Studies on the Mechanism of the Conversion of Coupling Factor 1 from Chloroplasts to an Active Adenosine Triphosphatase*

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ABSTRACT: Coupling factor 1 from spinach chloroplasts has latent ATPase activity which becomes expressed upon heat treatment. Optimal conditions for this conversion were established. The heat-activated ATPase was further stimulated by incubation with thiol compounds. Subsequent removal of the thiol on a Sephadex column resulted in inhibition of ATPase activity without restoration of the coupling activity. The activation energy for the conversion from latent into manifest ATPase was found to be much lower in the presence than in the absence of thiol during heating and still lower and independent of thiols for the inactivation of coupling activity. In the presence of 8 M urea or 0.1% sodium dodecyl sulfate both CF_1 and ATPase were found to contain, after reduction, 12 SH groups per mole in agreement with data from amino acid analysis. When the reduction step was omitted only 8 SH groups were titrable in urea or sodium dodecyl sulfate in both CF_1 and ATPase. Measurements of SH groups with dithionitrobenzoate in the absence of denaturing solvents yielded values of 2 SH per mole for the native CF_1 and 3 to 4 SH per mole for the heat-activated ATPase.

N-Ethylmaleimide or iodoacetamide inhibited the activation of CF_1 by heat but not by trypsin. The catalytic activity of the activated enzyme was, however, not affected by iodoacetamide, nor was the coupling activity inhibited by either iodoacetamide or *N*-ethylmaleimide. These findings suggest that SH groups which can interact with iodoacetamide participate in the process of heat activation but not in the catalytic activity. It is concluded therefore that the conversion from latent into manifest ATPase represents a conformational change which may proceed *via* disulfide interchange, and that the loss of coupling activity and the activation to an ATPase are two independent processes, the latter being reversible without restoration of the former. The latency of ATPase activity in the chloroplast coupling factor must therefore be recognized as an intrinsic property of the protein.

It was shown in the preceding paper (Farron, 1970) that coupling factor 1 (CF_1)[1] from spinach chloroplasts can be isolated as a homogeneous protein with latent ATPase activity. Transformation of CF_1 to an active ATPase by mild heat treatment did not result in any changes in hydrodynamic properties. No changes could be detected in amino acid composition or in acrylamide gel electrophoresis. It was therefore concluded that, in contrast to mitochondrial coupling factor 1 (Pullman *et al.*, 1960) which forms a dissociable complex with a protein of low molecular weight that inhibits specifically mitochondrial ATPase (Pullman and Monroy, 1963), the chloroplast factor is a single protein with a latent ATPase which can be activated by changes in protein conformation.

It is the purpose of this paper to describe optimal conditions for the transformation of CF_1 to ATPase. This transformation is accompanied by the appearance of titrable SH groups. A partial reversal of this transformation was achieved but the resulting protein which contained latent ATPase activity was lacking coupling activity.

Experimental Procedures

Materials. $^{32}P_i$ was purchased from New England Nuclear Corporation and purified by ashing in 10% Mg $(NO_3)_2$ in ethanol, followed by hydrolysis in 2 N HCl for 30 min. Iodoacetamide-1-^{14}C was purchased from Nuclear Chicago Corporation and was used without further purification. *N*-Ethylmaleimide was obtained from Schwarz BioResearch, Inc., dithiothreitol was purchased from Calbiochem. All other chemicals were obtained from Sigma Chemical Co.

EDTA-treated chloroplasts were prepared as previously described (McCarty and Racker, 1966). CF_1 was prepared as described in the accompanying paper (Farron, 1970).

Methods. Cyclic photophosphorylation in the presence of pyocyanin was assayed as previously described (McCarty and Racker 1966). Ca^{2+}-dependent ATPase activity after activation by trypsin was assayed as described by Vambutas and Racker (1965). $^{32}P_i$ esterification was measured according to Lindberg and Ernster (1956); P_i was determined by the method of Lohmann and Jendrassik (1926); chlorophyll was determined as described by Arnon (1949). Protein concentrations of CF_1 were determined spectrophotometrically according to Warburg and Christian (1941). After comparison of this method with direct dry weight determinations of CF_1, the values obtained by spectral analysis were multiplied by 1.85 in order to convert them into dry weight of protein. The procedure of Lowry *et al.* (1951) was used when measurements of protein were made in the presence of ATP, and the value obtained was multiplied by a factor of 1.15 to obtain the equivalent dry weight.

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: CF_1, chloroplast coupling factor; NEM, *N*-ethylmaleimide.

