ACTIVATION ENERGIES OF DIFFERENT MITOCHONDRIAL ENZYMES: BREAKS IN ARRHENIUS PLOTS OF MEMBRANE-BOUND ENZYMES OCCUR AT DIFFERENT TEMPERATURES

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Summary

A series of mitochondrial enzymes exhibit breaks in Arrhenius plots from low to high activation energies upon temperature decrease. All membrane-bound enzymes tested except cytochrome c oxidase have shown these transitions, while soluble matrix enzymes (malic dehydrogenase and fumarase) did not have breaks or they had different characteristics. Triton-X-100 which solubilizes the mitochondrial membrane, induces a straightening of the plot for ATPase. The observation that the breaks in the Arrhenius plots for membranous enzymes fall at different temperatures suggests caution in their correlation with phase transitions of membrane phospholipids.

Several membrane activities have been found to depend upon the physical state of the membrane phospholipids. Studies of membrane activities of omeothermic and poikilothermic animals (1-3) and of a E. coli auxotroph requiring unsaturated fatty acids for growth and grown on media containing different fatty acids (4-6), have shown that the activation energies are not constant with temperature, but distinct breaks in the slopes of Arrhenius plots have been found at well defined temperatures. It has been suggested that these temperatures coincide with the transition temperatures of the phospholipids from a liquid-crystalline to a crystalline phase (7). Although a correlation was found in the case of mitochondrial respiration in omeothermic animals and in chilling-sensitive plants, with breaks in the mot-

ion of added spin labels (3), the data on the E. coli auxotroph appear to point out that breaks of the Arrhenius plots for proline uptake and succinic dehydrogenase occur at different temperatures (5) and do not coincide with transition temperatures of the phospholipids measured by X-ray diffraction.

In this investigation we have studied a range of mitochondrial activities; both soluble enzymes and membrane-bound lipid-dependent activities have been tested in order to ascertain if a correlation may be found between the existence of the breaks and the lipid requirement, and if the breaks for different enzymes fall at the same or different temperatures.

Methods

Beef heart mitochondria (BHM) have been prepared with the method of Smith (8). The following enzymic activities have been assayed spectrophotometrically in a Zeiss PMQ II equipped with a thermostatic cell: succinate-cytochrome c reductase (9), cytochrome c oxidase (10); malate dehydrogenase (11), β-hydroxy butyric dehydrogenase (12), and fumarase (13). Temperatures in the assay media were directly controlled by immersing a thermometer in the cuvettes. ATPase activity and respiration were assayed as described elsewhere (14, 15). Protein was determined with a biuret method (16).

Results

Figure 1 shows the Arrhenius plots for different mitochondrial activities in intact frozen mitochondria. The total Mg^{++}-dependent ATPase, succinic-cytochrome c reductase, β-hydroxybutyric dehydrogenase, and succinoxidase, show evident breaks in the slopes; in contrast with these membrane-bound enzymes which are known to depend upon lipids for activity (17-20), soluble matrix enzymes like malic dehydrogenase do not show changes in activation energies with changing temperatures. Fumarase however appears to

