The Proton Adenosinetriphosphatase Complex of Rat Liver Mitochondria. Temperature-Dependent Dissociation–Reassociation of the F_1-ATPase Subunits†

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ABSTRACT: The soluble F_1 moiety of the rat liver mitochondrial proton ATPase dissociates into two easily separable fractions when cold treated and then warmed. One fraction is soluble in potassium phosphate buffer, pH 7.4, whereas the other is insoluble. Neither of these two fractions alone can catalyze ATP hydrolysis under assay conditions optimal for the native F_1-ATPase. The insoluble fraction when resolved via sodium dodecyl sulfate–polyacrylamide gel electrophoresis is shown to be composed of only α and γ subunits. When this fraction is chromatographed on Sephadex G-75, it is resolved into an $\alpha\gamma$ complex and into the α subunit alone. The soluble fraction when resolved in the same electrophoretic system is shown to contain the remaining subunits, β, δ, ϵ, and some γ. This fraction is resolved into two major components by chromatography on Sepharose CL-6B, a $\beta\gamma$ complex and β subunit alone. The cold-dissociated enzyme can be readily associated when the temperature is raised to 20 °C. In the presence of either ATP or MgATP the enzyme completely regains its original ATPase specific activity. In contrast, Mg^{2+} is only about 15% effective in restoring ATPase activity. The results presented here define conditions for the dissociation and reassociation of the major subunits comprising the F_1-ATPase of rat liver and thus provide a unique system among mammalian enzymes for testing the function of individual subunits. In addition, they strongly indicate that neither the α nor β subunits, nor complexes of these subunits with the γ subunit, are capable of catalyzing ATP hydrolysis. Finally, they provide evidence that the γ subunit interacts with both the α and β subunits. As the most likely subunit stoichiometry of the rat liver F_1 is $\alpha_3\beta_3\gamma\delta\epsilon$ [Catterall, W. A., Coty, W. A., & Pedersen, P. L. (1973) *J. Biol. Chem. 248*, 7427–7431], these results suggest that the γ subunit may "tag" one out of three $\alpha\beta$ pairs in the molecule. The possible significance of this unusual type of subunit interaction to the function of the mitochondrial proton ATPase is discussed.

The proton adenosinetriphosphatase (EC 3.6.1.3) is a complex multisubunit enzyme composed of two major portions designated F_0 and F_1 [for recent reviews, see Senior (1979), Dunn & Heppel (1981), Fillingame (1981), Senior & Wise (1983), Amzel & Pedersen (1983), and Wang (1983)]. F_0 is the hydrophobic, membrane integrated sector that functions in directing proton flux across the membrane. F_1 is water soluble, projects into the mitochondrial matrix, and contains the active site. When associated with the mitochondrial membrane, the complete F_0F_1-ATPase complex is capable of reversible ATP synthesis from ADP and P_i. In soluble form, however, the F_1 headpiece has been shown to perform only Mg^{2+}-dependent ATP hydrolysis.

F_1-ATPases have been isolated from such diverse sources as chloroplasts (Howell & Moudrianakis, 1967; Farron, 1970), bacteria (Schnebli & Abrams, 1970; Bragg & Hou, 1972; Adolfson et al., 1975), yeast (Tzagoloff & Meager, 1971), and mammalian cells (Pullman et al., 1960; Gautheron et al., 1964; Catterall & Pedersen, 1971; Lambeth & Lardy, 1971; Beechey et al., 1975). The F_1's from these sources are all composed of at least five distinct subunits designated α, β, γ, δ, and ϵ that appear to be present in a 3:3:1:1:1 stoichiometry (Catterall & Pedersen, 1971; Senior, 1973; Catterall et al., 1973; Esch & Allison, 1979; Foster & Fillingame, 1982). Although considerable effort has been invested in determining the function of these subunits [for reviews, see Senior (1979), Dunn & Heppel (1981), Senior & Wise (1983), and Amzel & Pedersen (1983)], much critical information is lacking.

