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Effects of some alcohols on the conformation of mitochondrial H^+-ATPase complex and F_1-ATPase from pig heart

Yao-jun Guo, Ying Zhang, Yi-xian Liu and Zhi-huan Lin

Institute of Biophysics, Academia Sinica, Beijing (China)

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The conformations of the H^+-ATPase complex and F_1-ATPase in low concentrations of methanol, ethanol, *n*-propanol, *iso*-propanol and *t*-butanol were studied by circular dichroism. For F_1-ATPase, all but methanol first increased and then decreased the circular dichroism magnitude of helical bands as the alcohol concentration was increased. With ethanol, *n*-propanol, *iso*-propanol and *t*-butanol, the α-helix content reached a maximum at about 5% alcohol and began to decrease at 10%. The content of β-sheet showed the opposite effect, reaching a minimum at 5% and increasing slightly at higher concentrations. None of the alcohols studied had a significant effect on the conformation of the H^+-ATPase complex. This difference implies that the alcohols had a greater effect on free F_1-ATPase than on the membrane-bound F_1-ATPase. The hydrophobic protein F_0 and the membrane lipids in the H^+-ATPase complex may stabilize and protect F_1 from the effects of the alcohols.

Introduction

Alongside advances in the study of structure-function relationships of enzymes, increasing attention is being paid to the conformation of enzymes in solution in relation to their biological function. F_1-ATPase is a soluble portion of the H^+-ATPase complex associated with the inner membrane of mitochondria. It is active in the hydrolysis and synthesis of ATP, and plays a central role in the energy transduction of mitochondria. Linnett and Beechey [1] used aurovertin as a fluorescent probe to examine changes in the conformation of the H^+-ATPase complex and F_1-ATPase molecules after they had been linked together by different ligands. Recktenwald and Hess [2] determined the effects of several ions, such as Mg^{2+}, SO^{2-}, and HSO_3^-, on the dynamic properties of yeast mitochondrial F_1-ATPase. Lin et al. [3,4] studied the change in conformation between the activated and inhibited states of mitochondrial soluble F_1-ATPase from pig heart, and investigated the effects of organic solvents such as methanol and ethanol on the hydrolytic activity and conformation of the enzyme.

We have here measured the far ultraviolet CD spectra of the H^+-ATPase complex and F_1-ATPase in low concentrations of methanol, ethanol, *n*-propanol, *iso*-propanol and *t*-butanol, and have analysed the changes in conformation of F_1-ATPase in the two states.

Materials and Methods

The mitochondrial H^+-ATPase complex was prepared and purified from pig heart according to the method of You and Yang [5]; it was stored below $-50\,^\circ C$. The F_1-ATPase was prepared by

Abbreviation: CD, circular dichroism.

Correspondence: Y.-j. Guo, Institute of Biophysics, Academia Sinica, Beijing, China.

the method of Lin et al. [6]; the product was precipitated with ammonium sulphate at 52.5% saturation, and stored at 4° C. Protein concentrations were determined by the method of Lowry et al. [7]. Methanol, ethanol, *n*-propanol, *iso*-propanol and *t*-butanol were redistilled, and other reagents were of analytical grade.

The CD analysis was based on the method of Chang et al. [8]. All CD spectra were measured on a Jobin Yvon Mark III Dichrograph, with the following parameters: cell pathlength, 0.1 mm; sensitivity, $5 \cdot 10^{-6}$ ΔA/mm; time constant, 2 s; chart speed, 10 nm/cm; scan speed, 12 nm/min; bandwidth, 2 nm. Samples were kept at 30° C for 20 min before scanning. An appropriate blank was subtracted from the spectra. The mean residue weight of the enzymes was taken as 115.

Results and Discussion

Circular dichroism spectra of the H^+-ATPase complex and F_1-ATPase

The far ultraviolet CD spectra of both the F_1-ATPase and H^+-ATPase complex showed one positive and two negative peaks (Figs. 1 and 2).

The CD spectrum of the soluble F_1-ATPase is typical of a protein containing some α-helix, with a double minimum at 220 nm and 208 nm (the latter being the higher) and a positive peak at 192 nm. In the CD spectrum of the H^+-ATPase, the two negative peaks were not as distinct as those of the F_1-ATPase, and all the bands were red-shifted: this is because the H^+-ATPase consists of a soluble F_1 and a hydrophobic F_0, both of which contribute to the CD spectrum of the H^+-ATPase complex.