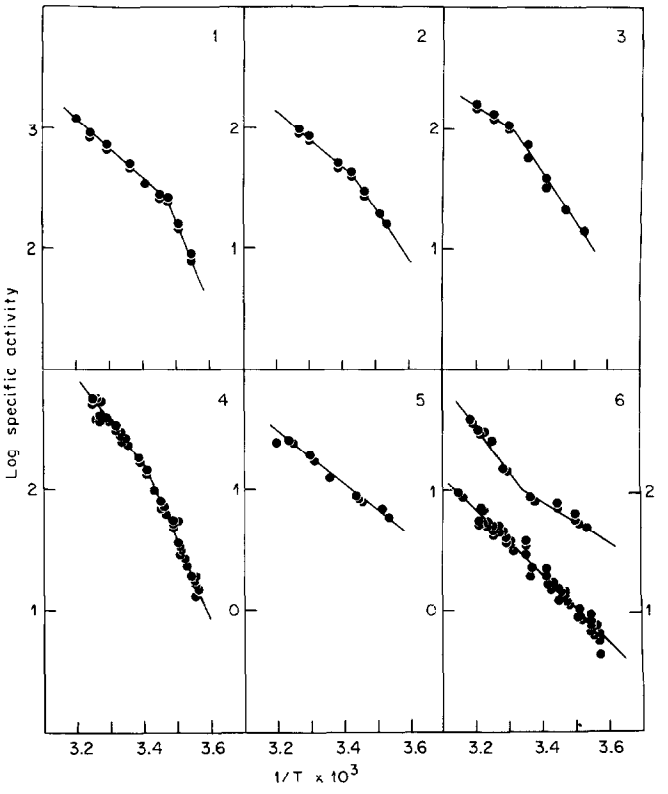

Fig. 1 - Arrhenius plots of various mitochondrial activities.
1. ATPase (nmoles Pi hydrolysed/min.mg protein);
2. β-hydroxybutyric dehydrogenase (nmole NAD reduced/min. mg protein);
3. Succinoxidase (natoms O_2 consumed/min.mg protein);
4. Succinate-cytochrome c reductase (nmoles cytochrome c reduced/min.mg protein);
5. Cytochrome c oxidase (nmoles reduced cytochrome c oxidised/min.mg protein);
6. Upper plot, right side: fumarase (nmoles fumarate formed/min.mg protein) - Lower plot, left side: malate dehydrogenase (μmoles NAD reduced/min.mg protein).

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have an inverted break, as is known in the literature (21). Surprisingly, cytochrome c oxidase has no break in the temperature range studied. Raison et al. (2) found however a transition also in cytochrome oxidase in rat liver mitochondria using a manometric assay with ascorbate as substrate.

Table I - Activation Energies of Different Mitochondrial Enzymes.

Activity	Break temperature (°C)	Activation above transition (kcal/mole)	Activation below transition (kcal/mole)
ATPase	17.7	9.8	27.1
β-Hydroxybutyric dehydrogenase	18.5	10.9	18.2
Succinate oxidase	27.0	9.1	17.0
Succinate-cytochrome c reductase	20.0	15.7	32.8
Cytochrome c oxidase	-	9.3	
Malate dehydrogenase	-	13.0	
Fumarase	18.5*	16.7	7.8

*Below 18.5°C the slope in the plot indicates a lower activation energy.

The breaks in the Arrhenius plots for the membranous enzymes fall at different temperatures, averaging 17.7° for ATPase, 20° for succinic-cytochrome c reductase, 27° for succinate oxidase, 18.5° for β-hydroxy-butyric dehydrogenase.

The activation energies in kcal per mole are reported in Table I; the largest increase occurring below the break temperature is that of the ATPase reaction.

The ATPase has been further investigated in relation to the state of the mitochondria (fresh, frozen, disrupted by detergent). Some results are reported in Fig. 2. The residual oligomycin insensitive ATPase of intact mitochondria also shows a biphasic Arrhenius plot. On the other hand the detergent Triton-X-100, at the concentration of 2.5 mg/ml, which results in well evident solubilization of the mitochondrial membrane and makes the ATPase oligomycin insensitive, induces a straightening of the plot, with concomitant increase of the temperature at which there is a change of the slope.

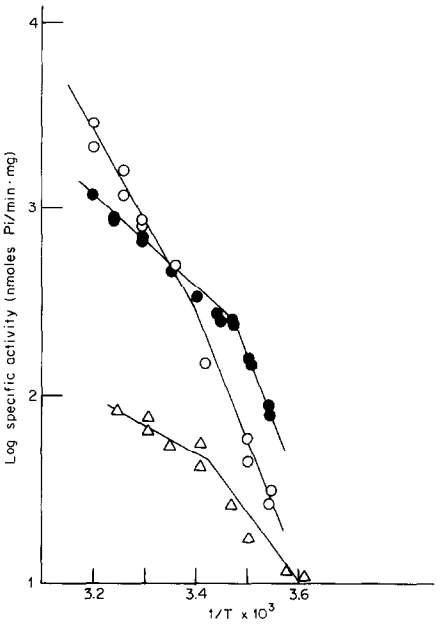

Fig. 2 - Arrhenius plot of mitochondrial ATPase in frozen BHM. ●——●, under standard conditions; ——— , + oligomycin (1 mg/ml); o——o, + Triton-X-100 (2.5 mg/ml).

Discussion

Several membrane-bound activities show atypical activation energy curves with evident breaks in the Arrhenius plots. Data for mitochondrial or equivalent activities are already available in the literature (1-3, 22-24).