It is known that only the α and β subunits bind adenine nucleotides (Ohta et al., 1980; Dunn, 1980) with the presumed active site for ATP synthesis and hydrolysis involving at least the β subunit binding site (Wagenvoord et al., 1980; Grubmeyer & Penefsky, 1981a,b; Williams & Coleman, 1982; Matsuoka et al., 1982). The δ (Bragg et al., 1973; Futai et al., 1974) and ϵ subunits (Dunn & Heppel, 1981) are generally believed (though not proven) to be required for F_1 binding to the F_0 portion. Several lines of evidence indicate that γ has an important role in organizing the F_1 complex (Dunn & Heppel, 1981), probably in binding the ϵ subunit (Dunn, 1982), and may be required for ATPase activity (Smith & Wilkowski, 1978). In addition, Yoshida et al. (1977) postulated on the basis of reassociation studies on the $\gamma\delta\epsilon$ portion of the thermophilic bacterium F_1-ATPase that γ acts as a proton gate. However, Dunn & Heppel (1981) did not confirm this result using the isolated *Escherichia coli* $\gamma\delta\epsilon$ complex, nor did Khananshvili & Gromet-Elhanan (1982), who found that γ does not function as a H^+ gate in *Rhodospirillum rubrum*. It seems clear, therefore, that work in a number of laboratories involving a variety of F_1-ATPase preparations has not yet provided conclusive role assignments for the F_1-ATPase subunits.

In an attempt to better understand both the structure of the liver F_1-ATPase and the function of the individual subunits comprising this structure, we have focused on the well-known sensitivity of the enzyme to cold (Penefsky & Warner, 1965; Rosing et al., 1975). In this paper we show that under defined conditions cold inactivation of the liver enzyme is quite mild and can be used as an experimental tool for obtaining useful information about both the structure of F_1 and the relationship between the structure of the enzyme and its catalytic activity.

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Experimental Procedures

Materials

Animals. Adult, male CD retired breeder rats obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA), were fed on a Rockland diet (Teklad, Winfield, OH) ad libitum.

Reagents. The following chemicals were obtained from the indicated sources: D-(+)-mannitol, HEPES,[1] defatted bovine serum albumin, pyruvate kinase, lactic dehydrogenase, phosphoenolpyruvate, and Trizma base from Sigma Chemical Co.; Cellex D (standard capacity), acrylamide, methylenebis(acrylamide), and Coomassie Brilliant blue R-250 from Bio-Rad; Sepharose CL-6B and Sephadex G-25 and G-75 from Pharmacia Fine Chemicals; TEMED from Eastman Kodak Co.; ATP and NADH from P-L Biochemicals; urea (ultrapure) from Schwarz/Mann; dithiothreitol from Calbiochem (San Diego, CA).

Methods

Isolation of Mitochondria. Rat liver mitochondria were isolated by the high-yield method of Bustamante et al. (1977). The isolation medium contained 220 mM D-mannitol, 70 mM sucrose, 2 mM HEPES, and 0.5 mg/mL defatted bovine serum albumin, pH 7.4.

Preparation of F_1. F_1 was prepared from rat liver mitochondria essentially according to Catterall & Pedersen (1971) with modifications as described in Pedersen & Hullihen (1978). F_1 was stored lyophilized in 250 mM potassium phosphate–5.0 mM EDTA, pH 7.5, at −20 °C.

Cold Treatment. F_1 was resuspended to a final protein concentration of 2–2.5 mg/mL in 250 mM potassium phosphate–5.0 mM EDTA, pH 7.5, just prior to incubation at 4 °C.

When removed from the cold, the enzyme was either immediately applied to a Sepharose CL-6B column or was warmed at 37 °C for $^1/_2$ h. Samples, after being warmed, were then centrifuged in order to sediment the precipitate that forms upon warming. The resulting supernatant was removed by Pasteur pipet and saved either for further purification or for SDS gel electrophoresis. The sediment was resuspended twice in 5.0 mM Tris-HCl–1.0 mM EDTA, pH 8.0, following precipitation with 3.5 mM ammonium sulfate–10 mM Tris-HCl–1 mM EDTA, pH 7.5. This resuspended fraction was then further purified on Sephadex G-75. Alternatively, the sediment was resuspended in deionized water, precipitated with ammonium sulfate, and then prepared for SDS gel electrophoresis.

Column Chromatography. Native F_1 and the supernatant fraction resulting from cold treatment were applied to a Sepharose CL-6B column (1.2 × 54 cm) and eluted with 250 mM potassium phosphate–5.0 mM EDTA, pH 7.5.

The pellet fraction resulting from cold treatment was separated on a Sephadex G-75 column (1.2 × 24 cm) with an elution buffer containing 10.0 mM Tris–5.0 mM $MgCl_2$, pH 8.0.

