Estimation of the Turnover Number of Bovine Heart F_oF_1 Complexes for ATP Synthesis†

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ABSTRACT: In mitochondria and submitochondrial particles (SMP), the rate of ATP synthesis is restricted by the rate of energy production by the respiratory chain. Fractional inactivation of the ATP synthase complexes (F_oF_1) of bovine heart SMP by covalent modifiers increased the rate of ATP synthesis per mole of active F_oF_1. Thus, by use of SMP containing fractionally inactivated F_oF_1 complexes, a synthetic rate of 420 mol of ATP (mol of F_oF_1·s)$^{-1}$ was measured, which extrapolated to a V_{max} of 440 s^{-1}. At this extrapolated point, the turnover rate of F_oF_1 complexes was independent of the rate of energy production by the respiratory chain. These results have been discussed in relation to the effect of fractional inactivation of the F_oF_1 complexes of SMP on the steady-state free energy of the system. The above rate of ATP synthesis is comparable to the rate of ATP hydrolysis by SMP (400–520 s^{-1}) in the absence of energy coupling constraints and control by the ATPase inhibitor protein. More interestingly, this rate is also comparable to the rate of ATP synthesis by chloroplast F_oF_1 under high light intensity (~420 s^{-1}). Under the conditions specified, bovine heart SMP and chloroplasts show similar apparent K_m values for ADP. Thus, it appears that the mammalian and chloroplast ATP synthase complexes are similar not only in structure but also in catalytic efficiency for ATP synthesis.

Previous studies (Matsuno-Yagi & Hatefi, 1986) have shown that in submitochondrial particles (SMP)[1] the kinetics of oxidative phosphorylation can be analyzed in terms of a minimum of two kinetic modalities. One mode is characterized by low apparent K_m values for ADP (6–10 μM) and P_i (≤0.25 mM) and a low rate of ATP synthesis [~500 nmol (min·mg of protein)$^{-1}$]. This kinetic mode of ATP synthesis is expressed when the rate of energy production relative to the number of functional ATP synthase complexes is low. The second kinetic mode is characterized by a 10-fold higher apparent K_m for ADP and P_i and an overall measured rate of ATP synthesis of ~2800 nmol (min·mg of protein)$^{-1}$. This mode of ATP synthesis predominates when the coupled activity of the respiratory chain relative to the number of functional ATP synthases is high.

The above findings suggested, among other things, that the turnover capability of F_oF_1 complexes in the direction of ATP synthesis might be considerably higher than the rates normally measured. In other words, it appeared that, under the normal oxidative phosphorylation assay conditions, the rate of energy production may be too low to allow the turnover potential of the ATP synthases to be realized.

When relieved of energy coupling constraints and control by the ATPase inhibitor protein, preparations of SMP catalyze ATP hydrolysis at a rate of 10–13 μmol (min·mg of protein)$^{-1}$, which corresponds to an F_oF_1 turnover rate of 400–520 s^{-1}. This paper shows that, under optimal conditions, the ATP synthases of bovine heart SMP can synthesize ATP with a comparable turnover rate.

Materials and Methods

Preparations and Assays. SMP (Matsuno-Yagi & Hatefi, 1985), complex V (Stiggall et al., 1979), and F_1-ATPase (Wong et al., 1984) were prepared from bovine heart mitochondria as in the references given. Antibodies against complex V were raised in rabbits, and the IgG fraction was purified as described previously (Robbins et al., 1981). Protein concentration was determined by the method of Lowry et al. (1951). Oxidative phosphorylation activity was assayed according to Matsuno-Yagi et al. (1985), and ATPase activity was measured according to Stiggall et al. (1979) with the modification given in Matsuno-Yagi and Hatefi (1984). In both assays, the temperature was 30 °C, and the pH was 7.5.

Immunoquantitation of F_1 in SMP Preparations. The method employed was essentially the same as reported by others (Vaessen et al., 1981; Mathur & Grimshaw, 1986). Samples were prepared and electrophoresed on 12% SDS–polyacrylamide gels by the method of Laemmli (1970). Protein electrotransfer onto nitrocellulose was carried out at 100 V for 70 min essentially according to Towbin et al. (1979), using a Bio-Rad Mini Trans-Blot apparatus. The nitrocellulose sheets were immunostained with antibodies against complex V followed by decoration with *Staphylococcus aureus* ^{125}I-labeled protein A [(2–5) × 10^5 cpm/mg]. The radioactive bands were located on nitrocellulose sheets by autoradiography, and the segments of the sheets corresponding to the α and β subunits of F_1 were excised and counted in a Packard Crystal 5400 Series γ counter. A standard curve was obtained by using purified F_1. At relatively high amounts of F_1 (>0.3 μg), the radioactivity tended to deviate from linearity, presumably because of the limited binding capacity of the nitrocellulose sheets. Addition of a fixed amount of SMP to variable amounts of F_1 gave rise to a constant increase in the radioactivity. This indicated that, although protein transfer was not complete under the conditions employed, the transfer efficiency was the same for purified F_1 and F_1 in SMP prep-

