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KINETICS OF HYDROGEN-DEUTERIUM EXCHANGE IN ATPase FROM A THERMOPHILIC BACTERIUM PS3

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SUMMARY: The kinetics of the hydrogen-deuterium exchange reaction in a stable ATPase (TF_1) from a thermophilic bacterium PS3 was followed by infrared absorption measurements. The rates of the hydrogen-deuterium exchange reactions decreased in following order; free form, TF_1·ADP, TF_1·ATP and TF_1·AMP-P(NH)P. TF_1 does not dissociate into subunits even in the absence of nucleotides, thus differences in exchange likely reflect differences in conformations of subunits. These results indicate that the structure is most restricted when ATP or AMP-P(NH)P is bound to the enzyme.

INTRODUCTION: In oxidative phosphorylation and photophosphorylation, coupling factor 1 (F_1) is known to catalyze the synthesis of ATP from ADP and inorganic phosphate (1). Recently, two of us (M.Y. and Y.K.), in collaboration with Sone and Hirata, purified an ATPase (TF_1) from a thermophilic bacterium PS3 (2). This ATPase is stable enough to carry out a reconstitution of proteoliposome (3) and the other experiments related to oxidative phosphorylation (4-6).

During the phosphorylation, the trans-membrane electrochemical potential energy of protons provides the energy source for synthesizing ATP (1) and Boyer has suggested that ATP

Abbreviations used are AMP-P(NH)P, adenylyl-imidodiphosphate; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; TF_1, soluble ATPase, i.e., coupling factor 1 obtained from a thermophilic bacterium PS3; CF_1, chloroplast coupling factor 1; HMM, heavy meromyosin from rabbit muscle; EF-Tu, polypeptide chain elongation factor Tu.

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formation is accomplished through energy-linked conformational changes in the ATPase complex (1). The hydrogen isotope exchange method is a useful technique to study a protein conformational change or a dynamic structural fluctuation of biopolymers. Ryrie et al. showed that illumination of chloroplast induced conformational changes in CF_1 of chloroplasts as assessed by tritium exchange (7). In this communication, we report the effects of various adenine nucleotides on the kinetics of the hydrogen-deuterium exchange reaction of peptide groups of TF_1. Mesophilic F_1 dissociates easily, making exchange results difficult to interpret. But thermophilic F_1 is sufficiently stable that no dissociation takes place even in the absence of a nucleotide (8).

MATERIALS AND METHODS: TF_1 was prepared according to the method of Yoshida et al. (2). AMP, ADP, ATP and an ATP-analogue, AMP-P(NH)P were purchased from Boehringer. Hepes was obtained from Nakarai Chemicals, Kyoto, and sodium azide (NaN_3) was from Wako Chemicals, Osaka. The hydrogen-deuterium exchange experiment was carried out as follows; 200 µl of a TF_1 solution (7.5 mg/ml) in 5 mM Hepes/Na (pH 7.5) (when indicated with 1.25 mM sodium azide) was lyophilized, and this solid was dissolved at time zero in 50 µl ^2H_2O solution of 200 mM Hepes/Na + $MgCl_2$ + the indicated amount of a nucleotide. The amount of the peptide NH groups of TF_1 remaining undeuterated at a given time was determined by an infrared absorption measurement (9). This was performed with a Perkin-Elmer 621 infrared spectrophotometer and a Digilab FTS-14 Fourier Transform Spectrophotometer. The amount of undeuterated peptide groups of TF_1 was taken as propotional to the ratio of the absorbance of amide II (at 1550 cm^{-1}) to that of amide I (at 1650 cm^{-1}), i.e., A(amide II)/A(amide I). The baseline of the amide II band was taken as the absorption curve of a solution of completely deuterated TF_1, which was obtained by incubation in ^2H_2O at pH 7.6 and 57°C for 3 days. The A(amide II)/A(amide I) value for completely undeuterated protein was assumed to be 0.45 (10-12). Infrared difference spectra were obtained by the use of an on-line computer system of the Digilab FTS-14, and were output on an XY-plotter. Details of such experiments are described in our previous papers (11,13,14).