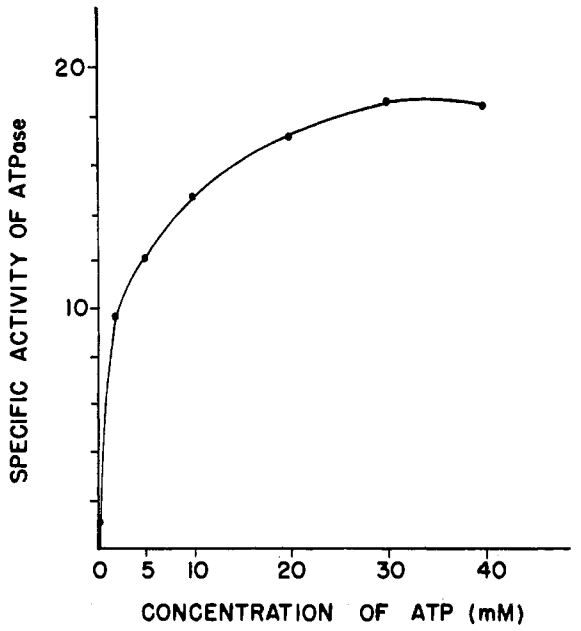

FIGURE 1: Effect of ATP concentration during heat activation on yield of ATPase. An aliquot of enzyme [stored in 2 M $(NH_4)_2SO_4$] was centrifuged and desalted on a 1 × 10 cm Sephadex G-50 column equilibrated with 40 mM Tris-Cl, pH 8.0, and 2 mM EDTA. To an aliquot of 180 μg of CF_1 an amount of ATP was added to a volume of 0.30 ml to give the final concentration indicated in the figure. The reaction mixture was immersed in a water bath at 64°. Heat activation was stopped by addition of 3 volumes of Tris-Cl–EDTA buffer, pH 8.0, kept at 20°. Samples (3 μg) were assayed for ATPase activation as described under Methods.

Definition of Unit and Specific Activity. A unit of ATPase is defined as the amount of enzyme which catalyzes the hydrolysis of 1 μmole of ATP per min under the specified assay conditions. Specific activity is expressed as units per milligram of dry weight of protein.

Results

Activation of ATPase by Heat. Vambutas and Racker (1965) observed that crude extracts of chloroplasts containing coupling activity exhibited ATPase activity after heating to 65° for 2 min in the presence of 1–4 mM ATP. However, the ATPase activity thus obtained was always lower than that after trypsin treatment. It can be seen from Figure 1 that 30–40 mM ATP was required to obtain maximal ATPase activity by heat treatment at 64°. Studies at various temperatures (Figure 2) revealed that (a) ATP appeared to be required for the stabilization of CF_1 and ATPase rather than for the activation, since measurable activation occurred in the absence of added ATP (lowest curve); and (b) less ATP was required for stabilization at lower temperatures. A meaningful kinetic analysis of this system was difficult since the observed velocity of enzyme activity was the resultant of activation and denaturation proceeding simultaneously but apparently at very different relative rates depending on ATP concentration and temperature.

When the time course of heat activation was measured at 62° at optimal ATP concentration and over a range of pH values from 6.3 to 8.5 (pH was measured at 62°), the initial rates of activation as well as the final activity after about 8 min increased with increasing pH. Prolonged periods of heating (up to 120 min) at pH values between 6.3 and 7.8 gave no further activation but at higher pH the activity declined

TABLE I: Protection of CF_1 by Various Nucleotides During Heat Activation.[a]

Addition to Heat Activation Mixture	ATPase, μmoles of P_i/min per mg of Protein	% of Control
None	1.2	10.4
ATP	11.65	100.0
CTP	4.3	37.0
GTP	2.0	17.0
ITP	2.3	20.0
UTP	5.8	50.0
PP_i	0.99	8.5

[a] Conditions and preparation of enzyme were as described in the legend to Figure 1, except that nucleotides were added to give a final concentration of 4 mM. ATPase activity obtained with ATP is referred to as 100%.

TABLE II: Reversal of Heat Activation.[a]

Sequential Treatment of CF_1	ATPase, μmoles of P_i/min per mg	Photophosphorylation, μmoles of P_i Esterified/hr per mg of Chlorophyll above Control
None	1.1	407
Heating, Sephadex	22.0	177
50 mM dithiothreitol	38.2	159
Sephadex	3.6	102
Trypsin	17.6	
No CF_1 added (control)		67

[a] CF_1 (1.5–1.8 mg/ml) was heated under the conditions described in the legend to Figure 1 and cooled on ice. The enzyme was precipitated with an equal volume of saturated ammonium sulfate, centrifuged, and passed through a 1 × 15 cm column of Sephadex G-50 (fine) equilibrated with 40 mM Tris-Cl, pH 8.0. Dithiothreitol was added to give a final concentration of 50 mM. After 1 hr the total incubation mixture was again passed through a 1 × 15 cm Sephadex column to remove the thiol. After maximum inhibition was obtained, an aliquot was reactivated by trypsin as described by Vambutas and Racker (1965). The amount of trypsin necessary to give maximal reactivation of the remasked enzyme was found to be one-fourth of that necessary to give maximal activation of the native enzyme in the same time.