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[1] Abbreviations: SMP, submitochondrial particles; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; Δψ, membrane potential; F_1 and F_o, catalytic and membrane sectors, respectively, of the mitochondrial ATP synthase complex (F_oF_1); CF_1, chloroplast F_1; SDS, sodium dodecyl sulfate; STA buffer, buffer containing 0.25 M sucrose and 50 mM Tris–acetate, pH 7.5; TBT-Cl, tributyltin chloride; Tris, tris(hydroxymethyl)aminomethane.

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arations. Increasing amounts of SMP used alone also gave a linear plot up to 2 μg of protein loaded. The amount of F_1 present in SMP preparations could be estimated either from the increment of radioactivity caused by addition to F_1 of a fixed amount of SMP or from the slope of the line obtained with variable amounts of SMP. The value obtained by either procedure was 0.15 mg of F_1/mg of SMP. When a molecular weight of 360 000 was used for bovine heart F_1 (Lambeth et al., 1971), the F_1 content in SMP was, therefore, estimated to be 0.42 nmol of F_1/mg of SMP.

Estimation of [^{14}C]DCCD Incorporation at the DCCD Binding Protein. SMP at 10 mg/mL were incubated on ice for 3 h in the presence of variable concentrations (1.7–17 μM) of [^{14}C]DCCD (26 μCi/μmol) in a buffer containing 0.25 M sucrose and 50 mM Tris–acetate, pH 7.5 (STA buffer), and then passed through Sephadex centrifuge columns (G-50 fine) (Penefsky, 1977). The eluate was diluted with 6 volumes of a buffer containing 0.25 M sucrose, 50 mM Tris–acetate, pH 8.3, and 10 mM succinate and further incubated at 30 °C for 30 min to achieve complete activation of ATPase. The SMP samples were then centrifuged in a Beckman airfuge for 5 min and resuspended in 0.25 M sucrose containing 6 mM sodium phosphate, pH 7.2. The ATPase activity was measured, and aliquots were subjected to 12.5% SDS–polyacrylamide gel electrophoresis for determination of [^{14}C]DCCD bound at the DCCD binding protein as detailed previously (Matsuno-Yagi & Hatefi, 1986). The ATPase activity of control SMP was determined after the particles had been treated as above in the absence of DCCD.

Materials. Nitrocellulose paper was obtained from Schleicher & Schuell. XAR film was from Kodak. ^{125}I-Labeled protein A was a gift of John Hural. The sources of other materials used were the same as indicated elsewhere (Matsuno-Yagi & Hatefi, 1985).

Results

As was mentioned above, our previous studies suggested that, under normal oxidative phosphorylation conditions, the synthetic turnover rate of F_oF_1 complexes is restricted by the rate of energy production by the respiratory chain. Suggestive evidence was also obtained that the rate of ATP synthesis per mole of F_oF_1 complex could be considerably increased by increasing the rate of energy production relative to the number of functional ATP synthase complexes (Matsuno-Yagi & Hatefi, 1986). Therefore, it was of interest to measure the synthetic turnover rate of F_oF_1 complexes under optimal conditions.

Experimentally, the problem could be approached in two ways. One way was to increase the rate of energy production. When this was done with the use of NADH as respiratory substrate, our highly active SMP preparations exhibited a rate of ATP synthesis of about 2800 nmol (min·mg)$^{-1}$, which at saturating [ADP] amounted to ≥3000 nmol (min·mg)$^{-1}$. Although this rate of ATP synthesis was 1 order of magnitude greater than the rates reported for SMP by others [see, for example, Penefsky (1974), Thayer and Hinkle (1975), Hackney and Boyer (1978); McCarthy and Ferguson (1983), and Herweijer et al. (1985)], it was clear that this rate was still far below the turnover capability of the ATP synthases. It was also clear that the limiting factor was the rate of energy production, even with NADH as the respiratory substrate, and that no other oxidizable substrate could be used to obtain a higher rate. The second way was to diminish the number of functional ATP synthase complexes. This could be done with the use of covalently reacting or high-affinity inhibitors of either F_o or F_1. Our previous studies had shown that fractional