RESULTS: The infrared absorption spectrum of TF_1 changes as its deuteration prodeeds (Fig. 1). Apparently, amide II band at 1550 cm^{-1} (undeuterated peptide N^1H groups) decreased, while amide II' band at 1450 cm^{-1} (deuterated peptide N^2H groups) increased. Some

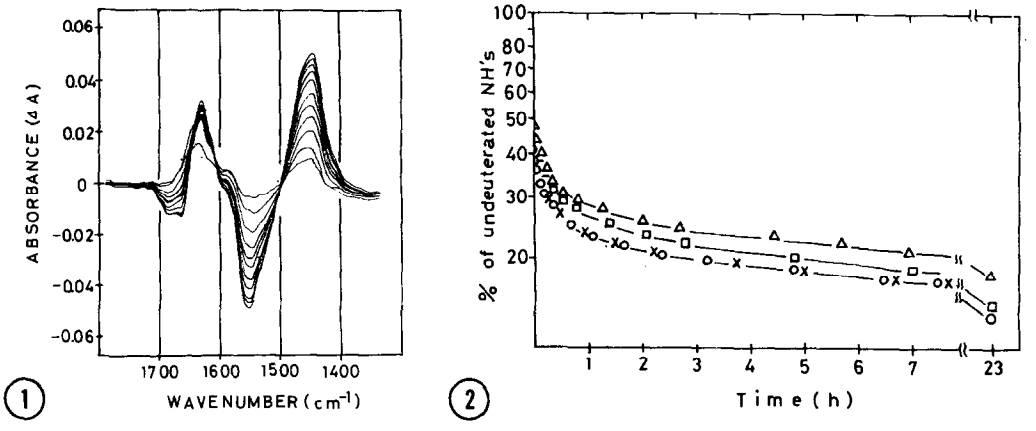

Figure 1. A series of difference spectra showing a time dependent lowering of the 1550 cm^{-1} absorption peak and a rising of the 1450 cm^{-1} peak in a course of deuteration of TF_1 at 25°C and pH 7.60. Infrared absorption spectra were stored at 5, 11, 20, 35, 47, 68, 105, 135, 170, and 235 min after TF_1 was dissolved in ^2H_2O soluiton, the spectrum at 2.5 min was subtracted from each of these spectra, and the results were recorded on an XY recorder. The solution contained 3% (w/v) TF_1, 20 mM AMP-P(NH)P, 20 mM $MgCl_2$ and 5 mM NaN_3 in Hepes/Na buffer (200 mM).

Figure 2. Semilogarithmic plots of the hydrogen-deuterium exchange of the peptide hydrogen in TF_1 at 40°C and pH 7.52 in 200 mM Hepes/Na buffer. (○), TF_1 with 5 mM $MgCl_2$, (×), with 20 mM AMP and 20 mM $MgCl_2$, (□), with 20 mM ADP and 20 mM $MgCl_2$, (△), with 20 mM AMP-P(NH)P and 20 mM $MgCl_2$.

examples of kinetic plots obtained from infrared absorption measurements are shown in Fig. 2. The exchange rates of peptide hydrogens were slower in the presence of ADP and AMP-P(NH)P. In addition, the kinetics was followed at the various concentrations of ADP or AMP-P(NH)P from 0 to 20 mM. From this titration, the dissociation constant of AMP-P(NH)P with TF_1 was roughly estimated as 3-8 x 10^{-4}M. Therefore, in the presence of 20 mM ADP or 20 mM AMP-P(NH)P, almost all of the TF_1 molecules would be in the form of the TF_1·ADP or TF_1·AMP-P(NH)P complex respectively.

In order to express the rate of exchange reaction quantitatively, the ρ value defined as the probability of solvent exposure of the peptide hydrogens was used (11,15,16). The percent distribution

Table I

Probability of solvent exposure of peptide hydrogens of TF_1 under various conditions.

