MECHANISM OF DISSOCIATION OF PIGEON LIVER FATTY ACID SYNTHETASE COMPLEX INTO HALF-MOLECULAR WEIGHT SUBUNITS AND THEIR REASSOCIATION TO ENZYMATICALLY ACTIVE COMPLEX*

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SUMMARY

Pigeon liver fatty acid synthetase multienzyme complex has been quantitatively dissociated in low ionic strength glycine-tris buffer into two subunits of identical or nearly identical molecular weight. A total of 5.5 ± 1.0 readily titratable -SH groups/molecule are lost during the formation of the subunits. The dissociated enzyme is reconstituted to an active enzyme in high ionic strength buffer in the presence of dithiothreitol. The extent of reassociation is a function of the concentration of subunits. Under optimum conditions a complete recovery of enzyme activity is obtained.

The multienzyme fatty acid synthetase complexes of pigeon and rat liver consist of at least seven different functional protein units (1). These complexes have not been fractionated into individual proteins whereas systems obtained from bacteria (2) and plants (3) readily separate into individual enzymes. However, Butterworth et al. (4) showed that the pigeon liver complex can be dissociated into two subunits of nearly identical molecular weight. The process employed in these studies (4) entailed reaction of the sulfhydryl groups of the enzyme with carboxymethyl disulfide or potassium maleate. Treatment with carboxymethyl disulfide gave a mixture containing about 75% dissociated complex. These subunits could be partially recombined to yield active enzyme (4).

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We wish to report in this communication the complete dissociation of the pigeon liver fatty acid synthetase complex into subunits of nearly identical molecular weight in the presence of low ionic strength buffer and 2-mercaptoethanol. We also report the conditions required for the quantitative reassociation of these subunits into active complex, and the results of a preliminary investigation on the mechanism of the dissociation and reassociation processes.

MATERIALS AND METHODS

The fatty acid synthetase complex of pigeon liver was prepared according to Hsu et al. (5) and assayed for activity as described previously (1, 6). The purified enzyme was either used fresh or stored in 0.2 M potassium phosphate, pH 7.0, containing 1 mM EDTA and 10.0 mM DTT† at -12°. The stored enzyme was stable for at least a month, provided that it was incubated at 30° with 10 mM DTT just prior to use.

Dissociation of the complex (10-20 mg protein/ml in 0.2 M phosphate buffer, 1 mM EDTA and 10 mM DTT) was effected by dialysis of the enzyme against 250 ml of 35 mM glycine (Nutritional Biochemical Corporation), 5 mM Tris (Fisher Scientific), 1 mM EDTA (Matheson, Coleman and Bell), and 1 mM 2-mercaptoethanol (Eastman Organic Chemicals), pH 8.3, (glycine-tris buffer) for a period of 24-36 hours at 4° with three changes of buffer. The dissociated enzyme had an activity of less than 1 unit (a unit equals 1 mμmole of palmitic acid formed/min/mg of protein) whereas the intact complex had an activity of approximately 100.

Reassociation of the dissociated enzyme (10-20 mg/ml or less) was effected by dialysis against a 125 ml solution of 0.2 M potassium phosphate,

† DTT - Dithiothreitol.

pH 7.0, containing 1.0 mM EDTA and 10 mM DTT for a period of 6-8 hours, with a buffer change at 3 hours. Sedimentation velocity analysis was carried out with a Spinco Model E analytical ultracentrifuge equipped with Schlieren optics.

RESULTS AND DISCUSSION

Sedimentation velocity experiments (Fig. 1) demonstrate that treatment of the complex (Fig. 1A, $s_{20,w} = 14$ S) with glycine-tris buffer, pH 8.3 ($\mu = 0.008$) containing 2-mercaptoethanol results in the slow dissociation of the complex into species with an $s_{20,w}$ of approximately 8.5 S (Fig. 1C). The meniscus depletion sedimentation equilibrium method of Yphantis (7) yielded a molecular weight value of 230,000 for the dissociated enzyme*, which is approximately half the value reported for the complex (8). The ultracentrifuge pattern in Fig. 1B shows the relative proportion of the two enzyme species when 58% of the activity for fatty acid synthesis was lost. Thus, the loss of enzymatic activity of the complex corresponds quantitatively to the appearance of half-molecular weight subunits. Similar results were obtained at various stages of dissociation. The completely reassociated enzyme appears as a single species with an $s_{20,w}$ value of 14 S (Fig. 1D).