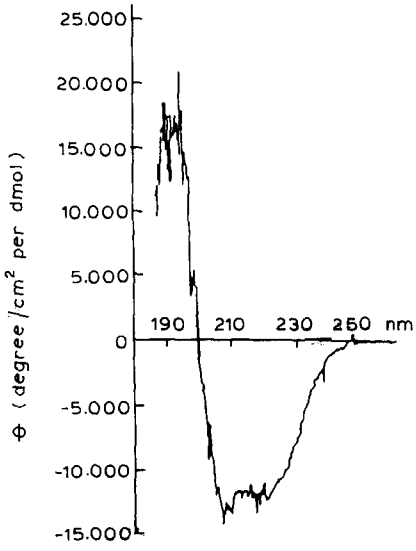

Fig. 1. Typical CD spectra of F_1-ATPase. The measurement was made in the cell with an optical path length of 0.1 mm at 0.75 mg protein/ml in 40 mM Tris-SO_4^{2-} (pH 8.0).

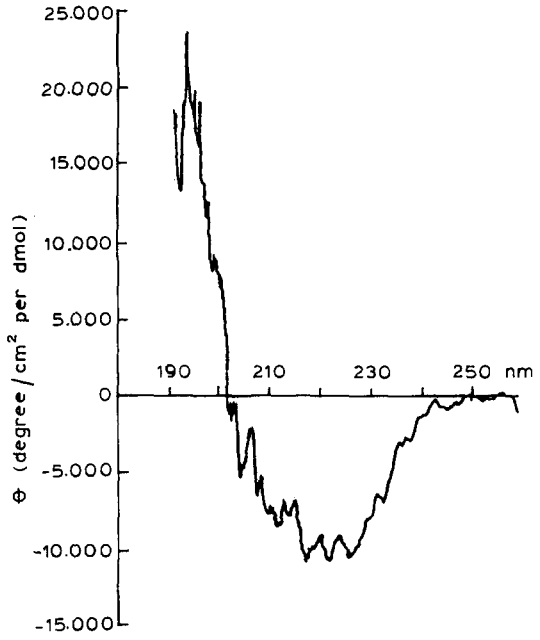

Fig. 2. Typical CD spectra of H^+-ATPase complex. The measurement was made in the cell with an optical path length of 0.1 mm at 0.6 mg protein/ml in 250 mM sucrose, 10 mM Tris-SO_4^{2-} (pH 8.0) and 0.2 mM EDTA.

Effects of alcohols on the conformation of the H^+-ATPase

The effects of the addition of 5, 10 and 20% methanol, ethanol, *n*-propanol, *iso*-propanol and *t*-butanol, respectively, on the $\theta_{208\ nm}$ of the H^+-ATPase complex appeared to be insignificant (Table I and Fig. 3), suggesting that the α-helix content was not significantly altered by the presence of alcohol. Lenaz et al. [9] have also shown that *n*-propanol has a slight effect on the conformation of the ATPase complex.