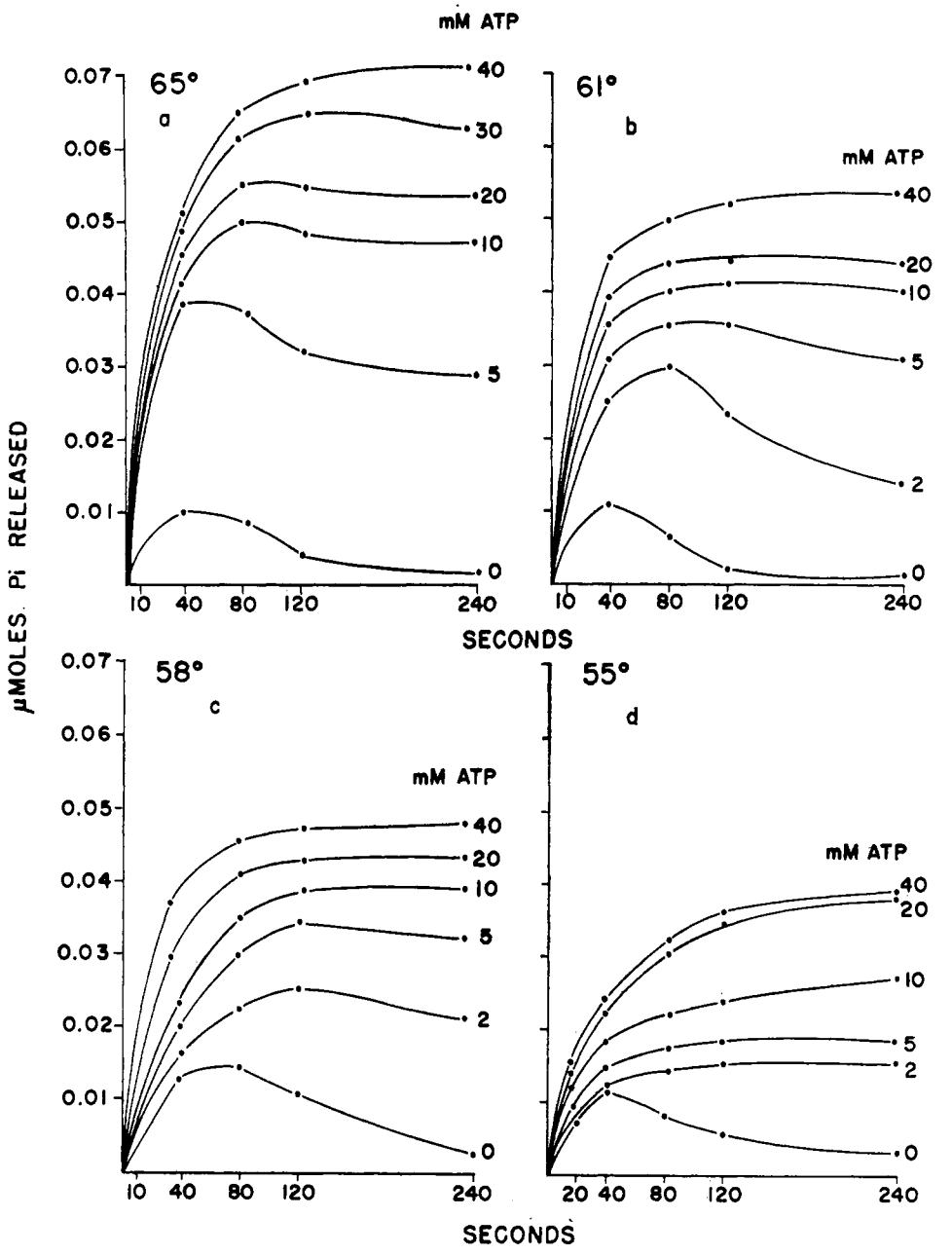

FIGURE 2: Effect of ATP concentration on the rate of heat activation. The enzyme was prepared as described in the legend to Figure 1; aliquots were assayed at the times indicated. (a) The enzyme was activated at 65°; (b) the enzyme was activated at 61°; (c) the enzyme was activated at 58°; (d) the enzyme was activated at 55°.

rapidly. This inactivation at alkaline pH was even more pronounced at 65°.

Heat activation at optimal ATP concentration and at optimal pH was independent of protein concentration between 0.5 and 2.0 mg per ml. At concentrations higher than 2.0 mg/ml aggregation occurred with slight loss in activity, below 0.5 mg/ml activity declined rapidly. The latter effect could not be offset by addition of bovine serum albumin to dilute solutions of CF_1.

The effectiveness of nucleotides other than ATP during heat activation was investigated. UTP was 50% as effective as ATP, all other nucleotides tested were less effective and pyrophosphate was completely ineffective. The data are summarized in Table I.