The eluant in both cases was monitored at 280 nm with an ISCO UA-5 UV monitor (Lincoln, NE), and peak fractions were pooled and either lyophilized or precipitated with ammonium sulfate.

SDS–Polyacrylamide Gel Electrophoresis. Cylindrical SDS–polyacrylamide electrophoresis was performed in the phosphate system as described by Weber & Osborn (1969) and modified by Catterall et al. (1973). Gels were subjected to electrophoresis at 4 mA/tube for $^1/_2$ h and then increased to 8 mA/tube until termination of the run. Gels were stained first in 10% (v/v) acetic acid, 25% (v/v) methanol, and 0.05% (w/v) Coomassie blue for 6–9 h and then in 10% (v/v) acetic acid, 10% (v/v) methanol, and 0.005% (w/v) Coomassie blue for an additional 12–18 h. The gels were then destained by diffusion in 10% acetic acid and scanned at 560 nm by using a Gilford spectrophotometer equipped with a scanning device.

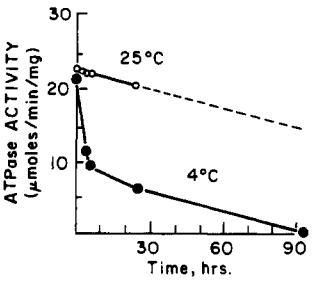

FIGURE 1: Effect of temperature on the ATPase activity of liver F_1. Incubation of F_1-ATPase (~500 μg) was carried out in 250 mM potassium phosphate–5 mM EDTA, pH 7.5 (PE buffer), in a volume of 100 μL at the temperature indicated. At the time points shown 1-μL aliquots were removed and assayed for ATPase activity.

Assay for ATPase Activity. Soluble F_1-ATPase activity was determined by the coupled spectrophotometric assay of Pullman et al. (1960) as modified by Catterall & Pedersen (1971). Initial rates were employed in all activity calculations.

Determination of Protein. Soluble protein was determined by the Lowry procedure (Lowry et al., 1971) employing bovine serum albumin as the standard protein.

Results

Cold Dissociation of the F_1-ATPase. The F_1-ATPase from rat liver mitochondria exhibits a time-dependent loss of ATP hydrolytic activity when incubated in the cold (4 °C). Although at room temperature in potassium phosphate buffer (250 mM, 5 mM EDTA, pH 7.5) the enzyme is very stable, showing only a 9% loss in ATPase activity after 17 h, at 4 °C in the same buffer system 70% of the initial activity is lost (Figure 1). Treatment of the enzyme under these conditions for 90 h results in a complete loss of catalytic activity. This activity loss is dependent on both protein and buffer concentration, with low concentrations of protein and buffer resulting in more rapid inactivation.

In addition to a loss in enzymatic function in the cold, the rat liver F_1-ATPase (2–2.5 mg/mL) dissociated into two major fractions. Upon being warmed to 37 °C (30 min), the cold-dissociated enzyme in phosphate buffer separates into a soluble fraction and an insoluble fraction. The insoluble fraction (a white precipitate) can be removed from the suspension by centrifugation (10 000*g*, 20 min, 25 °C). The resulting sediment (fraction I) contains two polypeptides that upon SDS gel electrophoresis exhibit mobilities identical with those of the α and γ subunits of the F_1-ATPase (see Figure 2). This fraction shows no ATP hydrolytic activity when assayed under optimal assay conditions.

The supernatant (fraction II), which was separated from the insoluble fraction I, was electrophoresed in the Weber and Osborn system described above. Comparison of the gel pattern of this fraction (Figure 3B) relative to untreated F_1 led to identification of the component subunits: β, δ, ε and a small amount of γ. To check the identification of fraction II, the supernatant resulting from a short cold treatment (20 min, 4 °C) was electrophoresed in SDS to determine which of the

[1] Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; PE buffer, 250 mM potassium phosphate–5 mM EDTA, pH 7.5.