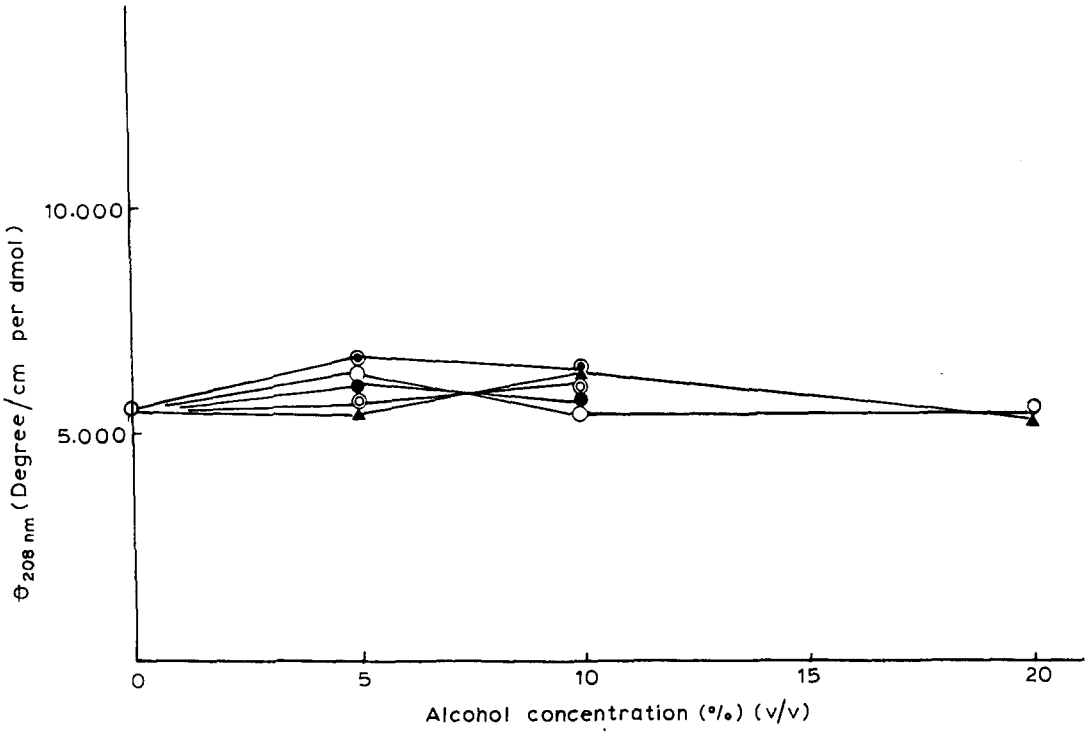

Fig. 3. Changes in the molar ellipticity of H^+-ATPase in different concentrations of alcohols. ○——○, methanol; ▲——▲, ethanol; ●——●, *n*-propanol; ◎——◎, iso-propanol; ⊙——⊙, *t*-butanol:

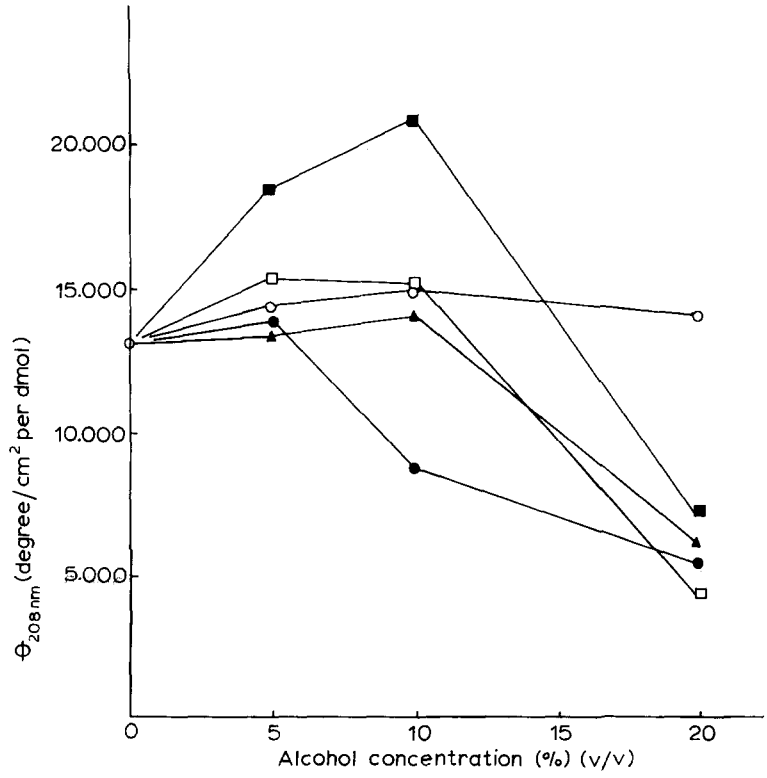

Fig. 4. Changes in the molar ellipticity of F_1-ATPase in different concentrations of alcohols. ○——○, methanol; ▲——▲, ethanol; ●——●, *n*-propanol; □——□, *iso*-propanol; ■——■, *t*-butanol.

Effects of alcohols on the conformation of the F_1-ATPase

The effects of the five alcohols on the $\theta_{208\ nm}$ of the F_1-ATPase were more prominent than the effects on the H^+-ATPase complex (Table I and Fig. 4; compare with Fig. 3).

For all the alcohols, except methanol, a rise in concentration first increased and then decreased the magnitude of CD at 208 nm (and thereby the helix content): the CD reached its maximum value at 5%, alcohol, and began to fall at about 10% (Table II). The opposite was true for the content of the β-sheet: it reached its lowest point at about 5% alcohol, and increased slightly at higher alcohol concentrations. No general conclusions could be drawn about the β-turns and unordered forms.

It can be concluded that addition of alcohol affects some change in the conformation of the F_1-ATPase. However, at methanol concentrations of 5–20%, and lower concentrations of the other alcohols the enzyme maintains its enzymatically active conformation. As the chain length, degree of branching, and concentration of the alcohol increase, the F_1-ATPase molecules begin to relax considerably. This may explain the results of Lin and coworkers [10], who found that 5–20% methanol increased the hydrolytic activity of the F_1-ATPase, but that the enzyme was inhibited by the other four alcohols and with increasing effect as the alcohol concentration was increased.

A suitable secondary structure is required to maintain the highest activity of F_1-ATPase. There is evidence that the hydrolytic activity was reduced when the α-helix content of F_1-ATPase was decreased. However, that is not the only factor. Although there was a parallel relationship between the α-helix content and hydrolytic activity at 5–20% methanol, there is no close relationship when the concentration of the other alcohols is 5% (the α-helix content was increased but hydrolytic activity was reduced). It may be that the addition of 5–20% methanol creates a suitable environment for the active site of the enzyme and thereby makes hydrolysis easier, whereas the other alcohols do not have this effect.