Figure 3 shows the effect of varying the temperature in the presence and absence of dithiothreitol on the activation of ATPase. At all temperatures below 65°, maximum activation was achieved within 6 to 8 min and further heating up to 2 hr did not change this value. The rates of activation as well as the plateau values increased with the temperature except at 65° when denaturation was rapid relative to activation and marked losses were incurred after 3 min. Dithiothreitol at 5 mM nearly

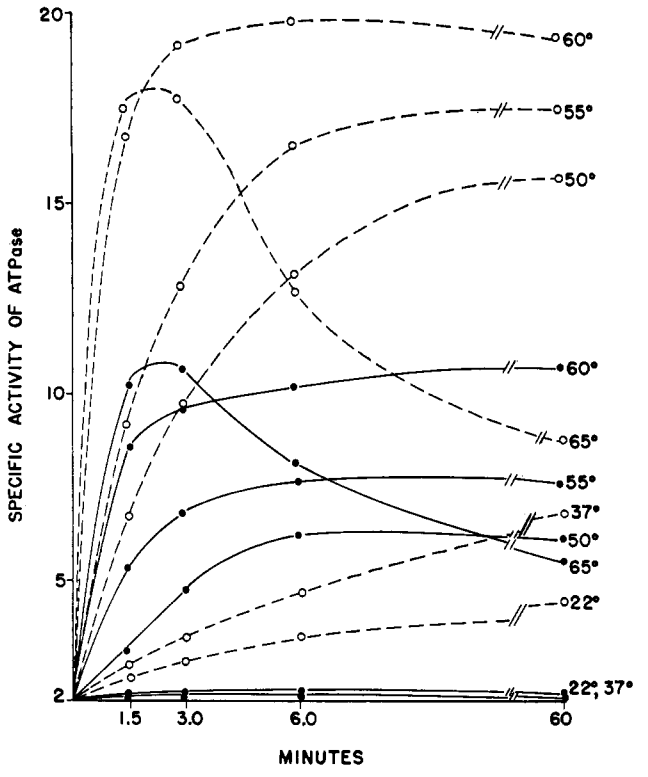

FIGURE 3: Effect of temperature on heat activation of ATPase. The enzyme was prepared as described in the legend to Figure 2 and heated in the presence or absence of 5 mM dithiothreitol at the temperatures indicated; aliquots were assayed for ATPase activity at the times indicated: (-○-) with dithiothreitol; (-●-) without dithiothreitol.

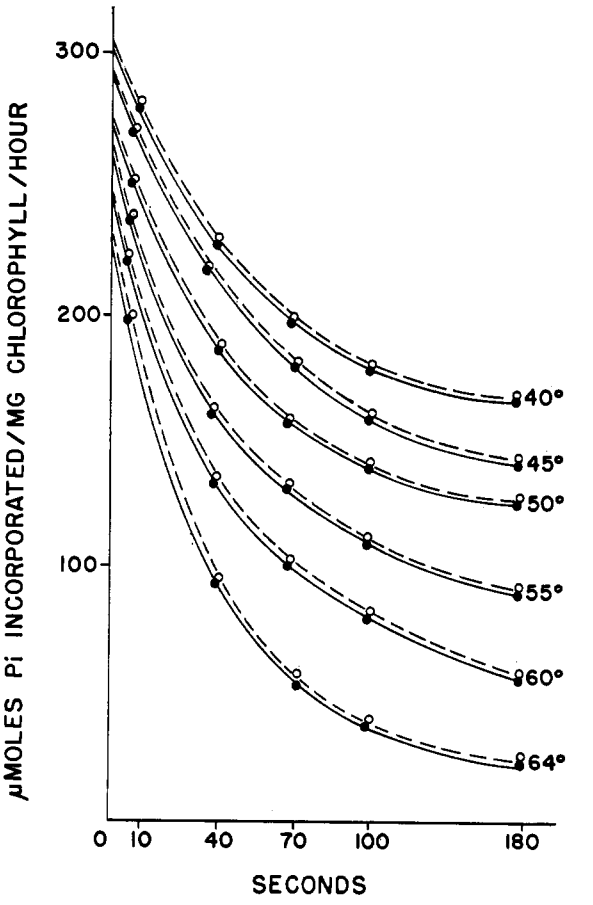

FIGURE 4: Effect of temperature on loss of coupling activity. The enzyme was prepared and heated under conditions identical with those described in the legend to Figure 3; aliquots were assayed at the times indicated for coupling activity: (-○-) with dithiothreitol; (-●-) without dithiothreitol.

doubled the rates of heat activation as well as the maximal ATPase activity obtained without altering the shape of the curves.

Since activation of the chloroplast factor to an ATPase accompanied by a loss of its ability to stimulate photophosphorylation, this inactivation was followed as a function of temperature. The results summarized in Figure 4 show that under the test conditions neither the rate nor the extent of inactivation were affected by the presence of 5 mM dithiothreitol during heating.