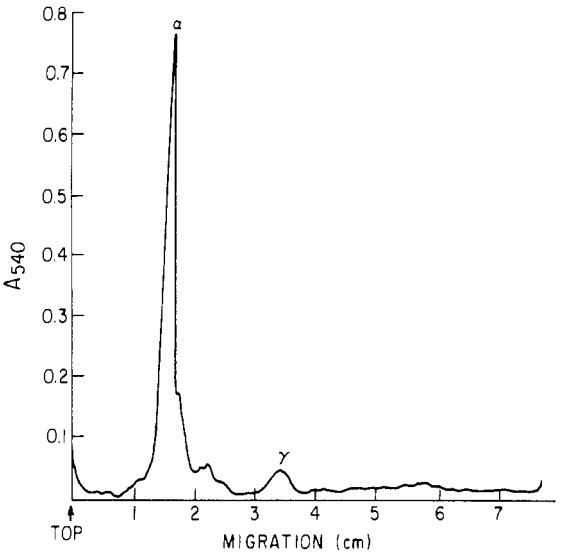

FIGURE 2: SDS–polyacrylamide gel patterns of the insoluble fraction formed upon warming of cold-treated liver F_1. The soluble F_1-ATPase (250 μg/100 μL) in PE buffer was cold treated for 17 h (4 °C). It was then removed from the cold and warmed to 37 °C in a water bath for 30 min. The insoluble fraction that formed was removed from suspension and prepared for SDS–PAGE as described under Methods. Twenty micrograms of sample was applied to the cylindrical gels for electrophoresis.

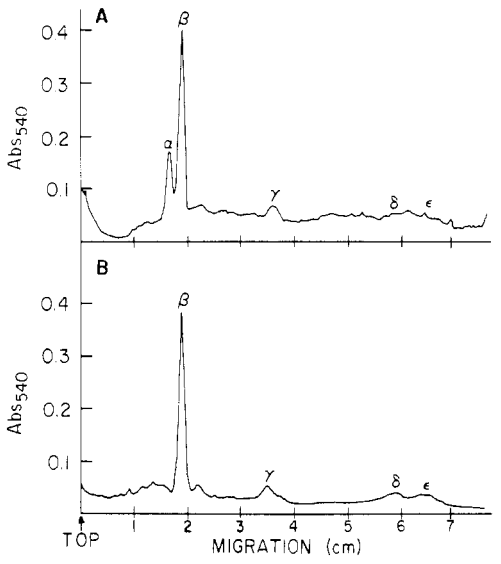

FIGURE 3: SDS–PAGE pattern of the soluble fraction of cold-dissociated liver F_1. The soluble fraction remaining after removal of the sediment was prepared for SDS–PAGE as described under Methods. Panel A shows the gel pattern of the soluble fraction when the enzyme was incubated for 20 min at 4 °C. Only about 40% of the precipitable protein was removed from the complex. Panel B shows the electrophoretic pattern of the soluble fraction after 17 h at 4 °C. The α subunit is completely absent from this fraction. Ten micrograms of protein was applied to the gels in each case.

two bands α or β disappeared with time from the soluble fraction. Figure 3A shows that after 20-min incubation at 4 °C the α band of the rat liver F_1-ATPase has substantially diminished whereas the β band has remained unchanged. The 17-h cold-treated fraction containing only β, γ, δ, and ϵ is not capable of supporting ATP hydrolytic activity under our assay conditions, indicating that the α subunit is required for catalytic activity.

Separation of Fraction I. Fraction I (the sediment) can be further purified by gel filtration using Sephadex G-75. This fraction containing α and γ was washed twice (see Methods),

