Vol. 111, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1983 Pages 366-372

A TEMPERATURE-DEPENDENT STRUCTURAL CHANGE OF MITOCHONDRIAL ATPase

G. Parenti Castelli, A. Baracca, R. Fato and A. Rabbi

Istituto di Chimica Biologica, Università di Bologna
Via Irnerio 48, 40126 Bologna, Italy

Received January 5, 1983

SUMMARY: The temperature dependence of the intrinsic tryptophan fluorescence in either bovine heart submitochondrial particles or oligomycin-sensitive ATPase isolated therefrom shows a discontinuity at near 25°C, which coincides with the temperature where a break in the Arrhenius plot of ATPase activity is found. Addition of n-butanol to submitochondrial particles induces a decrease of tryptophan fluorescence in the whole temperature range. The discontinuity is interpreted as a temperature-dependent structural change and related to a viscosity-induced phase separation of the intrinsic mitochondrial proteins.

The activity of many membrane-bound enzymes has an anomalous temperature dependence, as shown by their Arrhenius plots undergoing breaks or discontinuities at critical temperatures, with sudden increases of activation energy below the breaks (1). Although such breaks are not unique to membrane enzymes (2), there is some agreement that lipids are involved in the phenomenon.

Mitochondrial ATPase (3) is a complex enzyme, which is lipid-dependent when membrane-bound, although its catalytic portion (F_1) is located in contact with the aqueous medium (4). It has been shown that the Arrhenius plot of the isolated enzyme in its vesicular membrane-bound form has a break in contrast with that of soluble F_1 (5); the temperature at which the break occurs is related to the physical state of the lipids, investigated by means of spin labels (5). Other studies on mitochondrial ATPase from yeast have shown that the break is shifted to increasing temperatures by incorporating more rigid lipids in the mitochondria (6). Other kinetic parameters, such as cooperativity of allosteric enzymes, have been shown to vary as a function of lipid fluidity either *in vivo* or *in vitro* (7).

Few studies with membrane-bound enzymes (8, 9) including mitochondrial ATPase (10) have revealed that the break in the reaction velocity is also accompanied by a sudden change in the apparent K_m of the enzymes for their substrates. Such

0006-291X/83 $1.50

change may be indicative of a conformational modification of the enzyme at the critical temperature.

A conformational role of lipids in the activity of membrane-bound enzymes has been previously advanced on the basis of studies performed in our laboratory (1, 11); according to such hypothesis, lipids may be required to prevent access of water molecules to peptide linkages, thus favoring maximal interpeptide hydrogen bonding and assuring maximal contents of ordered conformations such as α-helix.

The present investigation shows that the average environment of the tryptophans in submitochondrial particles and in the isolated ATPase undergoes a major change at a temperature of 25°C; such change, detected by the anomalous temperature dependence of tryptophan fluorescence, is indicative of a temperature-dependent structural change of the enzymic protein.

METHODS

Submitochondrial particles ETP from beef heart were prepared as described by Beyer (12) and oligomycin-sensitive ATPase (Complex V) was isolated and purified according to Stiggall *et al.* (13). Protein was assayed according to Lowry *et al.* (14) and lipid phosphorus according to Marinetti (15). ATPase activity was determined either by assaying P_i liberated by ATP hydrolysis (16) or by an ATP-rigenerating system and continuous recording of NADH (13).

For the determination of tryptophan fluorescence, both submitochondrial particles and the isolated enzyme were suspended in 0.25 M sucrose, 10 mM Tris-Cl buffer at pH 7.8; the fluorescence spectra of tryptophan were recorded between 300 and 370 nm after excitation at 295 nm, a wavelength where the contribution of tyrosine is negligible, using a Perkin-Elmer MPF-4 spectrofluorimeter.

RESULTS

The phospholipid content of the purified oligomycin-sensitive ATPase was 0.5 mg per mg protein, and the basal activity of the enzyme was stimulated only 50% by addition of exogenous phospholipids. A break in the Arrhenius plot of ATPase activity in either submitochondrial particles or the isolated ATPase occurs at 25°C with a large increase of activation energy below the break (Table 1).

Fig. 1 shows the fluorescence emission spectra of the enzyme tryptophans at 17°C and 32°C after excitation at 295 nm; if we except a large increase of quantum yield at the lower temperature, no major changes in the shape of the spectra are apparent, with an emission maximum at 326-328 nm, to be compared with 348 nm for free tryptophan in water (17), indicating that the majority of the tryptophan

Table 1. Some properties of isolated oligomycin sensitive ATPase in comparison with submitochondrial particles ETP.