Plotting the plateau values obtained at each temperature for ATPase activity gained and for coupling activity lost against temperature (Figure 5), it can be seen that coupling activity declines at temperatures lower than those necessary to elicit ATPase activity and that activation of ATPase occurs over a much narrower range of temperatures than the loss of coupling activity, suggestive of a "melting out" process. Plotting the logarithm of the initial rates (Figure 6) of either process against the reciprocal of the absolute temperature one obtains a value of 35 kcal/mole of enzyme for the $\Delta\epsilon^{\ddagger}$ for the heat activation of ATPase in the absence of dithiothreitol and a $\Delta\epsilon^{\ddagger}$ of 17 kcal/mole in the presence of 5 mM dithiothreitol, and a $\Delta\epsilon^{\ddagger}$ of 6–7 kcal/mole for the process responsible for the loss of coupling activity under either conditions.

Reversal of ATPase Activity. Attempts to reverse heat activation by slow cooling were completely unsuccessful, but since SH groups appeared to be involved in the activation process, the effect of thiol on reversal was investigated. It was found that significant inhibition of ATPase activity could be achieved by incubating the heat-activated ATPase with 50 mM dithiothreitol at room temperature, usually for 1 hr, and subsequent removal of the thiol on a Sephadex column equilibrated with 40 mM Tris-Cl–2 mM EDTA buffer, pH 8.0. During the incubation with dithiothreitol the ATPase activity increased to values ranging from 140 to 170% (taking the activity obtained on heating as 100%). Upon removal of the thiol the enzyme emerged from the column with a residual activity of 30 to 50%, which further decreased to 15–20% on standing at room temperature for an additional hour. Passage of the heat-activated enzyme through Sephadex did not change the activity of control preparations which had not been previously incubated with dithiothreitol. Full ATPase activity was regained upon treatment with trypsin and partial reactivation was achieved by a second heat treatment. The inhibition of ATPase activity was not accompanied by a recovery of coupling activity. These results are summarized in Table II.

Attempts to regain coupling activity included, in addition to slow cooling, short exposure to 6 M urea as well as 1-hr incubation with thiol at 20° on slow removal of the reagents by dialysis against 40 mM Tris-Cl–2 mM EDTA buffer, pH 8.0, containing 4 mM ATP. However, these treatments resulted not only in complete loss of coupling activity but in an irreversible loss of ATPase activity as well.

TABLE III: Determination of Cysteine and Cystine Content of CF_1 and ATPase.[a]

Treatment of CF_1	Additions during Incubations	Protein, μg	OD_{412}	cpm	mμmoles of SH/330 μg of Protein
Experiment 1					
None	8 M urea	320	0.108		8.15
		640	0.211		8.05
	8 M urea, 0.05 M dithiothreitol	300	0.151		12.2
		600	0.326		12.0
Experiment 2					
None	0.01 M iodoacetamide-^{14}C for 1 hr	388		452	2.02
		209		259	2.15
	0.01 M iodoacetamide-^{14}C for 2 hr	380		425	1.95
		280		318	1.95
63°, 3 min	0.01 M iodoacetamide-^{14}C	83		159	3.3
		166		340	3.5
63°, 3 min	8 M urea, 0.01 M iodoacetamide-^{14}C	108		577	9.2
		216	1104	1104	8.9
8 M urea	8 M urea, 0.01 M iodoacetamide-^{14}C	580		3095	9.2
		1160		6596	9.8
8 M urea–0.05 M dithiothreitol	8 M urea, 0.01 M iodoacetamide-^{14}C	197		1440	12.7
		197		1503	13.2

[a] For titration of SH with dithionitrobenzoate (expt 1) solutions of CF_1 containing 3.7 mg of protein per ml in 50 mM Tris-Cl_1–2 mM EDTA, pH 8.0, were incubated for 1 hr in 8 M urea or 8 M urea and 50 mM dithiothreitol, precipitated at 2 M ammonium sulfate, resuspended in a minimal volume of the same Tris–EDTA buffer containing 8 M urea, and passed through a 1 × 15 cm Sephadex G-50 (fine) column equilibrated with 8 M urea. The effluent fractions were assayed for SH content. The small amount of dithiothreitol which precipitated with the protein was separated completely on the column. For titration of SH with iodoacetamide-^{14}C (expt 2) aliquots of enzyme before and after heating were incubated with iodoacetamide-^{14}C (specific activity = 192 cpm per mμmole) under the same conditions as in expt 1, precipitated with an equal volume of saturated ammonium sulfate, redissolved in the original volume of buffer containing iodoacetamide-^{12}C, reprecipitated at 2 M ammonium sulfate, and passed through a column as indicated above.