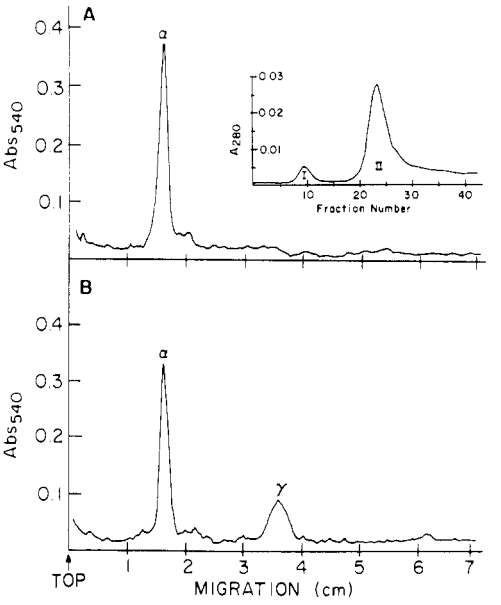

FIGURE 4: SDS gel patterns of the Sephadex G-75 fractions. The peaks eluted from the Sephadex G-75 column (see inset) were lyophilized and prepared for SDS gel electrophoresis. Panel A shows that peak I contains only the α subunit. Panel B is the pattern of peak II containing α and γ subunits. Eight and ten micrograms of protein, respectively, were employed on each gel. Inset: Sephadex G-75 chromatography of the sediment resulting from cold treatment of liver F_1. Fraction I (the sediment) was further separated into two components on a Sephadex G-75 column (1.2 × 24 cm), eluted with 10 mM Tris–5 mM $MgCl_2$, pH 8.0. Absorbance at 280 nm was determined with a UA-5 monitor (ISCO, Lincoln, NE) and 1-mL fractions were collected.

resuspended in 200 μL of elution buffer, and applied to the Sephadex G-75 column (1.2 × 24 cm). The two peaks were eluted with 10 mM Tris–5 mM $MgCl_2$, pH 8.0 (Figure 4, inset) because the sediment is not soluble in the high ionic strength buffer usually employed for rat liver F_1-ATPase. Identification via SDS gel electrophoresis shows that peak I contains only the α subunit (Figure 4A) and peak II contains the $\alpha\gamma$ complex (Figure 4B). [The finding that the $\alpha\gamma$ complex elutes after the smaller α subunit reflects a greater adherence of the complex to the gel filtration matrix for reasons that are currently unknown. The finding that cold treatment of F_1 in the presence of dithiothreitol also gives a precipitate containing α and γ subunits rules out the possibility that precipitation (and hence $\alpha\gamma$ complex formation) results because of disulfide bond formation.]

Separation of Fraction II. Fraction II was purified by using the high ionic strength buffer (250 mM potassium phosphate–5 mM EDTA, pH 7.5) since its solubility is similar to that of native F_1-ATPase. A Sepharose CL-6B column (1.2 × 54 cm) was employed to separate fraction II into its components. Figure 5, inset, shows both the native F_1 and fraction II elution profiles. Native F_1-ATPase elutes as a single major peak though not strictly a Gaussian profile (Figure 5A, inset). The cold-dissociated fraction II, by contrast, elutes as two distinct peaks (see Figure 5B, inset). When electrophoresed in the SDS–phosphate system, the first peak was found to contain $\beta\gamma$ complex (Figure 5A) while the second peak contained only β (Figure 5B).

Reassociation of Cold-Dissociated Enzyme. Figure 6 shows that the cold-induced dissociation of the F_1-ATPase is a reversible phenomenon. The enzyme was incubated at 4 °C for a period of 20 min in 5 mM Tris-HCl, pH 8.0, containing either ATP, MgATP, or Mg^{2+} alone. It was assayed for activity at 5-min intervals during that period. Under these

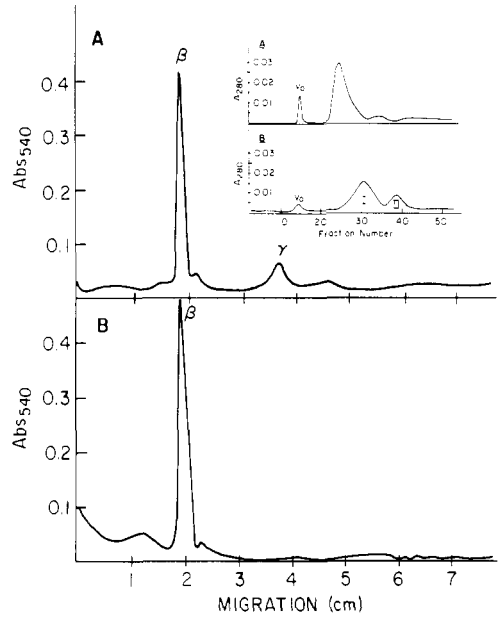

FIGURE 5: SDS–PAGE patterns of the Sepharose CL-6B fractions. The gel patterns of the two peaks eluted from the Sepharose CL-6B column (see inset) are shown. Panel A shows peak I containing β and γ subunits. Panel B shows peak II containing only β subunit. Ten micrograms of protein was employed for each gel. Inset: Sepharose CL-6B chromatography of native liver F_1 and of the soluble fraction following cold inactivation. Chromatography was performed on a Sepharose CL-6B column (1.2 × 54 cm) with PE as the elution buffer. Panel A shows the elution profile of the native enzyme. Panel B shows the profile of the soluble fraction of the cold-dissociated enzyme. Peaks were pooled, lyophilized, and prepared for SDS gels.