	ATPase	ETP
Phospholipid content, mg/mg protein	0.50	0.45
ATPase activity at 20°C, $\mu mol \cdot min^{-1} mg^{-1}$	8.0	1.15
ATPase activity at 20°C, after addition of 1 mg/mg egg lecithin, $\mu mol \cdot min^{-1} mg^{-1}$	12.0	-
Discontinuity in Arrhenius plot of ATPase activity	25°C	25°C
Activation energy above discontinuity, $kcal \cdot mol^{-1}$	6.5	12.4
Activation energy below discontinuity, $kcal \cdot mol^{-1}$	22.0	21.8

residues in the enzyme protein are buried in a rather hydrophobic environment, and suggesting that no major changes in the environment polarity occur at temperatures below and above the break in the temperature dependence of the enzymic activity.

By plotting the fluorescence intensity of the endogenous tryptophan as a function of temperature (Fig. 2), it is found that it decreases monotonically from 5°C to 26°C, but no further decrease occurs up to 30°C; above the latter temperature

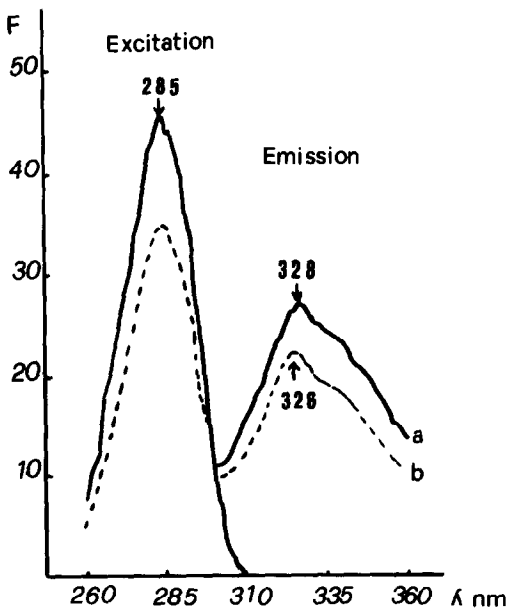
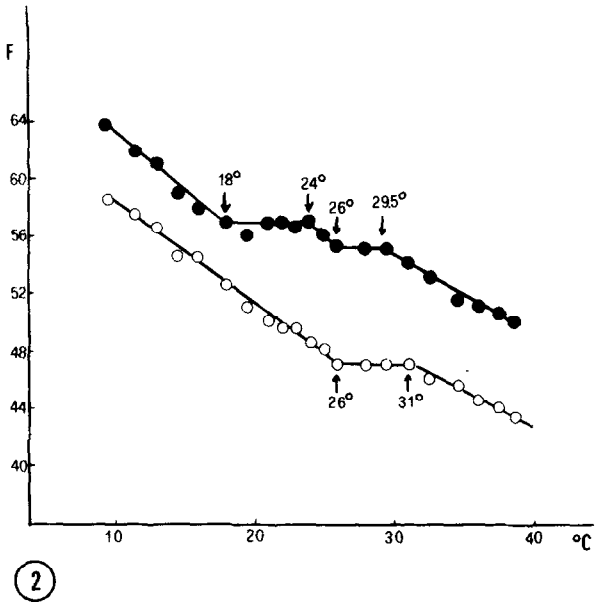


Fig. 1 - Intrinsic tryptophan fluorescence of mitochondrial oligomycin sensitive ATPase. The cuvette contained 165 µg of ATPase protein per ml. Curve *a*, spectrum at 17°C; curve *b*, spectrum at 32°C. The spectra were corrected for instrumental wavelength responses. The emission spectra were obtained after excitation at 295 nm.



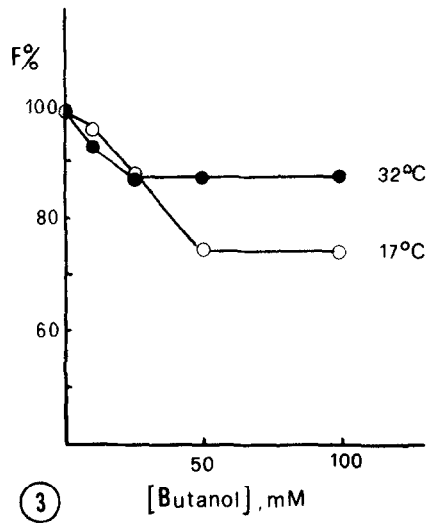


Fig. 2 - Temperature dependence of the intrisic tryptophan fluorescence of mitochondrial oligomycin sensitive ATPase. The fluorescence intensity at the emission maximum of 328 nm, is reported in arbitrary units. o—o, ATPase, 180 µg/ml, without exogenous phospholipids; •—•, same, with addition of egg lecithin (200 µg/ml).