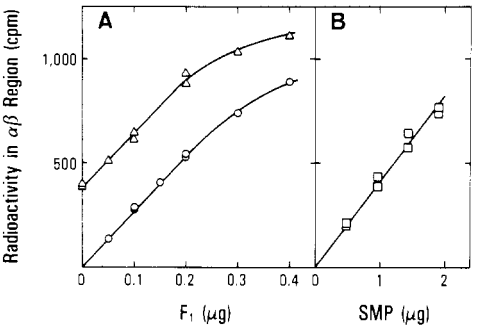

FIGURE 1: Quantitation of F_1 in SMP by immunoblotting. The indicated amounts of purified F_1 (○) and SMP (□) were electrophoresed on 12% SDS–polyacrylamide gels according to Laemmli (1970) and transferred onto nitrocellulose. In (△) of panel A, 0.98 μg of SMP was added to the indicated amounts of F_1. The papers were immunostained with antibody to the ATP synthase complex (complex V) and subsequently with ^{125}I-labeled protein A. Autoradiography was carried out overnight at −70 °C to locate the radioactive bands. The region of the nitrocellulose papers corresponding to the α and β subunits of F_1 was cut out, and their radioactivity was measured in a γ counter.

inhibition of ATP synthases at either F_o or F_1 would favor ATP synthesis in the high-K_m–high-turnover mode (Matsuno-Yagi & Hatefi, 1986), which was the kinetic modality needed for realizing the turnover potential of the ATP synthase complexes.

Therefore, the strategy used was as follows. Preparations of SMP were treated with an appropriate ATP synthase inhibitor in order to inactivate different fractions of these enzyme complexes. The modified SMP were assayed for oxidative phosphorylation activity at high rates of energy production. Then the rate of ATP synthesis determined was calculated in terms of the number of *uninhibited* ATP synthase complexes in the modified SMP used. Thus, if energy were the limiting factor in SMP, one would expect the rate of ATP synthesis *per functional ATP synthase molecule* to increase as increasing fractions of the F_oF_1 complexes were rendered nonfunctional. As will be seen below, this was found to be the case, with excellent agreement between the results for two ATP synthase inhibitors and two respiratory substrates.

Concentration of ATP Synthases in SMP. The concentration of F_oF_1 complexes in SMP was determined immunochemically, using an antibody to the purified ATP synthase complex (complex V). This antibody (rabbit serum IgG) reacts with several ATP synthase subunits, and particularly strongly with α and β. Thus, by the procedure described under Materials and Methods, it was shown that radioiodinated protein A could be used to quantitate the amount of antibody that had reacted with α/β in F_1 or SMP. These results are shown in Figure 1. Panel A shows the relationship between the amount of purified F_1 used and radioactivity due to ^{125}I-labeled protein A in the α/β region of immunoblots. It also shows that addition of a fixed amount of SMP to the same increasing concentrations of F_1 resulted in a parallel line moved up on the ordinate. These results indicated that in each experiment a constant fraction of the α/β subunits of F_1 and SMP was transferred from SDS–polyacrylamide gels to nitrocellulose and that the iodinated protein A detection method is quantifiable, at least up to a certain level of antigen. Beyond this level of antigen, the data deviated from linearity (Figure 1A). Furthermore, the distance on the ordinate between the two parallel lines indicated that the added SMP contained 0.42 nmol of F_1/mg. Panel B demonstrates the relationship between increasing amounts of SMP and protein A radioactivity in the α/β region of immunoblots. It also shows that, when used alone, the same amount of SMP that was added to F_1 in the

