Biochimica et Biophysica Acta, 363 (1974) 135–140

BBA 76714

TWO TEMPERATURE-INDUCED CHANGES IN MITOCHONDRIAL MEMBRANES DETECTED BY SPIN LABELLING AND ENZYME KINETICS

JOHN K. RAISON and EDWARD J. McMURCHIE

Plant Physiology Unit, CSIRO Division of Food Research and School of Biological Sciences, Macquarie University, North Ryde, N.S.W., 2113, Sydney (Australia)

(Received March 25th, 1974)

SUMMARY

1. Changes in the structure and function of membranes, as a function of temperature, were investigated using spin labels and measurements of the Arrhenius activation energy of membrane-associated enzymes.

2. The lipid components of mitochondrial membranes exhibit abrupt changes in molecular ordering at two temperatures. For mitochondria from sheep liver the changes occur at 29 and 17 °C and for mitochondria from rat at 24 and 8 °C. Coincident with these changes the Arrhenius activation energy of succinate oxidase of both mitochondrial preparations, increased as the temperature was lowered.

3. Similar changes in molecular ordering were observed at two temperatures with membranes of the endoplasmic reticulum.

INTRODUCTION

The application of spin labels to probe the physical state of the complex lipid components of biological membranes has provided useful information on temperature-induced changes in the fluidity and molecular ordering which are related to membrane function [1, 2]. Spin labels infused into the membranes of rat liver mitochondria, for example, exhibit a sudden change in the temperature-dependent response of motion at about 23 °C [1], the same temperature at which a change is evident in the Arrhenius activation energy (E_a) of the succinate oxidase system of these mitochondria [3]. This change in spin-label motion occurs within an experimental temperature range of about 1 C degree [1], and is indicative of a phase change in the membrane lipids [2]. However, thermal phase transitions in these same mitochondrial membranes, detected by differential scanning calorimetry, occur over a range of about 30C degrees [4]. Because of phospholipid heterogeneity, in regard to both acyl fatty acid and polar head group composition, the complete transformation of lipids from a "cogel" to a "liquid–crystalline" structure during heating would be expected to occur over a considerable temperature range [5]. The change detected by spin labelling was therefore considered to represent only one of the many thermotropic and lyotropic mesomorphic changes which would occur during the thermal transition [1].

As shown in this paper a more detailed examination of spin-label motion in membranes, as a function of temperature, has revealed a second change at a lower temperature than the change previously observed. The E_a of succinate oxidase also increases below the temperature of the lower phase change indicating that this change in the lipids also influences the conformation of membrane proteins.

MATERIALS AND METHODS

Mitochondria were isolated from adult rats (Carsworth Farm) and from sheep (supplied by CSIRO Division of Animal Physiology, Prospect, N.S.W.) by methods described previously [3]. Succinate oxidase activity was measured polarographically using six electrode vessels (Rank Bros., Cambridge, U.K.), each maintained at separate temperatures. The electrodes were calibrated as previously described [6], and the reactions were carried out in medium containing 0.25 M sucrose, 0.01 M Tris, 0.01 M K_2HPO_4, 0.005 M $MgCl_2$, 0.005 M EDTA and 0.5 mg/ml bovine serum albumin adjusted to pH 7.2 with HCl. Succinate was used as the substrate for oxidation at a concentration of 5 mM. Additions of ADP (200 nmoles), were made to the reaction vessel (total volume 3.0 ml) in 2-μl amounts.

The endoplasmic reticulum fraction was obtained by centrifugation of the supernatant remaining after sedimentation of mitochondria, at 65 000 $\times g$ for 60 min. The pellet was resuspended in 1 ml Tris buffer (pH 7.6).

Electron spin resonance (ESR) spectra were recorded with a Varian E4 spectrometer fitted with a temperature controlled cell housing. The temperature of the sample was controlled using an electronic regulator designed and constructed by Mr H. F. Symmons of the CSIRO Division of Physics, Sydney. The temperature of the gas flow purging the sample cavity was determined with a Mettler Digital Thermometer (Model M-15), and fluctuations in the sample temperature determined with a thermocouple. The sample temperature was maintained at $\pm$ 0.1 C degree of the set temperature. The spin labels, M12NS (3-oxazolidinyloxy-2-(10-carbmethoxydecyl)-2-hexyl-4,4-dimethyl) and 5N10 (3-oxazolidinyloxy-2-butyl-2-pentyl-4,4-dimethyl) were prepared by the general synthesis of Keana et al. [7]. The spin labels dissolved in ethanol were added to membranes (30 mg of mitochondrial protein/ml) to give an approximate bulk concentration of $1 \cdot 10^{-4}$ M of spin label. The motion of the spin label (correlation time, τ_0) was calculated from measurements of the width and height of the absorption lines of a standard first-derivative spectrum, as described [2].