The dissociation and inactivation of the fatty acid synthetase complex in glycine-tris buffer appears to occur because of an inherent instability of the complex in low ionic strength buffer. However, Wakil et al. (9) have proposed that phosphate ions may be required for the maintenance of a proper orientation of the active sites of the component enzymes of the complex and hence are essential for optimum enzyme activity. If so, the replacement

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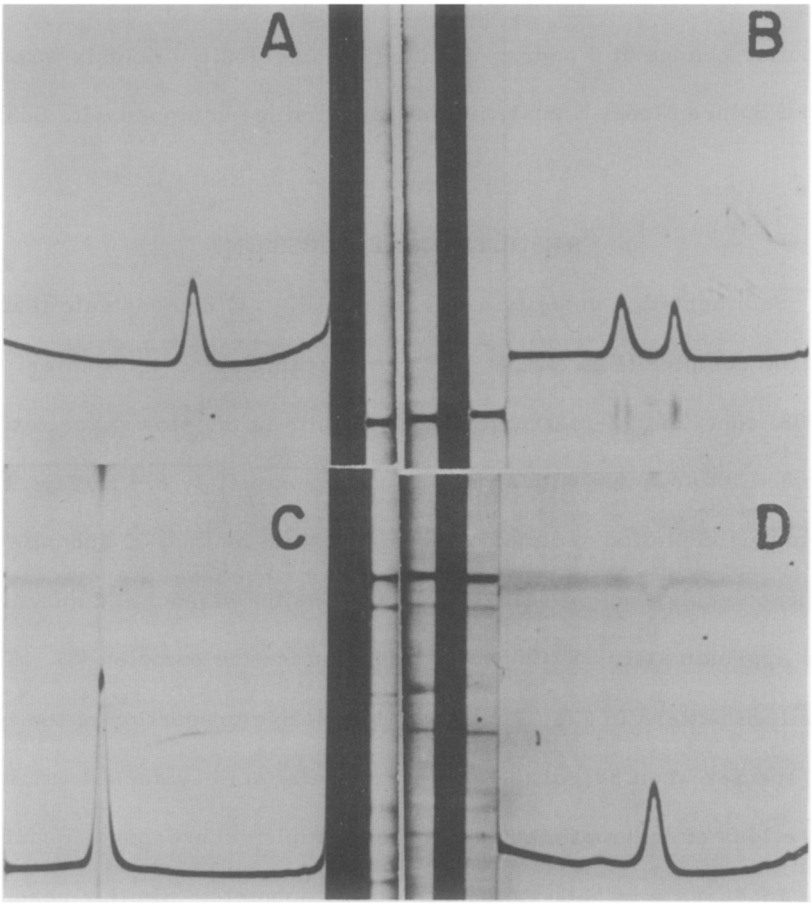

Fig. 1. Sedimentation velocity patterns of (A) fatty acid synthetase complex (5.0 mg protein/ml) in 0.2 M phosphate, pH 7.0, containing 1 mM EDTA and 1 mM DTT, (B) partially dissociated enzyme (5 mg protein/ml; 42% of the original activity for fatty acid synthesis) in glycine-tris buffer, (C) dissociated enzyme (7 mg protein/ml; no enzymatic activity) in glycine-tris buffer, and (D) reassociated enzyme (5 mg protein/ml). Centrifugation was carried out in Kel-F cells at 59,780 rpm. The rotor temperature was maintained at 20° and the bar angle was set to 70°. Photographs were taken approximately 32 min after reaching full rotor speed. Activity was measured at 30° in 0.2 M potassium phosphate, pH 7.0, containing 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.033 mM acetyl-CoA, 0.1 mM malonyl-CoA, 0.1 mM NADPH and 5-10 μg of enzyme protein in a total volume of 1.0 ml. The initial rate of NADPH oxidation was followed at 340 nm.

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of phosphate by other ions (glycine) would result in a progressive inactivation and simultaneous dissociation of the complex. Under such conditions

Table I

Recovery of Activity for Fatty Acid Synthesis as a Function of the Concentration of Subunits[a]

Reassociation conditions	Protein concentration (mg/ml)	Enzyme activity (% of control)
0.2 M phosphate[b]		
No DTT	5.0	< 1
10 mM DTT	0.1	40
	1.14	68
	3.8	82
	9.5	97

[a] Enzyme was dissociated as described in the text. In control experiments the complex was carried through every stage of the dissociation procedure in 0.2 M phosphate, 1 mM EDTA and 1 mM 2-mercaptoethanol instead of glycine-tris buffer. Activities for fatty acid synthesis were measured as described in the legend to Fig. 1.

[b] Enzyme was dialyzed against 0 or 10 mM DTT for 7-8 hours. No further increase in enzyme activity was observed on longer periods of dialysis.

substitution of phosphate for glycine-tris buffer should result in the regeneration of the complex.

To test the above possibility the reassociation of the dissociated subunits was carried out in 0.2 M phosphate, pH 7.0, containing 1 mM EDTA and 1 mM 2-mercaptoethanol. No appreciable conversion of the subunits to active complex occurred in a period of 6-7 hours or longer under these conditions (high ionic strength phosphate) (Table I). We concluded, therefore, that factors other than the concentration of phosphate ions are instrumental in effecting the dissociation and reassociation of the fatty acid synthetase.