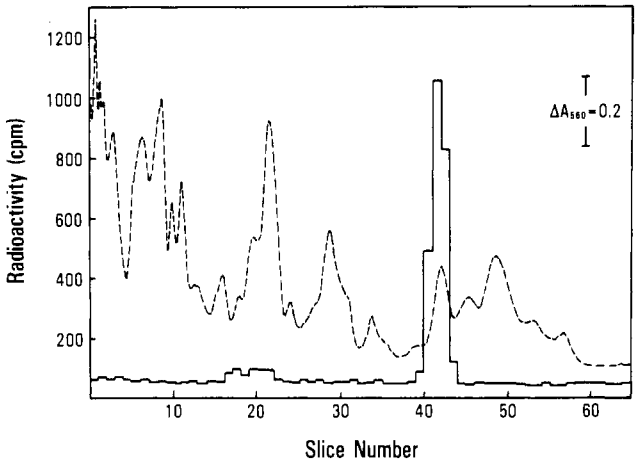

FIGURE 2: SDS–polyacrylamide gel electrophoresis of [^{14}C]DCCD-labeled SMP. SMP at 10 mg/mL in STA buffer were incubated at 0 °C for 3 h with 20 μM [^{14}C]DCCD and electrophoresed on a 12.5% SDS gel by the method of Weber and Osborn (1969). The gel was stained for protein with Coomassie Blue and destained, and the protein stain was traced in a densitometer. It was then sliced, digested, and counted for radioactivity. The inhibition of ATPase activity was 93%. The major peak of radioactivity (solid line) at M_r 17 000 corresponded to the position of the DCCD-modified proteolipid subunit of F_o on Weber–Osborn gels (Stekhoven et al., 1972; Kiehl & Hatefi, 1980). The dashed line is the Coomassie Blue absorbance at 560 nm.

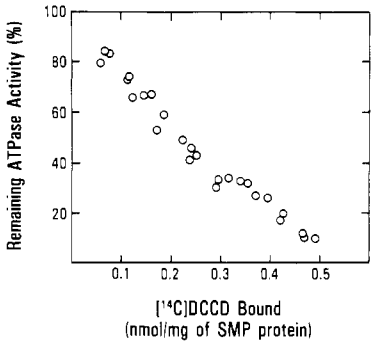

FIGURE 3: Correlation between [^{14}C]DCCD bound at the DCCD binding protein and oligomycin-sensitive ATPase activity of SMP. SMP were treated at 0 °C for 3 h with variable concentrations of [^{14}C]DCCD (1.7–17 μM) and passed through Sephadex centrifuge columns. Aliquots were diluted and incubated at 30 °C in a buffer containing 0.25 M sucrose, 50 mM Tris–acetate, pH 8.3, and 10 mM succinate. After 30 min of incubation, SMP were centrifuged and resuspended in STA buffer and assayed for ATPase activity in the presence of 5 μM CCCP. Incorporation of radioactivity into the DCCD binding protein of the SMP samples was determined as in Figure 2.

experiment of panel A acquired the same level of protein A radioactivity as in panel A. Since our preparations of SMP do not contain any soluble oligomycin-insensitive ATPase, their F_1 concentration, as estimated above, could be considered the same as their F_1F_o concentration. This extrapolation agreed with the concentration of F_o in SMP as suggested by the DCCD titration results shown below. Therefore, the ATP synthase concentration of SMP employed in this study was 0.42 nmol/mg of protein. The range determined in several experiments was 0.4–0.45 nmol/mg of SMP protein, which is very close to the value published by others for pig heart SMP (Penin et al., 1986).

Fractional Modification of F_oF_1 Complexes and Determination of the Extent of Inhibition. The inhibitors selected for fractional modification of F_oF_1 complexes were DCCD and TBT-Cl. Both compounds react covalently and, under the conditions used, specifically at F_o. The DCCD inhibition is irreversible, and the TBT-Cl inhibition occurs at low reagent concentration and is stable to dilution and washing of the particles. Thiol compounds reverse the TBT-Cl inhibition, but thiols were not employed in our assays. As checked in other studies, neither reagent, as used, caused any detectable uncoupling (Matsuno-Yagi & Hatefi, 1986). The inhibition of ATPase activity by TBT-Cl was diminished slightly after energization of SMP. Therefore, the values used below for the extent of inhibition of ATPase activity by TBT-Cl were those determined after the particles had been subjected to energization and then assayed for ATPase activity in the presence of an uncoupler.