Fig. 3 - Effect of n-butanol on the intrinsic tryptophan fluorescence of mitochondrial oligomycin sensitive ATPase at two different temperatures. o—o, at 17°C; •—•, at 32°C.

the fluorescence decreases again but with a lower slope that in the low temperature range. When sonicated vesicles of egg lecithin are added to the enzyme, the activity is enhanced by about 50% and the plot of tryptophan fluorescence as a function of temperature shows an additional plateau between 18° and 24°C besides the plateau between 26° and 30°C shown when no lipids are added.

Addition of n-butanol to submitochondrial particles has been shown to modify ATPase activity with changes in the cooperative character of the oligomycin inhibition and of activation energy (18). Fig. 3 shows that addition of n-butanol lowers tryptophan fluorescence at both 17° and 32°C in isolated ATPase with a slightly stronger relative effect at the higher temperature. A plot of the temperature dependence of tryptophan fluorescence in presence of n-butanol shows a decrease of fluorescence in the whole temperature range in both isolated ATPase and submitochondrial particles (cf. Fig. 4 for submitochondrial particles).

DISCUSSION

We have shown that a discontinuity in the temperature dependence of mitochondrial ATPase activity is accompanied by an anomalous behavior of the fluorescence

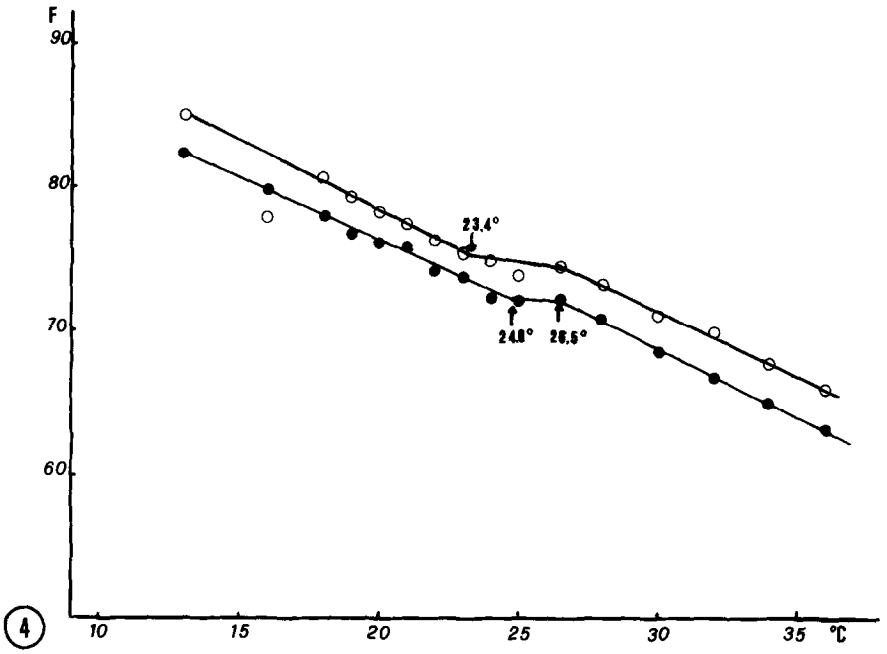


Fig. 4 - Effect of n-butanol on the temperature dependence of intrinsic tryptophan fluorescence of submitochondrial particles ETP; cf. Fig. 2 for details. o—o, no addition; ●—●, + n-butanol, 50 mM.

of the tryptophans of the enzymic protein in the same temperature region. A separate study has shown a discontinuity in the temperature dependence of a hydrophobic lipid spin label (5), indicating that a major change in the membrane structure occurs at a critical temperature in the environment of ATPase.

The present study does not allow to understand what is the nature of such a structural change, but points out that a parameter pertaining to the protein moiety of the membrane, namely intrinsic tryptophan fluorescence, is modified concomitantly with the change detected in the lipids. The decreased quantum yield in the 25° to 30°C region with respect to the extrapolation of the slope from higher temperatures indicates that the tryptophan residues of the enzymic protein experience a less rigid environment than predicted from a linear temperature dependence when the temperature is lowered from 30° to 26°C. The shape of the emission spectra, however, is not different below and above the break, suggesting that the polarity of the tryptophan environment is not altered. The change in tryptophan environment is not unique to the isolated ATPase, but is rather similar in whole submitochondrial particles, where most of the proteins are not part of the ATPase complex. This fact indicates that the change is not an intrinsic property of the ATPase pro-

tein but a general membrane phenomenon, where all the proteins appear to behave in a cooperative fashion.

The effect of n-butanol of a constant fluorescence decrease in the whole range of temperature, with maintenance of the break, suggests that the solvent, at the concentration used of 50 mM, induces a fluidity increase in the protein environment without major shifts in the temperature dependence. The effects of butanol are already detectable on the ATPase environment at concentrations as low as 5 mM, which is much below the concentration considered to exert a general anesthetic effect (19); this high sensitivity enphasizes the advantage of using intrinsic tryptophan fluorescence rather than extrinsic probes (20) for detecting fluidity changes upon anesthetic addition.