Titration of SH Groups in CF_1 and ATPase. In view of the apparent participation of SH groups in activation and re-masking of ATPase activity, it became important to establish whether a net change in the number of free SH groups took place during either process. SH groups were therefore measured before and after heat activation (a) in buffer, (b) in 8 M urea or 0.1% sodium dodecyl sulfate, (c) after reduction in 8 M urea, in two independent ways as described in the legend to Table III. The results summarized in Table III show that about 2 moles of SH per mole of enzyme was accessible to 5,5′-dithiobis(2,2′-nitrobenzoic acid) or to iodoacetamide in the native protein and that about 2 additional moles of SH per mole of enzyme reacted after heat activation. In the SH determination full color development with dithionitrobenzoate was obtained within 20 min both with the native and the heat-activated proteins. Under denaturing conditions 8 equiv of SH was found, whether measured before or after heating, indicating no net change in free sulfhydryl content during heat treatment. After reduction of the protein under denaturing conditions, 12 moles of SH per mole of enzyme was found indicating the presence of two disulfide bonds in the molecule.

Effect of SH-Specific Inhibitors. The data presented above indicated an involvement of SH groups in the activation and reversal of ATPase in contrast to coupling activity which shows no such participation. The effects of SH-specific inhibitors on either activity as well as on the activation process proper was therefore studied. The data summarized in Table IV show that when native enzyme was exposed to 0.01 M NEM for 1 hr at 20°, coupling activity remained unaffected. Yet, if this preparation was heat activated after removal of the excess inhibitor, only 29% ATPase activity of the control value was obtained. Even exposure of CF_1 to NEM for 3 hr did not further decrease the final ATPase activity. The low activity of this NEM-treated, heated enzyme appears to be largely due to inhibition of the activation process since up to 80% ATPase activity could be elicited from this preparation by exposure to trypsin.

The heat-activated ATPase had usually some (10 to 25%) residual coupling activity. When heat-activated ATPase was treated with 0.01 M NEM the ATPase activity was inhibited 87% whereas the residual coupling activity was not further reduced by this treatment. It should be pointed out, however, that coupling activity measured by stimulation of photophosphorylation in subchloroplast particles which contained residual CF_1 activity may be due to the fulfillment of a structural requirement for CF_1. It has been shown that compounds such as dicyclohexylcarbodiimide can partially substitute for CF_1 in this role (McCarty and Racker, 1967).

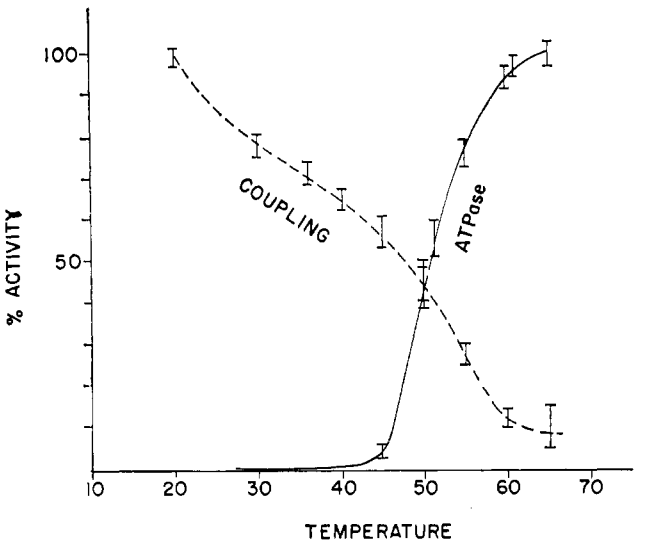

FIGURE 5: Appearance of ATPase activity and disappearance of coupling activity as a function of temperature. The plateau values obtained in the experiments shown in Figures 3 and 4 are plotted against the temperature of heat activation.

Since NEM inhibited the activated ATPase, the possibility was considered that treatment of the native protein with NEM prior to heat activation led to an inhibited, latent ATPase rather than to an interference with the activation process. However, two observations are in favor of the latter interpretation. (1) Iodoacetamide does not inhibit activated ATPase; however, if native CF_1 is incubated with 0.01 M iodoacetamide for 1 hr at room temperature, the excess iodoacetamide removed, and the alkylated enzyme heated, only 40 to 50% ATPase activity could be obtained. (2) Native alkylated CF_1 can be activated with trypsin to give 100% ATPase activity. The data are summarized in Table V. The question then arose whether NEM inhibited ATPase by virtue of its different molecular architecture or whether it reached cysteine residues that were not reached by iodoacetamide. Figure 7 shows that ATPase activity remained constant in the presence of 0.01 M iodoacetamide for 60 min (this time had been found to be sufficient for complete labeling with radioactive iodoacetamide) and declined rapidly after addition of 0.01 M NEM. Titration of SH equivalents in 0.1% dodecyl sulfate before and after reaction with NEM indicated the loss of one SH group per mole of enzyme (Table VI).