The correlation between the extent of F_oF_1 modification and the degree of inhibition of ATP synthases was studied by using [^{14}C]DCCD. As mentioned above, under appropriate conditions of temperature, pH, and DCCD concentration, this reagent specifically inhibits the mitochondrial ATP synthase by covalent and irreversible modification of the proteolipid subunit of F_o. As seen in Figure 2, essentially no other SMP polypeptide was modified by [^{14}C]DCCD under the conditions employed. The small amount of radioactivity observed in the gels around slice 20 was not associated with the $\alpha\beta$ subunits of F_1, which would band on these gels between slices 5 and 10. To determine the extent of inhibition by DCCD, it was necessary to use ATPase rather than ATP synthase activity as the parameter tested, because the latter activity, as mentioned above, depended on the rate of energy production and the number of functional ATP synthases. Moreover, it was necessary to preactivate the SMP preparations in order to obtain the highest ATPase activity of each SMP sample before addition of the inhibitor. This was done as described in the legend to Figure 3, and the highest ATPase activity achieved with several different preparations of SMP was 10–13 μmol of ATP hydrolyzed (min·mg of protein)$^{-1}$ at 30 °C. Assuming a concentration of 0.42 nmol of F_1/mg of SMP, this activity corresponds to an F_1 turnover rate of 400–520 s^{-1}, which is close to the turnover number of isolated F_1 (500–600 s^{-1}) (Cross et al., 1982; Wong et al., 1984).

Data regarding correlation of the extent of specific [^{14}C]-DCCD binding to F_o and the degree of inhibition of the ATPase activity of SMP are shown in Figure 3. It is seen that the extent of F_oF_1 modification by DCCD was reasonably well reflected in the degree of inhibition of ATPase activity and that the ATPase activity of partially modified SMP could be considered a measure of the unmodified ATP synthases present. Moreover, Figure 3 indicates that 100% extrapolated inhibition of ATPase activity corresponds to the uptake of 0.4–0.5 nmol of [^{14}C]DCCD/mg of SMP. Together with the results of Figure 1, this suggests that the binding of one molecule of DCCD at the F_o of one ATP synthase complex results in complete inhibition. A similar conclusion has been reached by others (Kopecky et al., 1981).

Turnover Rate of ATP Synthases in SMP Containing Fractionally Inhibited F_oF_1 Complexes. Figure 4 shows the results of two sets of experiments. In the first set, the ATP synthases of SMP were modified to various extents by [^{14}C]DCCD, and the degree of modification was determined from (a) the radioactivity bound to the DCCD binding protein of F_o and (b) the inhibition of ATPase activity. The respiratory substrates were NADH and succinate. In the second set, the respiratory substrates were the same, the ATP synthase modifier was TBT-Cl, and the extent of modification of F_oF_1 complexes was estimated from the degree of inhibition of ATPase activity.

In Figure 4, the lower panel (A) shows the oxidative phosphorylation activities of the inhibitor-treated particles plotted against their ATPase activities. It is seen that when

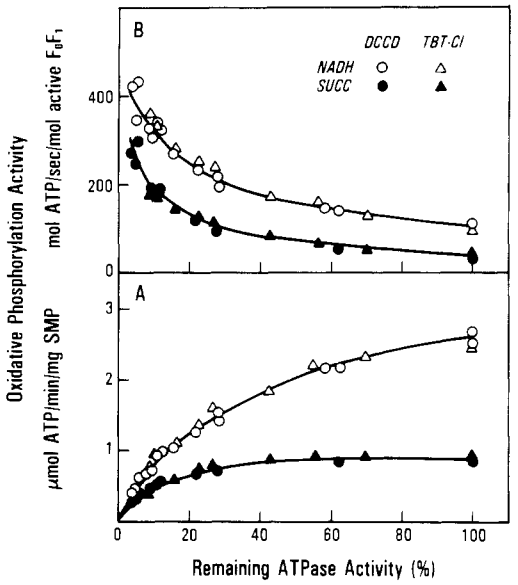

FIGURE 4: Effect of fractional inhibition of F_oF_1 complexes on the rates of ATP synthesis and hydrolysis. SMP were incubated at 0 °C with variable concentrations of DCCD (5–22 μM) for 3 h or with TBT-Cl (2–30 μM) for ≥30 min and passed through Sephadex centrifuge columns. The eluates were assayed for oxidative phosphorylation activity with NADH or succinate as the respiratory substrate. For the measurement of ATPase activity, SMP were further incubated at 30 °C for 4 min under oxidative phosphorylation assay conditions, followed by centrifugation and resuspension of the pellets in STA buffer. They were then assayed for ATPase activity in the presence of 5 μM CCCP. Oxidative phosphorylation activities in panel B are the synthetic turnover rates of F_oF_1 complexes calculated by dividing the measured rates of panel A by the corresponding concentrations of uninhibited F_oF_1.

