observed at pH 3.4. At pH 3, some dissociation of aldolase C4 was evident. Again, the fact that

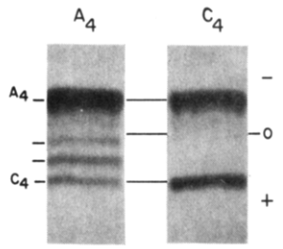

FIGURE 2: Base-mediated dissociation of aldolase A4. Solutions of chicken aldolases A4 (left) and C4 (right) at 0.5 mg/mL were titrated to pH 10 with 1 M Tris base. After 30 min, the solutions were neutralized in the presence of fully dissociated C or A subunits (previously titrated to pH 2.3) by rapid addition of the solutions to 9 volumes of 50 mM Tris-HCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 7.8 at room temperature. After concentration by ultrafiltration, the solutions were analyzed by electrophoresis on cellulose polyacetate strips. Greater than 65% of the initial enzymatic activities were recovered after neutralization.

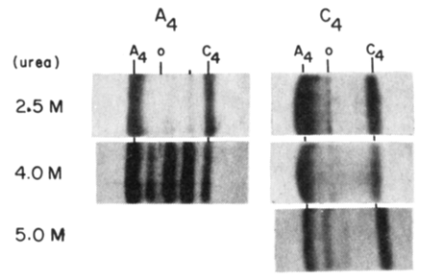

FIGURE 3: Urea-mediated dissociation of aldolases A4 and C4 at neutral pH. Solutions of chicken aldolases A4 and C4 were adjusted to the indicated concentrations of urea by addition of the appropriate volumes of 7.5 M urea. Final protein concentrations were 0.5 mg/mL. After incubation at room temperature for 30 min, the solutions were added to 9 volumes of the 100 mM Tris-HCl neutralization buffer described in the legend of Figure 1 along with fully dissociated C or A subunits. After concentration, the solutions were analyzed by electrophoresis. Greater than 70% of the initial catalytic activities were recovered.

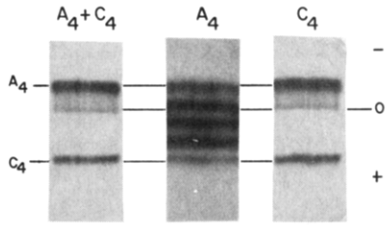

FIGURE 4: Dissociation of rabbit aldolase A4 in 1.5 M urea at pH 5. Samples of rabbit aldolase A4 and chicken aldolase C4 were dialyzed separately against 50 mM sodium acetate, 1 mM EDTA, 1 mM 2-mercaptoethanol, and 0.2 M NaCl, pH 5. Samples containing (left panel) both aldolase homotetramers, (middle panel) rabbit aldolase A4, and (right panel) chicken aldolase C4 were adjusted to 1.5 M urea with a 7.5 M urea stock solution prepared in the above sodium acetate buffer. Final protein concentrations were 0.5 mg/mL. After being stirred for 30 min at room temperature, the solutions were neutralized by dilution into the 100 mM Tris-HCl neutralization buffer described in the legend of Figure 1 either (1) in the absence of other aldolase subunits (left panel), (2) in the presence of fully dissociated chicken C subunits (middle panel), or (3) in the presence of fully dissociated rabbit A subunits (right panel). After concentration by ultrafiltration, the samples were subjected to electrophoresis as described under Materials and Methods.

the asymmetrical heterotetramer, A3C, and not A2C2, was the predominant new isoenzyme produced at this pH suggests that a C2 dimer is not an important intermediate in the H^+-mediated conversion of C4 tetramers to C subunits. Finally, as expected, extensive dissociation of aldolases A4 and C4 was observed at pH 2.3, as judged by the production of all possible heterotetramers composed of A and C subunits following treatment of the isoenzymes at this pH (Figure 1).