TABLE IV: Effect of *N*-Ethylmaleimide on Coupling and ATPase Activities.[a]

Treatment of CF_1	Coupling Activity, %	ATPase Activity, %
1. None	100	6.5
2. 0.01 M NEM	97	3.5
3. Heat activated	26	100
4. Heat activated, then 0.01 M NEM	22	13
5. 0.01 M NEM, heated after removal of excess NEM	15	29
6. As in 5, then trypsin (trypsin:enzyme 1:4 by weight)	Not assayed	79

[a] Aliquots of enzyme (1.5 mg/ml) were incubated with 0.01 M NEM in 40 mM Tris–2 mM EDTA buffer, pH 7.4, for 1 hr; the protein precipitated at 2 M ammonium sulfate, was redissolved in Tris–EDTA buffer, pH 8.0, and passed through a 1 × 15 cm Sephadex G-50 (fine) column before assay; 100% of coupling factor activity represents a rate of 295 μmoles of P_i esterified per hr per mg of chlorophyll and a 4.3-fold stimulation over the phosphorylation rate of EDTA–chloroplasts, without CF_1 added. 100% ATPase activity = 28 μmoles of P_i per min per mg of protein.

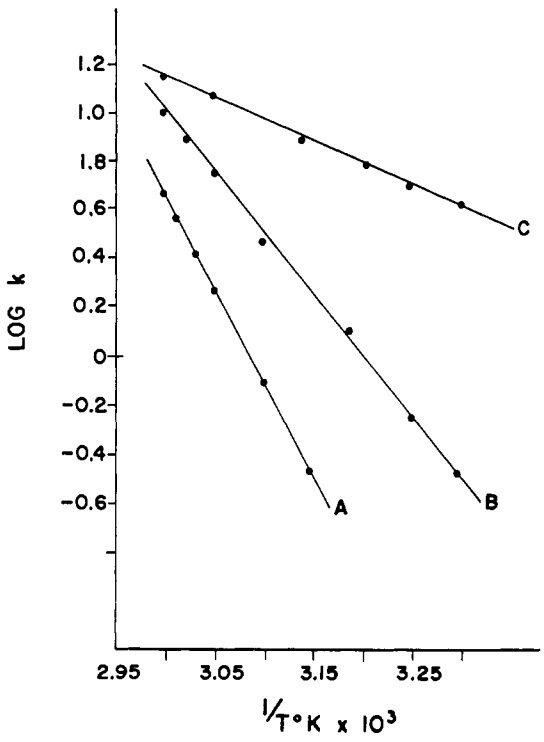

FIGURE 6: Determination of the activation energy for the conversion of CF_1 into ATPase: (A) activation of ATPase in the absence of thiol; (B) in the presence of thiol; (C) loss of coupling activity.

Discussion

The physical identity of F_1 from beef heart mitochondria with a Mg^{2+}-dependent ATPase has been established (Penefsky *et al.*, 1960). However, the hydrolytic activity could be inhibited by a low molecular weight protein of mitochondrial origin (Pullman and Monroy, 1963) without interfering with the coupling activity of F_1. The physical identity of CF_1 from spinach chloroplasts with a masked ATPase has now been established as documented in this and the preceding paper. In contrast to the F_1–inhibitor complex which can be dissociated by mild heat treatment without loss of coupling activity,

TABLE V: Inhibition of the Heat-Activation Process by Iodoacetamide.[a]

Treatment of CF_1	ATPase: μmoles of P_i/min per mg of Protein
64°, 3.5 min	19.2
0.01 M iodoacetamide, remove iodoacetamide, then 64°, 3.5 min.	9.1
64°, 3.5 min, then 0.01 M iodoacetamide	18.7
Controls with tryptic digestion:	
Trypsin-activated ATPase	19.0
0.01 M iodoacetamide, then trypsin treatment	18.7
Trypsin-activated ATPase, 0.01 M iodoacetamide	19.0

[a] Aliquots of CF_1 (1.5 mg/ml) were heated in 40 mM Tris-Cl, pH 7.8, 2 mM EDTA in the presence of 35 mM ATP for 3.5 min at 64° and after alkylation as indicated; all incubations with iodoacetamide were for 1 hr at 20°.

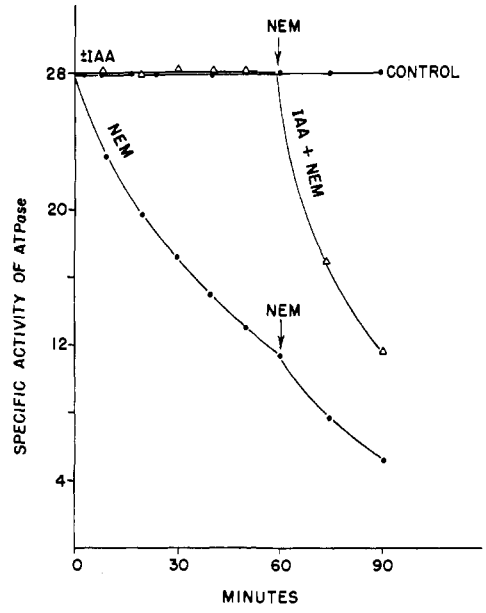

FIGURE 7: Effect of *N*-ethylmaleimide and iodoacetate on ATPase. The experimental conditions are described in the text.

heat treatment of CF_1, while activating a Ca^{2+}-dependent ATPase, results in a pronounced loss in coupling activity. Moreover, no dissociable small molecular weight protein could be detected after isolation of the heated protein on a Sephadex column.