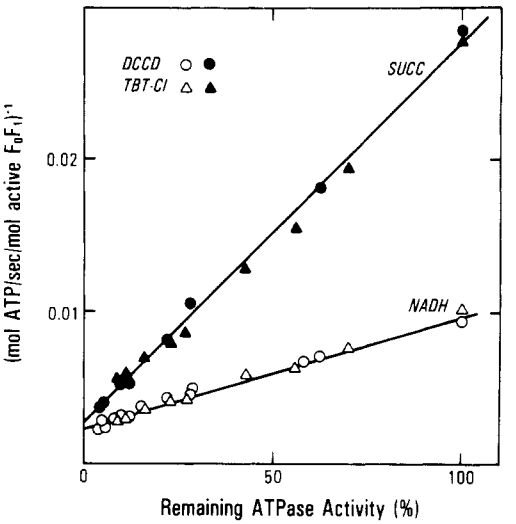

FIGURE 5: Replot of the data of Figure 4 for estimation of the maximum value for the synthetic turnover rate of F_oF_1 complexes when the remaining ATPase activity approaches zero and the rate of energy production by the respiratory chain is no longer a limiting factor in the turnover rate of the ATP synthase complexes (for details, see text).

succinate was the respiratory substrate, fractional inhibition of ATP synthases, as revealed by the diminishing ATPase activity of SMP, had little effect on the rate of ATP synthesis per milligram of SMP, until the ATPase activity was more than 80% inhibited. As detailed elsewhere (Matsuno-Yagi & Hatefi, 1986), this is because of the change in the kinetic modality of ATP synthesis. In uninhibited SMP, ATP synthesis supported by succinate oxidation occurs almost entirely in the low-K_m–low-turnover mode. As increasing fractions of the ATP synthases are inactivated, there occurs a change in the kinetic modality of ATP synthesis, resulting in greater contribution of the high-K_m–high-turnover mode to the overall kinetics of ATP synthesis. Thus, although a smaller number of ATP synthases are functional, they operate with greatly increased rates. Consequently, the rate of ATP synthesis per milligram of SMP remains high even though ATPase activity, which is a measure of the uninhibited F_oF_1 complexes, diminishes. Essentially the same explanation applies to the data for oxidative phosphorylation driven by NADH oxidation, except that in this case the rate of energy production via NADH oxidation is relatively high in the unmodified SMP, and ATP synthesis occurs largely in the high-K_m–high-turnover mode. As a result, fractional inhibition of the F_oF_1 complexes has a greater impact on the rate of ATP synthesis when NADH rather than succinate is the respiratory substrate.

The upper panel (B) of Figure 4 shows a replot of the data of panel A. In this figure, the rates of ATP synthesis on the ordinate have been calculated per mole of functional ATP synthase remaining at each point of inhibition. The concentration of F_oF_1 complexes used was 0.42 nmol/mg of SMP, as suggested by the data of Figure 1, and the fraction of functional ATP synthases at each stage of inhibition was calculated from the percent ATPase activity of the inhibitor-treated particles, as allowed by the results of Figure 3. Thus, it is seen in Figure 4B that as larger fractions of F_oF_1 complexes were inactivated, the rate of ATP synthesis *per active ATP synthase complex* was increased. At 96% inhibition of ATPase activity by DCCD, the rate of ATP synthesis supported by NADH oxidation was about 424 nmol (min·mg of SMP)$^{-1}$ (Figure 4A). This rate, calculated in terms of the 4% remaining, active ATP synthases, is about 420 mol of ATP (mol of F_oF_1·s)$^{-1}$ (Figure 4B). These data indicate that the mitochondrial ATP synthase is capable of synthesizing ATP at a rate comparable to the rate it can catalyze ATP hydrolysis. In the experiments of Figure 4, the inhibition of ATPase activity by DCCD or TBT-Cl was carried out only to the extent that allowed confidence in the accuracy of the results. The limit was 97% inhibition of ATPase activity with either DCCD or TBT-Cl. However, to the extent the modifications were carried out, it was reassuring to see that the data for the experiments with DCCD and TBT-Cl as the inhibitors fell on the same curve for either NADH or succinate as the respiratory substrate.

Calculation of Apparent V_{max} for ATP Synthesis. The reciprocal relationship shown in Figure 4B between the synthetic turnover rate of F_oF_1 complexes and the ATPase activity of the fractionally inhibited SMP preparations allowed the estimation of an apparent V_{max} for ATP synthesis. This was done by plotting the reciprocals of the F_oF_1 turnover rates from the ordinate of Figure 4B against the corresponding percent ATPase activities. As seen in Figure 5, the results were two straight lines for NADH and succinate as the energy source (each with DCCD or TBT-Cl as the ATPase inhibitor), and these lines converged at the same point on the ordinate. This point corresponds to a rate of 440 s^{-1}, which for the SMP preparation used could be considered the turnover number of F_oF_1 complexes in the direction of ATP.