Aldolase A4 has a considerably higher isoelectric point than aldolase C4 (Penhoet et al., 1969). As such, the greater resistance of C4 tetramers to dissociation at low pH could simply reflect lower electrostatic repulsion between C subunits within C4 tetramers than the repulsion which exists between A subunits in A4 tetramers at acid pH. Consequently, we compared the effect of increasing pH on the structures of chicken aldolases A4 and C4. As shown in Figure 2, aldolase A4 was more susceptible to dissociation in base than was aldolase C4. That is, partial dissociation of aldolase A4 was observed at pH 10. In contrast, no detectable dissociation of aldolase C4 was observed at this pH. The fact that aldolase AC3, and not A2C2, was the predominant heterotetramer produced when A4, treated at pH 10, was added to fully dissociated C subunits suggests that an A2 dimer is not an important intermediate in the base-mediated dissociation of A4 tetramers to monomers.

The quaternary structure of aldolase C4 was also found to be more stable to increasing concentrations of urea. At neutral pH, detectable dissociation of aldolase A4 occurred at urea concentrations of 2.5 M or above while detectable dissociation of aldolase C4 was observed only at urea concentrations of 5 M (Figure 3). Also, as indicated by electrophoretic analysis, no evidence for a stable dimeric intermediate was observed for the urea-mediated conversion of either aldolase homotetramer to monomers (Figure 3).

Masters & Winzor (1971) have previously reported that rabbit muscle aldolase A4 dissociates in 1.5 M urea at pH 5, and we reinvestigated this question using our modified subunit exchange assay. As shown in the left panel of Figure 4, no heterotetramers were formed after treatment of a mixture of rabbit aldolase A4 and chicken aldolase C4 under these conditions. However, this observation was a reflection of the high stability of aldolase C4 quaternary structure, rather than lack of dissociation of the A4 isoenzyme. This contention is based on our observation that a five-membered A–C isoenzyme set was generated when fully dissociated chicken C subunits were added to rabbit aldolase A4 which had been treated with 1.5 M urea at pH 5 (Figure 4, middle panel). In contrast, no heterotetramers were produced when fully dissociated A subunits were added to aldolase C4 which was treated under the same condition (Figure 4, right panel); in fact, at pH 5, urea concentrations as high as 2.5 M were required to elicit detectable dissociation of C4 tetramers (data not shown).

Hsu & Neet (1973, 1975) and Saunders & Weber (1975) presented ultracentrifugation data which suggested that high concentrations of magnesium chloride cause dissociation of rabbit aldolase A4. We reinvestigated this question using the subunit exchange assay. Initially, we did not observe appreciable dissociation of rabbit aldolase A4 in solutions containing magnesium chloride concentrations as high as 2 M, even at protein concentrations as low as 0.2 mg/mL. It is important to note that, under these ionic and protein concentrations, the ultracentrifugation data presented by Hsu & Neet (1973) would predict that rabbit aldolase A4 should be highly dissociated. To investigate the apparent conflict between the ultracentrifugation data and results of the present subunit exchange experiments, we dialyzed our aldolase preparations for extended periods of time against 1.2 M $MgCl_2$, a procedure used by Hsu & Neet (1973) before they performed their ultracentrifugation analyses. Under these conditions, we did detect some dissociation of rabbit aldolase A4 at protein concentrations of 0.05 mg/mL (Figure 5, left panel); however, negligible dissociation of rabbit A4 occurred in 1.2 M $MgCl_2$ at protein concentrations of 0.5 mg/mL (Figure 5, right panel).

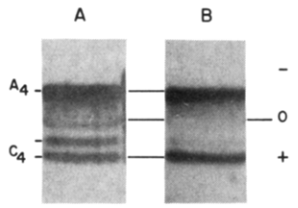

FIGURE 5: Effect of 1.2 M $MgCl_2$ on the quaternary structure of rabbit aldolase A4. Solutions of rabbit aldolase A4 at protein concentrations of 0.05 and 0.5 mg/mL were dialyzed against 0.2 M Tris-HCl and 1.2 M $MgCl_2$, pH 7.2 at room temperature, for 24 h. Then the solutions were diluted into 9 volumes of 100 mM Tris-HCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 7.7, along with fully dissociated chicken aldolase C subunits. After concentration by ultrafiltration, samples were analyzed by electrophoresis on cellulose polyacetate strips. At aldolase concentrations of both 0.05 and 0.5 mg/mL, essentially complete recovery of enzymatic activity was obtained after the magnesium chloride treatment.