A comparison between the native and the heat-activated preparations had established that the two protein species had identical amino acid compositions, molecular weights, and electrophoretic mobilities in polyacrylamide gel and absorption spectra (Farron, 1970). It was inferred from these findings that conformational changes are responsible for the appearance of ATPase activity and that the loss of coupling activity, brought about by controlled heating, must be small and localized. In addition, the present study indicates that two separate sites are independently affected during heating. Thus, coupling activity begins to decay at temperatures (30°) far too low to elicit ATPase activity. Neither the rates nor the extent of this inactivation is affected by the presence or absence of thiol compounds, whereas both rates and extent of ATPase activity gained are significantly potentiated by low concentrations of dithiothreitol during heating.

The reversal of ATPase activity follows the same pattern: preincubation with dithiothreitol was essential to inhibit ATPase activity, yet this treatment did not restore coupling activity. In addition, SH-specific inhibitors severely inhibited ATPase, again, without effect on coupling activity. From these observations it appears that activation and inhibition of the ATPase activity is associated with changes in the sulfhydryl distribution in the protein. The fact that the total number of SH groups is the same before and after heating indicates that no net reduction or net oxidation takes place during activation. It is therefore suggested that the changes responsible for the appearance of ATPase occur *via* disulfide interchange. It has been shown that low concentrations of thiol stimulate disulfide interchange (Givol *et al.*, 1964). Thus the above mentioned effects of thiol on activation and reversal are most plausibly explained by participation of disulfide interchange. This interpretation is strengthened by the observation that only heat activation, but not tryptic activation, was inhibited by alkylation of sulfhydryl residues prior to activation. That this inhibition was not complete is not surprising since only two out of a total complement of eight free SH groups per mole of enzyme could be alkylated. Some of the free SH groups (though inaccessible to the alkylating agent) may still participate in the disulfide interchange. The fact that preparations heated after alkylation of cysteinyl residues had only 30–40% ATPase activity but had as little coupling activity as

TABLE VI: Titration of Sulfhydryl Groups in Heat-Activated ATPase after Alkylation.[a]

Treatment	μmoles of SH/330 μg of Protein	ATPase: μmoles of P_i/min per mg of Protein
1. 0.01 M iodoacetamide for 1 hr	4.2	25
After trypsin treatment	4.0	28.7
2. 0.01 M iodoacetamide for 1 hr		
Then NEM	3.3	5.1
After trypsin treatment	3.2	23.5

[a] Samples were heat activated as described previously (Farron, 1970). Sulfhydryl groups were determined in 0.1% sodium dodecyl sulfate with dithionitrobenzoate after the unreacted alkylating agents had been removed on a 1 × 15 cm column of Sephadex G-50 and again after reactivation by trypsin.

100% active ATPase, would seem to substantiate the view that the conformational changes responsible for the two observable activity changes proceed not only at two different sites within the protein but also independently of each other.

It was shown by Bennun and Racker (1969) that following heat treatment CF_1 no longer attached to CF_1-deficient chloroplast particles. It therefore seems likely that activation of the ATPase activity *per se* did not destroy the coupling activity. This view is supported by the observation [McCarty and Racker (1968)] that activation of the Ca^{2+}-dependent ATPase of CF_1 by exposure to dithiothreitol instead of heat did not destroy the coupling activity. However, on addition of the dithiothreitol-activated CF_1 to CF_1-deficient chloroplast particles, the Ca^{2+}-dependent ATPase activity became masked, whereas the Mg^{2+}-dependent and light-induced ATPase activity described by Petrack *et al.* (1965), was stimulated (McCarty and Racker 1968; Bennun and Avron 1965).

It thus becomes increasingly clear that the ATPase activity of enzymes which participate in the phosphorylation mechanism is an artifact arising from the transphosphorylating capacity of the coupling factor. It is of interest to note that this undesirable side reaction with water is prevented in spinach chloroplasts by a device built into the enzyme itself, whereas in mitochondria a separate and dissociable protein is responsible for restricting the ATPase activity. It is conceivable that this difference in the control mechanism is related to the relative ease of reversal of phosphorylation (*e.g.*, $^{32}P_i$–ATP exchange) in the case of mitochondria and the apparent irreversibility of the phosphorylation system in chloroplasts. In line with this view is the observation that dithiothreitol not only activates the ATPase, but also allows a $^{32}P_i$–ATP exchange (McCarty and Racker, 1968) and an H_2O-^{18}O–P_i exchange (Skye *et al.*, 1967) to take place in chloroplasts. It may be hoped that a comparative analysis of the structure of F_1 and CF_1 may yield further information on different physiological properties of the energy-generating system of the organelles in which these proteins function.

Acknowledgments

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