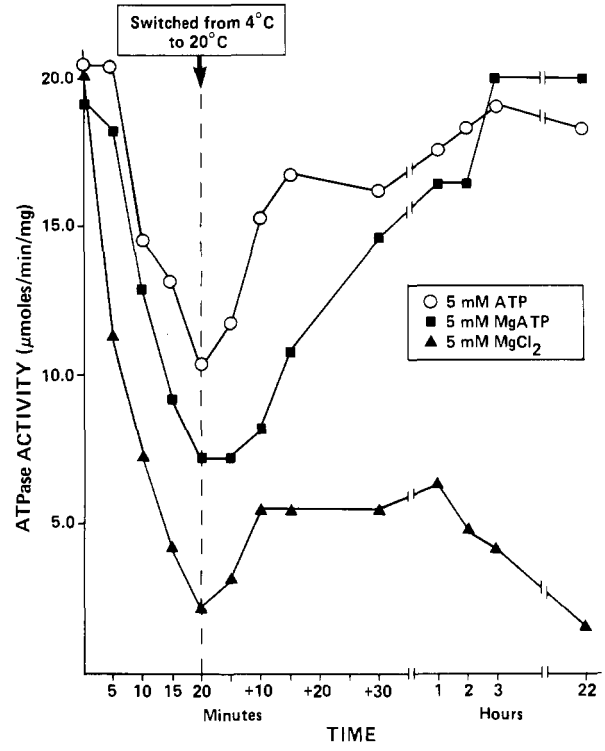

FIGURE 6: Reassociation of the cold-dissociated liver F_1-ATPase. Soluble F_1 (250 μg/100 μL) in 5 mM Tris-HCl buffer, pH 8.0, was incubated at 4 °C for 20 min and then placed in a 20 °C water bath. Aliquots were taken at the indicated time points for the ATPase assay.

conditions cold dissociation is very rapid, resulting respectively in 50%, 63%, and 90% losses of ATPase activity for the cases where ATP, MgATP, and Mg^{2+} alone are present. If the temperature is increased at the 20-min time point from 4 to 37 °C, the α and γ subunit complex precipitates as described in detail in the previous sections. However, if the temperature is increased from 4 to only 20 °C, complete reactivation of ATPase activity is observed in incubation systems containing ATP or MgATP. Only 15% reactivation of lost activity is observed in the system containing Mg^{2+} alone. These results show that the cold-dissociated F_1-ATPase of rat liver can be reassociated into a completely active complex.

Discussion

The isolated F_1 moiety of the mitochondrial proton ATPase has been known for many years to be cold labile, exhibiting a loss of ATP hydrolytic activity when cold treated (Penefsky & Warner, 1965; Rosing et al., 1975). In this study we have used this property of rat liver F_1-ATPase to obtain information about both its structure and the relationship of its structure to its catalytic activity.

Experiments described here show that the rat liver F_1-ATPase exhibits a reversible cold dissociation that upon warming to 37 °C yields two discrete fractions (Figures 2 and 3B). The first fraction displays decreased solubility in high-phosphate buffer and forms a white precipitate. Analysis by SDS–polyacrylamide gel electrophoresis shows this precipitate to be composed of both α and γ subunits (Figure 2). Further purification on Sephadex G-75 resolves this sediment into two peaks: one containing $\alpha\gamma$ and the other containing only α (Figure 4). The second fraction, which is soluble in high-phosphate buffer, was found to contain the remaining subunits: β, δ, and ϵ and some γ subunit (Figure 3B). When resolved on Sepharose CL-6B, the two major peaks were shown to be composed of $\beta\gamma$ complex and β alone (Figure 5).

Under these conditions where cold treatment is brief (20 min at 4 °C) rat liver F_1 is capable of reassociation to yield an active ATPase complex (Figure 6). When either ATP or MgATP is present, 100% of the ATPase activity lost in the cold is regained during incubation at 20 °C for 2 h. This experiment is particularly important since it demonstrates that cold dissociation does not result in irreversible denaturation of the enzyme. Rather, it represents a mild procedure for separating subunits or subunit pairs of the enzyme.