RESULTS

When infused into aqueous suspensions of mitochondrial membranes both M12NS and 5N10 partition predominantly into the lipid region of the membranes and exhibit restricted, isotropic motion. The motion of the spin label can therefore indicate changes in the molecular ordering of membrane lipids.

The change in motion (τ_0) as a function of temperature for 5N10 with sheep liver mitochondria and M12NS with rat liver mitochondria is shown in Fig. 1 as Arrhenius plots. With both mitochondrial preparations a change in slope (Arrhenius activation energy of motion) is evident at two temperatures; for mitochondria from the sheep at about 29 and 17 °C and from the rat at 24 and 8 °C. These temperatures

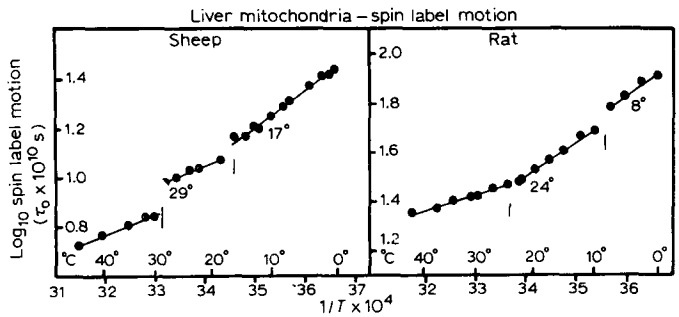

Fig. 1. Arrhenius plots of the motion of the spin labels 5N10 and M12NS infused into sheep and rat liver mitochondrial membranes, respectively. The calculated values for the Arrhenius activation energy of motion in each temperature range from 45 °C are for sheep, 15.5, 17.1 and 26.7 kJ/mole and for the rat 11.2, 24.6 and 26.7 kJ/mole.

are the approximate midpoints of the experimentally determined range at the zone of discontinuity. Similar changes in slope are also observed with the membranes of the endoplasmic reticulum from the sheep liver at 33 and 11 °C and from rat liver at 22 and 7 °C, as shown in Fig. 2.

The effects of temperature on the rates of succinate dependent oxidation for both sheep and rat liver mitochondria are shown in Fig. 3. With both mitochondrial samples the changes in the E_a are evident for both State 3 (substrate plus O_2 in the presence of ADP) and State 4 (substrate plus O_2 with ADP limiting) respiration. For mitochondria from sheep liver the changes in E_a were at about 29 and 16 °C and for the mitochondria from rat liver at about 24 and 8 °C; the E_a in each temperature range exhibits a progressive increase as the temperature is lowered. The temperatures of the changes in E_a correspond with the temperatures for the changes in spin label motion with these same mitochondria (Fig. 1).

Two further observations can be made (Fig. 3). The higher and lower changes in E_a are each at a higher temperature in the sheep. At any temperature, the succinate oxidase of the sheep has a lower E_a than that of the rat.

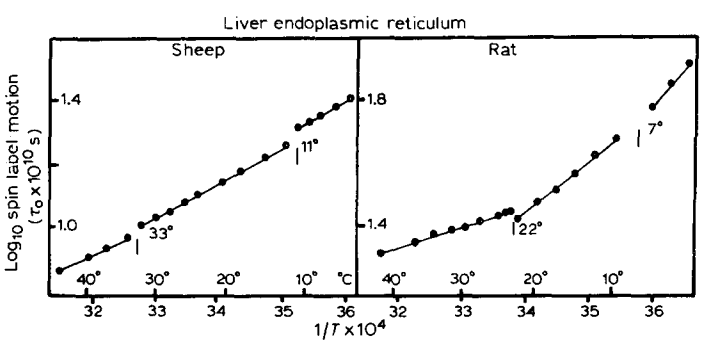

Fig. 2. Arrhenius plots of the motion of the spin label M12NS infused into sheep and rat liver endoplasmic reticulum membranes. The calculated values for the Arrhenius activation energy of motion in each temperature range from 45 °C are for sheep 18.3, 21.7 and 23.4 kJ/mole and for the rat 21.1, 30 and 41 kJ/mole.