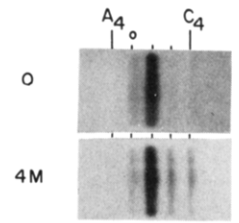

FIGURE 6: Mode of dissociation of chicken aldolase A2C2. A sample of aldolase A2C2 in 10 mM Tris-HCl, 1 mM EDTA, and 50 mM 2-mercaptoethanol, pH 7, was adjusted to 4 M urea by addition of an equal amount of an 8 M urea stock solution prepared in the above Tris buffer (final protein concentration 0.1 mg/mL). After incubation for 30 min at room temperature, the solution was added to 9 volumes of 50 mM Tris-HCl, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 7.6. After concentration by ultrafiltration, samples were analyzed by electrophoresis on cellulose polyacetate strips. Final recovery of aldolase activity after the urea treatment was greater than 65%. (Top panel) Control; (bottom panel) A2C2 treated with 4 M urea.

Also, the fact that aldolase AC3, and not A2C2, was the predominant heterotetramer produced at the lower protein concentration suggests that a stable dimer is not an important intermediate in the magnesium chloride mediated dissociation of aldolase A4 to individual A subunits.

Mode of Dissociation of Aldolase A2C2. The fact that interactions between C subunits in C4 tetramers are considerably stronger than those interactions which exist between A subunits in A4 tetramers (see above) allowed us to further investigate the premise that dimers are not important intermediates in the dissociation of aldolase tetramers to monomers. This test involved subjecting the symmetrical A2C2 heterotetramer to conditions which cause appreciable dissociation of aldolase A4 but which leave aldolase C4 tetramers intact. If aldolase A2C2 dissociates to 2A subunits plus a C2 dimer under these conditions, then a three-membered set of isoenzymes, in the form of A4, A2C2, and C4, should be produced. In contrast, if aldolase A2C2 dissociates to 2A and 2C subunits under these conditions, then asymmetrical members of the A–C set should be generated. Only slight dissociation of the A2C2 tetramer was observed at a urea concentration of 4 M (Figure 6), in contrast to the extensive dissociation of aldolase A4 under these conditions (see Figure 3). This observation suggests that the interactions which exist between aldolase subunits in A2C2 heterotetramers are stronger than those interactions which exist between A subunits in the A4 homotetramer. In fact, extensive dissociation of A2C2 heterotetramers was observed only at urea concentrations of 5 M (data not shown). More importantly, no evidence for the existence of a stable C2 dimer was observed when A2C2 was incubated in 4 M urea, as judged by the production of AC3 and the lack of production of A4 after incubation of this isoenzyme in 4M urea (Figure 6).

Discussion

The present work demonstrates the usefulness and reliability of a straightforward, highly sensitive subunit exchange assay for investigating the strengths of subunit–subunit interactions within oligomeric proteins and the modes of dissociation of oligomeric proteins. We used this subunit exchange assay to confirm a number of reports (based on ultracentrifugation data) that muscle aldolase dissociates under a variety of conditions in vitro. However, we were not able to confirm the reports by Hsu & Neet (1973, 1975) and Saunders & Weber (1975) that high concentrations of $MgCl_2$ cause extensive dissociation of muscle aldolase. The reason for this discrepancy between the interpretations based on subunit exchange and ultracentrifugation experiments is not known. However, as mentioned in the introduction, the subunit exchange assay, in contrast to ultracentrifugation analysis, does not require accurate measurements and/or estimates of physicochemical parameters or degrees of protein purity; erroneous views concerning the subunit structure and behavior of oligomeric proteins under certain conditions in vitro may result if the appropriate values of these parameters are not used in ultracentrifugation experiments. For example, interpretations based on some ultracentrifugation data (Stellwagen & Schachman, 1962; Deal et al., 1963) led to the suggestion that the aldolase oligomer was composed of three (not four) subunits and that aldolase oligomers dissociate at concentrations below 0.2 mg/mL (Kawahara & Tanford, 1966). However, data obtained in subunit exchange experiments (Penhoet et al., 1967; Lebherz, 1972) were instrumental in showing that neither of the above views was correct.