The finding that ATP is required for complete reactivation of the rat liver F_1-ATPase after cold dissociation differs from the finding for the bovine heart enzyme reported by Rosing et al. (1975). They report that ATP has little if any effect on the reactivation process. However, in their experiments potassium nitrate, which promotes dissociation of F_1 (Penefsky & Warner, 1965), and glycerol, which promotes the removal of bound nucleotides (Garrett & Penefsky, 1975), are both present. Significantly, Penefsky & Warner (1965) were able to achieve a 91% reactivation of the bovine heart enzyme in a buffer system containing both ATP and potassium nitrate but devoid of glycerol.

Unfortunately, these earlier studies provided no information about the effect of cold temperatures on the dissociation of the five different types of subunits of F_1. In experiments reported here it seems clear that when the rat liver enzyme is subjected to cold temperatures, the normal subunit forces that associate the α and β subunits are weakened sufficiently to favor dissociation of the complex with loss of enzymatic function. The δ and ϵ subunits remain with the β subunit whereas the γ subunit remains complexed to both subunits. Consistent with the observations made here, Begusch & Hess (1979) indicate in a preliminary report that cold dissociation of the yeast F_1 appears to dissociate the α subunit from the remaining part of the complex. However, they reported no experiments to indicate the fate of the remaining subunits.

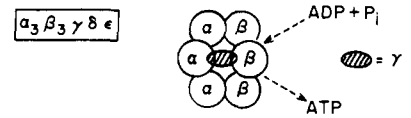

FIGURE 7: Proposed model of the structure of the rat liver F_1-ATPase emphasizing the binding of the γ subunit to a single $\alpha\beta$ pair. The above model is based on the data presented in this paper assuming an $\alpha_3\beta_3\gamma\delta\epsilon$ stoichiometry (Catterall & Pedersen, 1971; Catterall et al., 1973). It is also consistent with the crystallographic data for the rat liver enzyme (Amzel et al., 1982).

Also, in studies of the bovine heart enzyme Forrest & Edelstein (1970) reported that cold dissociation in dilute buffer results in a species sedimenting with an apparent molecular weight of 46 000. Identification of this species in terms of the five different types of F_1 subunits was never reported.

It seems important to emphasize that in the experiments reported here the γ subunit was shown to interact with both the α and β subunits. These complexes, $\alpha\gamma$ and $\beta\gamma$, were stable to repeated chromatography and ammonium sulfate precipitation, indicating a true subunit interaction and not simply coprecipitation. They were formed also in the presence of dithiothreitol, indicating that they are not simply the result of disulfide bond formation. Keeping in mind that the most likely subunit stoichiometry of the rat liver enzyme is $\alpha_3\beta_3\gamma\delta\epsilon$ (Catterall & Pedersen, 1971; Catterall et al., 1973), the simplest subunit model that can be depicted is presented in Figure 7. In this model γ is shown to be associated with one $\alpha\beta$ pair in the F_1-ATPase structure. These data are also consistent with the crystallographic model proposed by Amzel et al. (1982) since this arrangement would yield an apparent 2-fold axis of symmetry consisting of three equivalent masses at the current level of resolution.

The model depicted in Figure 7 suggests a potentially very important role for the γ subunit in the function of F_1-ATPase. The γ subunit (and possibly the other smaller subunits) may act to "tag" one $\alpha\beta$ pair. Indeed, this type of subunit association might provide a unique environment for an $\alpha\beta$ pair, perhaps altering the active site of that pair to a conformation more favored for catalysis. Significantly, the earlier work of Yoshida et al. (1977) indicating that the γ subunit in the thermophilic bacterial enzyme functions as a proton gate has not been confirmed for another bacterial system (Khananshvili & Gromet-Elhanan, 1982). Therefore, the model described here provides an alternate possibility for the role of this subunit.

More significant to our goal of understanding both the role of F_1-ATPase subunits and the structure of the enzyme complex are three additional findings. First, the individual α and β subunits and $\alpha\gamma$ and $\beta\gamma$ complexes can be rapidly purified. Second, neither the $\alpha\gamma$ nor the $\beta\gamma$ complexes are capable of supporting ATP hydrolytic activity contrary to an earlier report in the literature for the thermophilic bacterial enzyme (Yoshida et al., 1977). Third, the ability of the cold-dissociated liver enzyme to reassociate completely into a catalytically active complex provides a good system for testing the function of individual subunits.