When 10 mM DTT was added to the dissociated enzyme in 0.2 M phosphate buffer a progressive increase in enzymatic activity was observed, which leveled off after 7-8 hours. Further experimentation showed that the extent of reassociation was a function of protein concentration (Table I). At low protein concentrations (0.1 mg/ml or less), the maximum recovery of activity was 20-40% of the control, whereas at protein concentrations of 10 mg/ml or more, essentially complete reassociation was achieved as evidenced by the presence of one major peak on ultracentrifugation (Fig. 1D). Since the rate and extent of reassociation is dependent upon the protein concentration, it is evident that an interaction between the subunits of the fatty acid synthetase is of importance in their recombination.

The requirement for DTT for the reassociation of fatty acid synthetase subunits is surprising in view of the experimental conditions (1.0 mM 2-mercaptoethanol in glycine-tris buffer) employed to effect dissociation of the complex. Hence, the total number of free -SH groups was determined for the subunits and the intact complex. Fifty sulfhydryl groups/molecule of the complex are titratable at pH 7.0 with Ellman's reagent (10) in 70 min (Fig. 2). The reactivity of available -SH groups/molecule in the subunits (Fig. 2) is comparable to that in the complex, but a total of 5.5 ± 1.0 fast-reacting -SH groups/molecule are unavailable in the dissociated enzyme. Similar differences were obtained when the above titrations were carried out in the presence of 2.5 M guanidine hydrochloride. Under these conditions, the available -SH groups react at a much faster rate and some previously buried -SH groups are exposed to the reaction medium. Sixty-three -SH groups/molecule were titrated in the complex, and 57.5 ± 1.0 in the subunits. Most of the -SH groups which are lost during dissociation appear to

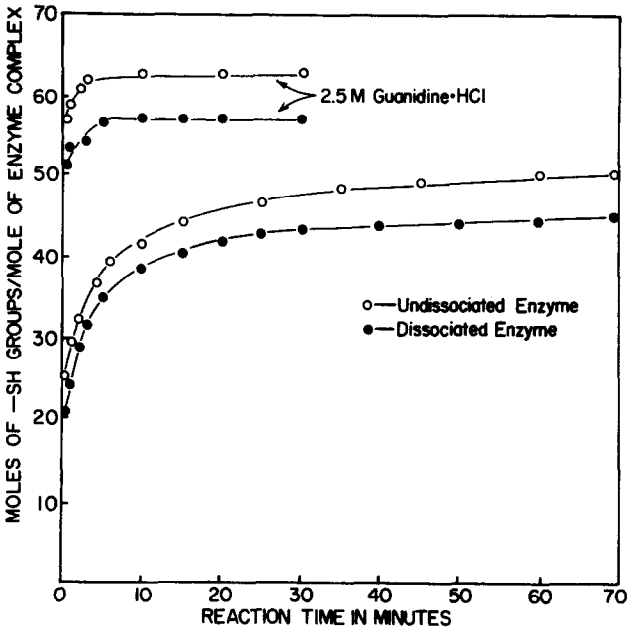

Fig. 2 Titration of total -SH groups of fatty acid synthetase complex and half-molecular weight subunits in the presence and absence of 2.5 M guanidine·HCl. Reactions were performed at 30° in 0.1 M potassium phosphate, pH 7.5, 1.0 mM 5,5'-dithiobis (2-nitrobenzoic acid) in the presence and absence of 2.5 M guanidine·HCl. The reaction was initiated by the addition of protein to a final concentration of 0.5 mg/ml. The optical density increase due to thiophenolate anion was measured at 412 nm. Extinction coefficients of $1.36 \times 10^4 M^{-1}cm^{-1}$ and $1.29 \times 10^4 M^{-1}cm^{-1}$ in the absence and presence of guanidine·HCl, respectively, were utilized to calculate the concentration of -SH groups. Corrections were applied for the reagent blanks in the absence of enzyme and for the fading of chromophore color with time in guanidine·HCl. Enzyme solutions were freed of DTT or mercaptoethanol by passing through a G-25 Sephadex column prior to titration. The results of three separate determinations for the number of -SH groups of the enzyme did not vary by more than one.

be highly reactive in the complex since a near constant difference in the number of -SH groups per molecule between the complex and the subunits exists at all times during titration (Fig. 2).

From the above results it is obvious that the dissociation of the fatty acid synthetase complex is accompanied by the loss of certain essential -SH groups which are not protected by 1 mM 2-mercaptoethanol in the presence of low ionic strength glycine-tris buffer. It may thus be suggested that con-

formational changes in the complex, under conditions of low ionic strength, modify the restrictive geometry and reactivity of these sulfhydryl groups. Such a modification may, in turn, promote subtle local configurational changes in the complex resulting in its dissociation. As an alternative, the dissociation of the complex may result from intramolecular electrostatic interactions in the presence of low ionic strength buffer. If so, the loss in -SH groups would be a by-product of this dissociation. It is not known whether DTT has a significant role in the formation of the proper configuration of the subunits prior to their recombination to form the enzyme complex or whether it acts in the conversion of an inactive complex to the active enzyme. Further studies are being carried out to investigate these possibilities.

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