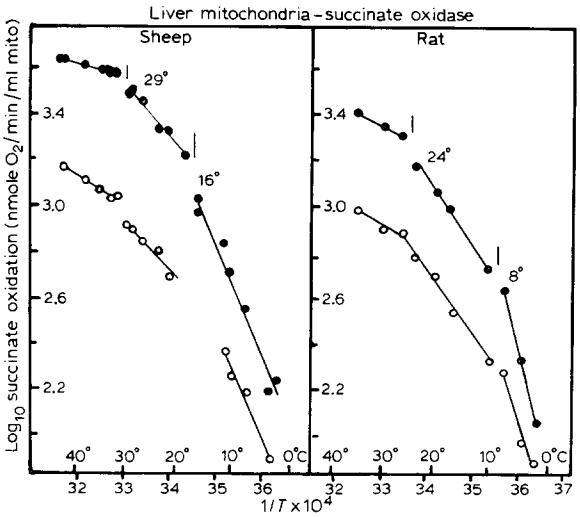

Fig. 3. Arrhenius plots of the State III (●) and State IV (○) rates of succinate oxidation by sheep and rat liver mitochondria. For the State III rates of respiration, values for the Arrhenius activation energy (E_a) within each temperature range from 40 °C are for the sheep 8.4, 41.8 and 96 kJ/mole and for the rat 16.7, 54.3 and 154 kJ/mole. The membrane preparations were those for which data is presented in Fig. 1.

DISCUSSION

Mitochondria from sheep and rat liver exhibit similar responses to changes in temperature. For both, the increases in the E_a of succinate oxidation (Fig. 3) occur at temperatures which coincide with the temperature at which the spin label reflects changes in the molecular ordering of the membrane lipids (Fig. 1). This coincidence between changes in the functional aspects of membrane-associated enzymes and changes in the physical state of membrane lipids has been noted previously with mitochondria from homeothermic animals [3, 8], and chilling-sensitive plants [9], and in all these mitochondria only one such change was evident. However, the present results indicate that changes occur in mitochondrial membranes and in endoplasmic reticulum at two distinct temperatures. At each point of change the physical state of the membrane lipids (Fig. 1) and the kinetics of oxidative enzymes are modified (Fig. 3). Similar changes in the physical state of membrane lipids, at two temperatures, have been reported for membranes of *Escherichia coli* ML [10] and for the "inner" membrane of fatty acid auxotrophs of *E. coli* K12 [11]. In the latter the temperatures of these changes correspond to the temperature at which changes are observed in both β-glucoside and β-galactoside transport systems [11].

The temperature-induced change in the E_a of membrane-associated enzymes is considered a direct consequence of a temperature-induced change in the physical state of the membrane lipids [12]. The coincidence of the temperature-induced changes in E_a (Fig. 3) and in the physical state of the lipids (Fig. 1) indicates that both the changes detected by spin labelling result in major alterations in the hydrophobic interactions between the lipid and protein components of membranes. Furthermore because the temperature-induced changes affect a number, if not all of the respiratory

enzymes associated with mitochondrial membranes [12], the changes in the physical state of the lipids evident at the two temperatures, probably involves a significant proportion of the lipids through co-operative interactions between lipid molecules.

The broad endothermic transition obtained by differential scanning calorimetry of rat liver mitochondria between −15 and 30 °C [4], and the transition detected by low angle, X-ray diffraction of beef heart mitochondria between −10 to 10 °C [13], are indicative of broad melting ranges for the fatty acid chains of the phospholipids during the "gel" to "liquid–crystalline" transition [14]. The temperature range between the changes detected by spin labelling (Figs 1 and 2 and [10] and [11]) is, considerably less and does not appear to be directly related to the physical events detected by the other methods. However, thermal transition ranges, similar to those in mitochondrial membranes, have been observed with binary mixtures of phospholipids where the beginning and end of the transition were detected by changes in the partitioning of the spin label 2,2,6,6-tetramethylpiperidine-1-oxyl between the hydrophobic and hydrophilic phases of aqueous dispersions [15]. The phase separation in this case was considered to be a two-dimensional (lateral) separation of the gel and liquid–crystalline phases. On lowering the temperature the point at which gel formation is initiated was referred to as T_f and the point at which all of the lipid is in the gel phase referred to as T_s [15]. Thus using this nomenclature with rat liver mitochondria (Fig. 1) T_f is at 24 °C and T_s is at 8 °C. Since the transition region between T_f and T_s is considered to represent a continual shift in the equilibrium between gel and liquid–crystalline phases (see [15]) it is probable that both the motion of a spin label and the E_a of a membrane-associated enzyme system might deviate from linear Arrhenius kinetics.