Discussion

Intact bovine heart mitochondria respiring on pyruvate plus malate synthesize ATP at a rate of about 800–900 nmol (min·mg of protein)$^{-1}$ with a P/O ratio close to 3.0 (Hatefi & Lester, 1958). Preparations of bovine heart SMP oxidizing NADH synthesize ATP at a rate of 2800–3000 nmol (min·mg of protein)$^{-1}$ with a P/O ratio of 1.5–1.6. The SMP rate of

ATP synthesis corresponds to an F_oF_1 turnover rate of 110–120 s^{-1}, which according to our results is considerably lower than the turnover capability of the bovine F_oF_1 complexes under optimal conditions. A major factor that limits the rate of ATP synthesis especially in SMP appeared to be the rate of energy production by the respiratory chain. It was found in the course of other studies (Matsuno-Yagi & Hatefi, 1986) that fractional inactivation of F_oF_1 complexes of SMP had a greater effect on inhibition of the rate of ATP hydrolysis than synthesis. The reason was discovered to be due to the fact that fractional inhibition of F_oF_1 complexes converted the kinetics of ATP synthesis to the high-K_m–high-turnover mode. Consequently, the turnover rate of the uninhibited fraction of F_oF_1 complexes for ATP synthesis was considerably increased. By comparison, as shown here, the unencumbered (i.e., by the ATPase inhibitor protein and proton back-pressure) ATPase activity of the modified SMP preparations reflected with reasonable accuracy the extent of inhibition of the F_oF_1 complexes.

These findings made it possible, therefore, to estimate the synthetic turnover rate of F_oF_1 complexes in SMP. Since the rate of energy production could not be increased beyond that which was afforded by NADH oxidation, the number of functional ATP synthases was diminished by fractional inhibition of F_oF_1 complexes, and the rate of ATP synthesis per mole of F_oF_1 was calculated at each level of inhibition. The results showed that with either NADH or succinate as the respiratory substrate, the turnover rate of the active F_oF_1 complexes for ATP synthesis could be increased as increasing fractions of these complexes were rendered inactive. The data allowed estimation of an apparent V_{max} for ATP synthesis, which was 440 mol of ATP (mol of $F_oF_1 \cdot s)^{-1}$ with either NADH or succinate as the respiratory substrate.

In other studies, we have shown that fractional inhibition of F_oF_1 complexes in SMP raised the steady-state level of the membrane potential ($\Delta\psi$). Although it is debatable whether $\Delta\psi$ is a kinetically competent intermediate for the energy-coupled reactions of mitochondria [see, for example, Williams (1978), Westerhoff et al. (1984), Rottenberg (1985), and Slater et al. (1985)], $\Delta\psi$ appears to be in equilibrium with the principal intermediate of the mitochondrial energy-linked reactions (Matsuno-Yagi & Hatefi, 1987). Therefore, it is possible that fractional inhibition of F_oF_1 complexes increases the steady-state free energy of the system. It has been suggested that the F_oF_1 complexes are a major route of proton leakage in respiration-energized SMP (Zoratti et al., 1986). The increase of steady-state free energy in SMP containing fractionally inactivated F_oF_1 complexes would provide an excellent rationale for the observed increase in the turnover rate of the uninhibited F_oF_1 complexes in these particles. This possibility would also provide an explanation for the converging straight line plots of Figure 5. Thus, the abscissa of Figure 5 (a measure of the number of functional F_oF_1 complexes) could be considered to represent the reciprocal of the steady-state free energy of the particles, i.e., the reciprocal of the concentration of a substrate of F_oF_1 complexes for ATP synthesis. In that case, convergence of the lines with succinate and NADH as respiratory substrates on the ordinate of Figure 5 would mean that at this extrapolated point energy is saturating and no longer a limiting factor in the rate of ATP synthesis.