ρ value	Percent of peptide hydrogens of different ρ values in presence at various additions						
	None†	NaN_3†	AMP*	ADP*	AMP-PNP*	AMP-PNP* NaN_3	ATP* NaN_3
$0<\rho<10^{-4}$	42	42	42	45	48	48	48
$0<\rho<10^{-5}$	33	33	34	36	38	38	38
$0<\rho<10^{-6}$	22	22	22	25	28	28	27

† the solutions contained 5 mM $MgCl_2$, and, where added 5 mM NaN_3.

* the solutions contained 20 mM nucleotide and 20 mM $MgCl_2$, and, where added 5 mM NaN_3.

The ρ value is the probability of solvent exposure of the peptide hydrogens, hence, ρ=0 means that the peptide hydrogens cannot be deuterated at all, whereas ρ=1 means that the peptide hydrogens are exposed to the solvent, that is, can be deuterated freely.

In general, the peptide groups undergo deuteration through the following scheme (10),

$$N \rightleftharpoons I \xrightarrow{k_e} \text{exchange},$$

where $k_e = 50(10^{-pH} + 10^{pH-6})10^{0.05(\theta-20)}$ min^{-1}, and θ is the temperature in degrees Celsius.

In this case, $\rho = [I]/([N]+[I])$. For details, see ref. (15).

of TF_1 peptide hydrogens with ρ values of given ranges under various conditions are summarized in Table I. As shown in Table I, sodium azide, an inhibitor, did not affect the exchange rates with or without added nucleotides. Therefore, the rate in TF_1·ATP with sodium azide was considered to be that in TF_1·ATP complex.

The fact that the rate is lower for TF_1·AMP-P(NH)P than that for TF_1·ADP is demonstrated in Fig. 3A by an infrared difference spectrum. In Fig. 3A, the difference spectrum between TF_1·AMP-P(NH)P and TF_1·ADP at an indicated time has a slightly higher peak at 1550 cm^{-1} and a lower peak at 1450 cm^{-1}. Fig. 3B indicates that sodium azide gives no effect on the exchange rates.

Our findings in TF_1 are in agreement with independent

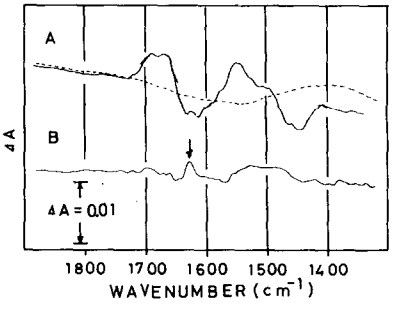

Figure 3. Infrared difference spectra of the following pairs.
(A) (TF_1 with 20 mM AMP-P(NH)P at 235 min)
- (TF_1 with 20 mM ADP at 235 min)
(B) (TF_1 with 20 mM AMP-P(NH)P at 105 min)
- (TF_1 with 20 mM AMP-P(NH)P and NaN_3 at 105 min)
All the solutions contained 3% TF_1 ($w/_v$), 20 mM $MgCl_2$ in 200 mM Hepes/Na buffer (pH 7.60).
The broken curve indicates the baseline between ^2H_2O and ^2H_2O, and the arrow indicates the absorption of adenine.

observations in CF_1 of spinach by Nebedryk-Viala et al. (16), except the following points; TF_1 gives the amide I peak at 1650 cm^{-1} whereas for CF_1 the peak is at 1633 cm^{-1} (at the protein concentration 154 μM). Therefore, TF_1 probably has a smaller amount of β-sheet than CF_1 (17). In contrast to observations with CF_1, the secondary structure of TF_1 seems to be independent of its protein concentration as long as in the range of 130 μM to 8 μM. Finally, the decrease in deuterium exchange rates on binding ATP and ADP were smaller with TF_1 than decreases of rates reported for CF_1 (16). This may reflect a partial dissociation of CF_1 into subunits in the absence of nucleotides, leading to increased hydrogen exchange.