The possibility of a conformational change occurring in the region between 30° and 26°C in the ATPase protein, resulting in a less compact structure of the enzyme below 26°C, deserves consideration. Fluorescence and circular dichroism studies of the effects of lipids and temperature on the structure of a serum apolipoprotein (apo-Lp-ala)(21) have revealed conformational differences of the protein below and above the lipid phase transition.

An alterantive explanation could be an aggregation of the ATPase complexes in the plane of the membrane, setting on when the temperature is decreased below 30°C and completed at 26°C in isolated ATPase; aggregation could result in a collisional quenching of tryptophan fluorescence, thereby explaining the anomalous decrease of quantum yield in the 30° to 26°C region.

Although no direct evidence for protein aggregation is given by our study, there are several indications that aggregation occurs when membranes are cooled below the phase transition of the lipids (22). On the other hand, circular dichroism studies have revealed major conformational changes at the level of secondary structure of membrane proteins when the lipids are rendered more rigid, e.g. by cholesterol addition (10). Of course, the two phenomena, i.e. aggregation and conformational changes, may be concomitant, both being the result of overcoming a threshold in the membrane viscosity. Vertical displacement of membrane integral proteins has been suggested by Borochov *et al.* (23) as a result of lipid rigidization. A working hypothesis deserving consideration is that a critical increase of membrane viscosity induces vertical displacement of the proteins; the change in their environment, with increased accessibility of water to peptide bonds, would both destabilize ordered secondary structure and induce aggregation of the proteins.

ACKNOWLEDGEMENTS

This study has been supported by grants from CNR and the Ministero della Pubblica Istruzione, Roma, Italy. We thank Dr. G. Lenaz for useful advice in the course of this work.

REFERENCES

1. Lenaz, G. (1979) Subcell. Biochem. 6, 233-343.
2. Massey, V., Curti, B., and Ganther, H. (1966) J. Biol. Chem. 241, 2347-2357.
3. Kagawa, Y. (1978) Biochim. Biophys. Acta 505, 45-83.
4. Kozlov, I.A., and Skulachev, V.P. (1977) Biochim. Biophys. Acta 463, 29-89.
5. Solaini, G.C., and Bertoli, E. (1981) FEBS Lett. 132, 127-128.
6. Bertoli, E., Finean, J.B., and Griffiths, D.E. (1976) FEBS Lett. 61, 163-165.
7. Farias R.N., Bloj, B., Morero, R.D., Sineriz, F., and Trucco, R.E. (1975) Biochim. Biophys. Acta 415, 231-251.
8. Sullivan, K.H., Jain, M.K., and Koch, A.L. (1974) Biochim. Biophys. Acta 352, 287-297.
9. Blazyk, J.F., Blazyk, J.M., and Kline, C.M. (1981) J. Biol. Chem. 256, 691-694.
10. Lenaz, G., Curatola, G., Fiorini, R.M., and Parenti Castelli, G. Proc. Symp. 13th International Cancer Congress, Seattle, 8-15 Sept. 1982, Alan Lyss, New York (in press).
11. Lenaz, G., Curatola, G., Mazzanti, L., and Parenti Castelli, G. (1978) Mol. Cell. Biochem. 22, 3-32.
12. Beyer, R.E. (1967) Methods Enzymol. 10, 186-194.
13. Stiggall, D.L., Galante, Y.M., and Hatefi, Y. (1978) 253, 956-964.
14. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
15. Marinetti, G.V. (1962) J. Lipid Res. 3, 1-20.
16. MacLennan, D.H., Smoly, J.M., and Tzagoloff, A. (1968) J. Biol. Chem. 243, 1589-1597.
17. Teale, F.W.J., and Weber, G. (1957) Biochem. J. 65, 476-482.
18. Parenti Castelli, G., Sechi, A.M., Landi, L., Cabrini, L., Mascarello, S., and Lenaz, G. (1979) Biochim. Biophys. Acta 547, 161-169.
19. Boggs, M., Yoong, T., and Hsia, J.C. (1976) Molec. Pharmacol. 12, 127-135.
20. Lenaz, G., Curatola, G., Mazzanti, L., and Parenti Castelli, G. (1978) Biochem. Pharmacol. 27, 2835-2844.
21. Trauble, H., Middelhoff, G., and Brown, V.W. (1974) FEBS Lett. 49, 269-275.
22. Shechter, E., Letellier, L., and Gulik-Krzywicki, T. (1974) Eur. J. Biochem. 49, 61-76.
23. Borochov, H., Abbott, R.E., Schachter, D., and Shinitzky, M. (1979) Biochemistry 18, 251-258.