To the extent examined, the design of the catalytic sector F_1 of the ATP synthase complex has remained essentially the same throughout phylogeny, and the structure of certain subunits of the F_o sector (e.g., subunits *b* and *c* of prokaryotes) has also been retained through evolution (Senior & Wise, 1983; Amzel & Pedersen, 1983; Hatefi, 1985). Particularly interesting is the 70% homology among species in the primary structure of the β subunit of F_1, which carries the catalytic site (Amzel & Pedersen, 1983). Thus, a question of interest is whether the catalytic efficiency of F_oF_1 for ATP synthesis and hydrolysis had also remained the same or been altered during evolution. Appropriate data from bacterial sources for comparison with our results are not available. However, at high light intensity, chloroplasts synthesize ATP at a rate of about 1400 μmol of ATP (mg of chlorophyll·h)$^{-1}$ (Vinkler, 1981). Under these conditions, the apparent K_m^{ADP} is about 60 μM (Vinkler, 1981), which is about the same as the apparent K_m^{ADP} of bovine SMP in the high-K_m–high-turnover mode. Furthermore, assuming a concentration of 0.37 mg of CF_1/mg of chlorophyll (Frasch et al., 1980) and a CF_1 molecular weight of 400 000 (Moroney et al., 1983), the above photosynthetic rate of ATP synthesis would amount to an F_oF_1 turnover rate of 420 s^{-1}. This value is essentially the same as the maximal rate (400 s^{-1}) calculated by Junesch and Gräber (1985) for ATP synthesis by spinach chloroplasts subjected to acid–base transition. Therefore, it appears that at least in chloroplasts and bovine mitochondria the F_oF_1 complexes might have comparable catalytic efficiencies for ATP synthesis.

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Kinetics of Long-Chain (Sphingoid) Base Biosynthesis in Intact LM Cells: Effects of Varying the Extracellular Concentrations of Serine and Fatty Acid Precursors of This Pathway†

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ABSTRACT: Serine palmitoyltransferase (EC 2.3.1.50) catalyzes the condensation of L-serine and palmitoyl-CoA to yield 3-ketosphinganine in the first unique reaction of long-chain (sphingoid) base biosynthesis. The kinetic effects of changing the extracellular concentrations of the precursors for this pathway were studied with LM cells by following the incorporation of L-[3-^{14}C]serine into the long-chain base (i.e., sphinganine and sphingenine) backbones of complex sphingolipids. [^{14}C]Serine was taken up by the cells and rapidly reached steady-state concentrations similar to those of the medium. From the cellular [^{14}C]serine concentrations and specific activities, the apparent V_{max} [14 pmol min^{-1} (10^6 cells)$^{-1}$] and K_m (0.23 mM) values for long-chain base synthesis were determined and found to be essentially identical with those for serine palmitoyltransferase assayed in vitro [i.e., 13 pmol min^{-1} (10^6 cells)$^{-1}$ and 0.27 mM, respectively]. The other precursor, palmitic acid, was also taken up rapidly and increased long-chain base biosynthesis in a concentration-dependent manner. This effect was limited to palmitic acid and matched the known specificity of serine palmitoyltransferase for saturated fatty acyl-CoA's of 16 ± 1 carbon atoms. These studies delineate the influence of extracellular precursors on the formation of the sphingolipid backbone and suggest that the kinetic properties of serine palmitoyltransferase govern this behavior of long-chain base synthesis in intact cells.

Sphingolipids are elaborations of a group of compounds referred to as long-chain (or sphingoid) bases, which encompass sphingenine, sphinganine, 4-D-hydroxysphinganine (phytosphingosine), and homologues of these compounds (Karlsson, 1970).[1] Long-chain base biosynthesis begins with the condensation of serine and palmitoyl-CoA to form 3-ketosphinganine [Snell et al., 1970; for recent reviews, see Kishimoto (1983) and Radin (1984)], which is catalyzed by serine palmitoyltransferase, a pyridoxal 5′-phosphate dependent enzyme. Serine palmitoyltransferase has been thought to be a control point for this pathway because (1) it catalyzes the first unique and committed step, (2) the reaction is essentially irreversible, (3) the reaction is rate limiting because free long-chain bases do not accumulate as biosynthetic intermediates in intact cells (Wang & Merrill, 1986), (4) the activity correlates with the approximate sphingolipid composition of tissues and changes when tissues undergo increased long-chain base biosynthesis (Williams et al., 1984a; Merrill et al., 1985), (5) mechanism-based inhibitors of serine palmitoyltransferase decrease the levels of some complex sphingolipids (Sundaram & Lev, 1985), and (6) other factors that decrease sphingolipid

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[1] The nomenclature used in this paper generally conforms to the recommendations of IUPAC/IUB; however, the terms sphingenine and sphinganine have been used for the long-chain bases with and without the 4-trans double bond, without specification of the alkyl chain length. Other frequently used names for sphingenine and sphinganine are sphingosine and dihydrosphingosine, respectively.

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