TABLE I

CHANGES IN THE MEAN RESIDUE ELLIPTICITY OF F_1-ATPase AND H^+-ATPase IN DIFFERENT CONCENTRATIONS OF ALCOHOLS

Additive	($\times 10^{-3}$ degree/cm^2 per dmol)			
	F_1-ATPase		H^+-ATPase	
	$\theta_{208\,nm}$	$\theta_{220\,nm}$	$\theta_{208\,nm}$	$\theta_{220\,m}$
0	13.2	12.3	5.5	5.3
5% MeOH	14.4	13.2	6.3	6.3
10% MeOH	14.9	13.8	5.3	7.4
20% MeOH	14.0	13.9	5.5	6.5
5% EtOH	13.4	12.4	5.5	6.1
10% EtOH	14.2	12.4	6.3	6.9
20% EtOH	6.1	9.4	5.3	6.5
5% *n*-PrOH	14.2	13.5	6.1	6.5
10% *n*-PrOH	8.9	10.4	5.7	5.7
20% *n*-PrOH	5.3	7.5	–	–
5% *i*-PrOH	15.4	14.3	5.7	5.7
10% *i*-PrOH	15.2	14.3	6.1	6.3
20% *i*-PrOH	4.3	8.1	–	–
5% *t*-BuOH	18.6	16.6	7.2	6.3
10% *t*-BuOH	21.0	18.3	6.3	6.7
20% *t*-BuOH	7.1	8.4	–	–

TABLE II

CHANGES IN SECONDARY STRUCTURE OF F_1-ATPase IN DIFFERENT CONCENTRATIONS OF ALCOHOLS

Additive	Helix	Beta	Beta-turn	Unordered
0	0.27	0.15	0.13	0.45
5% MeOH	0.36	0	0.10	0.54
10% MeOH	0.36	0	0.20	0.44
20% MeOH	0.37	0.10	0.13	0.40
5% EtOH	0.36	0.05	0.18	0.41
10% EtOH	0.30	0.25	0.03	0.42
20% EtOH	0.23	0.40	0.08	0.29
5% *n*-PrOH	0.28	0.25	0.01	0.46
10% *n*-PrOH	0.22	0.44	0	0.34
20% *n*-PrOH	0.16	0.35	0.13	0.36
5% *i*-PrOH	0.36	0	0.13	0.51
10% *i*-PrOH	0.36	0.05	0.12	0.47
20% *i*-PrOH	0.30	0.12	0.25	0.33
5% *t*-BuOH	0.35	0	0.16	0.49
10% *t*-BuOH	0.24	0	0.15	0.51
20% *t*-BuOH	0.24	0.15	0.17	0.44

A comparison of Fig. 3 and Fig. 4 shows that the five alcohols had less effect on the H^+-ATPase than on the F_1-ATPase. This difference suggests that the free F_1-ATPase was more affected by alcohol than the membrane-bound F_1-ATPase. It seems that the hydrophobic protein F_0 and the membrane lipids in the H^+-ATPase complex stabilize and protect F_1 from the effects of the alcohols. In order to demonstrate the stabilizing function of F_0, the H^+-ATPase complex was treated with EDTA and urea. The F_1 was detached from the ATPase complex, and an F_1-depleted F_0 with lipid particle was obtained. The addition of 5–20% methanol or *t*-butanol was found to have no significant effect on the CD spectra of the F_0 with lipid. Thus, alcohols seem to have less effect on the conformation of F_1 in the bound state than in the free state. This is further evidence that F_0 with membrane lipid has a stabilizing and protective function for F_1-ATPase.

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References

1 Linnett, P.E. and Beechey, P.B. (1979) Methods Enzymol. 55, 472–518
2 Recktenwald, D. and Hess, B. (1977) FEBS Lett. 76, 25–28
3 Lin, Z.H., Li, S.G. and Sun, S. (1984) J. Grad. Sch. (in Chinese) 1, 87–93
4 Lin, Z.H. and Zhang, Y. (1985) Acta Biochim. Biophys. Sin. (in Chinese) 17, 137–145
5 You, M.L. and Yang, F.Y. (1985) Acta Biochim. Biophys. Sin. (in Chinese) 17, 69–76
6 Lin, Z.H., Wu, E. and Sun, S. (1981) Biochim. Biophys. (in Chinese) 5, 53–56
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem., 193, 265–275
8 Chang, T.C., Wu, C.S. and Yang, J.T. (1978) Anal. Biochem. 91, 13–31
9 Lenaz, G., Curatola, G. and Masotti, L. (1975) J. Bioenerg. 7, 223–299
10 Zhang, Y., Li, S.G. and Lin, Z.H. (1986) Acta Biophys. (in Chinese) 2, 88–93