We have investigated some activities of beef heart mitochondria either membrane-bound like ATPase and respiration or soluble like malic dehydrogenase. All the membrane-bound enzymes have shown clear breaks in the Arrhenius plots, except cytochrome c oxidase, but the breaks fall at different temperatures; the value for ATPase is in accord with that of Sweetman and Griffiths (22, 23) for ATPase, ATP-dependent reversed electron transfer and energy-linked transhydrogenation in E. coli. It is of interest that succinate-cytochrome c reductase and succinate oxidase have breaks at 20° and 27° respectively, since the former is a part of

the latter activity. Furthermore cytochrome c oxidase, which is also a part of succinate oxidase, shows no break.

What is the possible interpretation of these findings?

Although the involvement of the lipid milieu in the transitions may be favored by the fact that lipid independent enzymes like malic dehydrogenase and the non-energy linked transhydrogenation (23) do not have breaks, and that Triton-solubilized ATPase undergoes a more linear Arrhenius plot, the fact that the breaks fall at different temperature suggests caution in this straightforward interpretation.

Calorimetric evidence (25) shows that in rat liver mitochondria the transition temperature of the lipids is centered at about 0°C, far away from the temperatures where the breaks occur.

Breaks could be the result of different rate limiting points in the temperature dependence of complex activities.

On the other hand it is suggestive to postulate that a specific distribution of different types of lipids in the mitochondrial membrane would create different hydrophobic microenvironments around specific enzymic activities. The breaks would be indicative of the temperature where "freezing" just begins.

Completely different types of analysis are compatible with a non-random distribution of lipids in biological membranes (26).

References

1. Lyons, J.M., and Raison, J.K., Comp. Biochem. Physiol. 37, 405 (1970).
2. Raison, J.K., Lyons, J.M., and Thomson, W.W., Arch. Biochem. Biophys. 142, 83 (1971).
3. Raison, J.K., Lyons, J.M., Mehlhorn, R.J., and Keith, A.D., J. Biol. Chem. 246, 4036 (1971).
4. Overath, P., Schairer, H.U., and Stoffel, W., Proc. Natl. Acad. Sci. USA 67, 606 (1970).
5. Esfahani, M., Limbrick, A.R., Knutton, S., Oka, T., and Wakil, S.J., Proc. Natl. Acad. Sci. USA 68, 3180 (1971).
6. Wilson, G., and Fox, C.F., J. Mol. Biol. 55, 49 (1971).
7. Chapman, D., Lipids, 4, 251 (1969).
8. Smith, A.L., Methods Enzymol. 10, 81 (1967).
9. Takemori, S., and King T.E., J. Biol. Chem. 239, 3546 (1964).
10. Smith, L., in: "Methods of Biochemical Analysis" (D. Glick,

ed.). Vol. II, p. 427 (Wiley, New York, 1955).
11. Englard, S., and Siegel, L., Methods Enzymol. 13, 99 (1969).
12. Fleischer, S., and Fleischer, B., Methods Enzymol. 10, 406 (1967).
13. Hill, R.L., and Bradshaw, R.A., Methods Enzymol. 13, 91 (1969).
14. Margolis, S., Baum, H., and Lenaz, G., Biochem. Biophys. Res. Commun. 25, 132 (1966).
15. Lenaz, G., and Beyer, R.E., J. Biol. Chem. 240, 3653 (1965).
16. Gornall, A.G., Bardawill, C.J., and David, M.M., J. Biol. Chem. 177, 751 (1949).
17. Bulos, B., and Racker, E., J. Biol. Chem. 243, 3891 (1968).
18. Brierley, G.P., Merola, A., and Fleischer, S., Biochim. Biophys. Acta, 64, 218 (1962).
19. Jurtshuk, P., Sekuzu, I., and Green, D.E., J. Biol. Chem. 238, 3595 (1963).
20. Fleischer, S., Brierley, G., Klouwen, H., and Slautterback, D.G., J. Biol. Chem. 237, 3264 (1962).
21. Massey, V., Biochem. J., 53, 72 (1953).
22. Sweetman, A.J., and Griffiths, D.E., Biochem. J. 121, 117 (1971).
23. Sweetman, A.J., and Griffiths, D.E., Biochem. J. 121, 125 (1971).
24. Kemp, A., Groot, G.S.P., and Reitsma, H.J., Biochim. Biophys. Acta, 180, 28 (1969).
25. Blazyk, J.F., and Steim, J.M., Biochim. Biophys. Acta, in press.
26. Lenaz, G., in: "The Role of Membrane Structure in Biological Energy Transduction". Plenum, in press.