We demonstrated a considerably higher stability of the quaternary structure of aldolase C4, as compared to aldolase A4, to increasing concentrations of a number of agents (H^+, OH^-, and urea) in vitro. These observations suggest that subunit–subunit interactions between C subunits in C4 tetramers are inherently stronger than those interactions which exist between A subunits in A4 homotetramers. Further, we showed that the symmetrical heterotetramer aldolase A2C2 was less susceptible to urea-mediated dissociation than was the A4 homotetramer. Thus, the presence of C subunits appears to have a stabilizing influence on the interactions which exist between subunits within aldolase tetramers, even when these subunits are not all of the same type. These observations are of interest in view of the report by Anderson & Weber (1966) that interactions between lactate dehydrogenase subunits are stronger in the M4 and H4 homotetramers than are those interactions which exist between subunits within heterotetramers.

Our work further suggests that a stable dimer is not an important intermediate during the dissociation of aldolase tetramers to monomers under a variety of conditions in vitro. This conclusion is in agreement with the report of Engelhard et al. (1976) which was based on physicochemical data obtained with rabbit aldolase A4 treated with increasing concentrations of H^+. The lack of a dimeric intermediate in the dissociation of aldolase tetramers to monomers is of interest in terms of the proposed structure of aldolase tetramers. On the basis of both X-ray crystallographic analysis (Heidner et al., 1971; Engles et al., 1969) and observation in the electron microscope (Penhoet et al., 1967), the aldolase molecule appears to have 222 point-group symmetry with the four subunits arranged in a tetrahedral structure. Thus, in the tetramer, each aldolase subunit can interact with all other subunits.

Dissociation of tetramers to dimers would necessitate disruption of two of the three subunit–subunit interactions within the tetramer. It would not be difficult to envision that disruption of these interactions would render a dimeric structure of the enzyme highly unstable, resulting in rapid dissociation of such dimers to monomers. Our inability to detect a dimeric intermediate in dissociation of the aldolase A2C2 heterotetramer is also consistent with this idea. The lack of long-lived dimeric intermediates in dissociation of aldolase tetramers in vitro also implies that the biogenesis of aldolase tetramers in vivo may result from the rapid association of four newly synthesized subunits into a tetrameric structure, rather than resulting from an initial assembly of newly synthesized subunits into long-lived dimers, followed by association of these dimers into tetramers.

The subunit exchange assay described here should be applicable to studying the dissociation and reassembly of oligomeric proteins in general. Although it is useful if the enzyme exists in multiple, electrophoretically distinct forms, the existence of naturally occurring isoenzymes is not a prerequisite for these analyses. The electrophoretic variants to be used in the subunit exchange experiments may be artificially created by chemical modification of a single "native" enzyme species. For example, Meighen & Schachman (1970a,b) used this approach to study the subunit structures of aldolase and glyceraldehyde-3-phosphate dehydrogenase. It can be argued that the chemical modifications necessary to produce charge alterations within the native enzyme may affect the stability of the quaternary structure of the oligomer and, therefore, that data obtained in subunit exchange experiments utilizing chemically modified enzymes should be interpreted with caution. However, if the chemically modified enzyme subunits are used solely to "trap" dissociated species of the native enzyme, then the above caution can be ignored. In conclusion, the subunit exchange assay described here is an inexpensive, straightforward, and highly sensitive method for investigating the disassembly and reassembly of oligomeric proteins in vitro.

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Registry No. Fructosediphosphate aldolase, 9024-52-6.

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