DISCUSSION: Buried peptide groups require a greater free energy of activation for exchange. The difference in activation free energy for buried peptide groups and exposed groups gives an approximation of the energy required for bringing a native conformation to a partially unfolded conformation. Our findings

in the kinetic experiments indicate that the above mentioned activation free energy is smaller for free TF_1 than for TF_1·ADP by 1.8 kJ/mole, and that for TF_1·ADP is also slightly smaller than for TF_1·ATP by 1.2 kJ/mole. The activation free energy in question gives rise to the fluctuation of the polypeptide chain. Thus, the free energy of TF_1·ATP is lower than of TF_1·ADP in the peptide groups which undergo the deuterium exchange. In brief, a more flexible conformation is higher in its free energy.

In contrast to TF_1, the hydrogen-deuterium exchange reaction was slower for heavy meromyosin (HMM) + ADP than for HMM + ATP system as reported previously (18). In addition, the relation of EF-Tu·GDP with EF-Tu·GTP was similar to the situation of HMM (14). Therefore, the free energy of HMM·ADP or EF-Tu·GDP seemed to be lower than that of the enzyme + nucleoside-triphosphate systems in the part of the protein which undergoes the hydrogen exchange.

Such differences in the relative stability of ATPase + ATP systems could to be related to the corresponding function; TF_1 is synthesizing ATP (reversible system), whereas HMM is consuming ATP.

REFERENCES

1. Boyer, P.D., Chance, B., Ernster, L., Michell, P., Racker, E. and Slater, E.C. (1977) Ann. Rev. Biochem. 46, 955-1026.
2. Yoshida, M., Sone, N., Hirata, H. and Kagawa, Y. (1975) J. Biol. Chem. 250, 7910-7916.
3. Sone, N., Yoshida, M., Hirata, H. and Kagawa, Y. (1977) J. Biol. Chem. 252, 2956-2960.
4. Yoshida, M., Sone, N., Hirata, H. and Kagawa, Y. (1977) J. Biol. Chem. 252, 3480-3485.
5. Yoshida, M., Okamoto, H., Sone, N., Hirata, H. and Kagawa, Y. (1977) Proc. Natl. Sci. U.S.A. 74, 936-940.
6. Wakabayashi, T., Kubota, M., Yoshida, M. and Kagawa, Y. (1977) J. Mol. Biol. in press.
7. Ryrie, I.J. and Jagendorf, A. (1972) J. Biol. Chem. 247, 4453-4459.
8. Kagawa, Y., Sone, N., Yoshida, M., Hirata, H. and Okamoto, H. (1976) J. Biochem. (Tokyo) 80, 141-151.
9. Blout, E.R., de Loze, C. and Asadourian,A. (1961) J. Amer. Chem. Soc. 83, 1895-1900.
10. Hvidt, A. and Nielsen, S.O. (1966) Adv. Prot. Chem. 21, 287-386.

11. Nakanishi, M., Tsuboi, M. and Ikegami, A. (1973) J. Mol. Biol. 75, 673-682.
12. Englander, S.W. (1975) Ann. NY. Acad. Sci. 244, 10-27.
13. Nakanishi, M. and Tsuboi, M. (1976) Biochem. Biophys. Acta. 434, 365-376.
14. Ohta, S., Nakanishi, M., Tsuboi, M., Arai, K. and Kaziro, Y. (1977) Eur. J. Biochem. 78, 599-608.
15. Hvidt, A. and Wallevik, K. (1972) J. Biol. Chem. 247, 1530-1535.
16. Nabedryk-Viala, E., Calvet, P., Thiery, J.M., Calmiche, J.M. and Girault, G. (1977) FEBS Lett. 79, 139-143.
17. Miyazawa, Y. and Blout, E.R. (1961) J. Amer. Chem. Soc. 83, 712-719.
18. Yamada, T., Shimizu, H., Nakanishi, M. and Tsuboi, M. (1977) J. Biochem. (Tokyo) 82, 139-144.