Work is currently under way in this laboratory to establish whether the two smaller subunits δ and ϵ interact with the γ subunit or interact separately with α, β, or an $\alpha\beta$ pair.

Acknowledgments

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Registry No. ATPase, 9000-83-3.

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Chromatin Structure of the β-Globin Gene Family in Murine Erythroleukemia Cells†

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ABSTRACT: We have analyzed the chromatin structure of the β-major globin gene and other related β-globin genes in induced and uninduced murine erythroleukemia (MEL) cell nuclei. Nuclei were digested with either DNase I or micrococcal nuclease, and the purified DNA was hybridized to a set of cloned genomic DNA fragments covering the β-globin gene region. This region consisted of two distinct domains as characterized by sensitivity to DNase I digestion. One domain was relatively sensitive and contained the potentially active or actively transcribed β-major and β-minor globin genes. The other, relatively insensitive domain contained the nontranscribed embryonic and β-globin homologous genes. The sensitivity of these domains was not altered during erythroid differentiation. In nonerythroid cells, the entire globin gene family, including the adult and embryonic globin genes, was contained in a single relatively resistant domain. Micrococcal nuclease (MNase) also defined two general domains of nuclease sensitivity that coincided with those of DNase I. However, the relatively sensitive MNase domain containing the β-major and β-minor genes became more sensitive upon chemically stimulated erythroid differentiation. A detailed examination of the β-major globin gene revealed that the actual coding region became increasingly sensitive to micrococcal nuclease after differentiation while the 5′-flanking DNA did not. Thus, micrococcal nuclease was able to accurately define the primary transcription unit of the β-major gene.

Recent studies have established that transcriptionally active genes are in chromatin structures with a conformation different from that of inactive genes. Genes that are expressed or have the potential to be expressed exhibit a preferential susceptibility to cleavage by nucleases. Two well-characterized examples are the globin genes in chicken erythrocytes (Weintraub & Groudine, 1976; Stalder et al., 1980a) and the ovalbumin gene in the hen oviduct (Garel & Axel, 1976; Bloom & Anderson, 1979; Bellard et al., 1980, Lawson et al., 1980; Anderson et al., 1983). This enhanced sensitivity is characterized either as a reduction of the sequence under study into small nonhybridizable fragments following extensive digestion or as site-specific cleavages introduced into unique hypersensitive sites near the gene under mild digestion conditions (Stalder et al., 1980a,b; Weintraub et al., 1981; Anderson et al., 1983). In these as in other cases, nuclease sensitivity or hypersensitivity correlates with the differentiated state of the cell rather than the actual transcription of the genes. Since there have been no reports of an expressed gene being in a nuclease-resistant state, the acquisition of nuclease sensitivity appears to be prerequisite to eukaryotic gene expression.

It has been recently shown in several specific systems that active genes reside within large DNase I sensitive domains that include not only the coding regions but also large regions of nontranscribed sequences as well. The ovalbumin gene and neighboring X and Y genes in hen oviduct nuclei are packaged into a DNase I sensitive domain approximately 100 kb in length (Lawson et al., 1982). These three genes are transcribed at different rates (Colbert et al., 1980; Lemur et al., 1981) and are equally sensitive to DNase I (Garel et al., 1977) and remain so during their transcriptional inactivation upon steroid hormone withdrawal (Lawson et al., 1982). The chicken α- and β-globin genes are also packaged into large DNase I sensitive domains in chicken erythroid cells (Stalder et al., 1980a,b) and remain sensitive following transcriptional termination in the quiescent red blood cell nucleus.

The extent of large DNase I sensitive domains containing active genes has been mapped in several cases. One such domain has been shown to extend as little as 3–4 nucleosomal DNA lengths beyond the 5′-end and 2–15 nucleosomal DNA lengths beyond the 3′-end of integrated adenovirus genes in transformed hamster cells (Flint & Weintraub, 1977). On the other hand, Bellard et al. (1980) have shown that DNase I sensitivity extends at least 30 nucleosomal DNA lengths beyond the 3′-side of the ovalbumin coding region. Recently, Lawson et al. (1982) mapped the DNase I sensitive domain containing the ovalbumin, X, and Y genes in hen oviducts and found this region to extend 30 kb to the 5′-side of the X gene and greater than 20 kb to the 3′-side of the ovalbumin gene.

Studies using micrococcal nuclease (MNase) have shown that the ovalbumin gene in hen oviduct nuclei is preferentially

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