The physiological significance of these temperature-induced changes in the molecular ordering of membrane lipids at T_f and T_s is clearly demonstrated by the corresponding change in E_a of the respiratory enzyme system associated with these membranes (Fig. 4). Furthermore, the change in E_a observed for adenine nucleotide translocase of rat liver mitochondria at about 10 °C [16], might also be a consequence of the change in molecular ordering of lipids at the lower temperature. Such changes in enzyme function need not be confined to mitochondrial membranes. Indeed the increase in E_a for both the $(Na^+ + K^+)$-activated and the Mg^{2+}-activated ATPase of rat brain endoplasmic reticulum at 20 and at 6 °C, as the temperature is lowered [17] is probably also a consequence of changes in the lipids, since changes in the liver endoplasmic reticulum were apparent at approximately similar temperatures (Fig. 2) to that of mitochondrial membranes. While the catalytic rate of these various membrane-associated enzymes decreases rapidly when the temperature is reduced below T_f there is measurable activity below T_s. However, in complex metabolic events, summation of the changes in all of the enzymic events which contribute to the overall function, can result in a sudden change in rate below T_f and complete cessation below T_s. Changes of this type have been noted in cardiac muscle function with homeothermic animals [8]. Thus in these animals the critical physiological temperature can be correlated with T_f. For microorganisms there is ample data showing that cells grow in the temperature region between T_f and T_s, i.e. when the membrane lipids are in the mixed gel–liquid–crystalline phase and the critical temperature appears to be associated with T_s [11].

REFERENCES

1 Raison, J. K., Lyons, J. M., Mehlhorn, R. J. and Keith, A. D. (1971) J. Biol. Chem. 246, 4036–4040
2 Mehlhorn, R. J. and Keith, A. D. (1972) in Membrane Molecular Biology (Fox, C. and Keith, A. D., eds) pp. 192–227, Sinauer Assoc., Stamford, Conn.
3 Lyons, J. M. and Raison, J. K. (1970) Comp. Biochem. Physiol. 37, 405–411
4 Blazyk, J. F. and Steim, J. M. (1972) Biochim. Biophys. Acta 266, 737–741
5 Ladbrooke, B. D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–367
6 Lyons, J. M., Raison, J. K. and Kumamoto, J. in Methods in Enzymology (Fleischer, S., Packer, L. and Estabrook, R., eds), Academic Press, New York, in the press
7 Keana, J. F. W., Keana, S. B. and Beetham, D. (1967) J. Am. Chem. Soc. 89, 3055–3056
8 McMurchie, E. J., Raison, J. K. and Cairncross, K. D. (1973) Comp. Biochem. Physiol. 44B, 1017–1026
9 Lyons, J. M. and Raison, J. K. (1970) Plant Physiol. 45, 386–389
10 Baldassare, J. J., McAfee, A. G. and Ho, C. (1973) Biochem. Biophys. Res. Commun. 53, 617–623
11 Linden, C. D., Wright, K. L., McConnell, H. M. and Fox, C. F. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2271–2275
12 Raison, J. K., Lyons, J. M. and Thomson, W. W. (1971) Arch. Biochem. Biophys. 142, 83–90
13 Gulik-Krzywicki, T., Rivas, E. and Luzzati, V. (1967) J. Mol. Biol. 27, 303–322
14 Luzzati, V. and Husson, F. (1962) J. Cell. Biol. 12, 207–219
15 Shimshick, E. J. and McConnell, H. M. (1973) Biochemistry 12, 2351–2360
16 Pfaff, E., Heldt, H. W. and Klingenberg, M. (1969) European J. Biochem. 10, 484–493
17 Gruener, N. and Avi-Dor, Y. (1966) Biochem. J. 100, 762–767