THE EFFECT OF THE *OUT-COLD^{TS}* MUTATION ON TEMPERATURE INDUCED CHANGES IN THE ARRHENIUS ACTIVATION ENERGY OF SUCCINATE-CYTOCHROME *c* REDUCTASE ACTIVITY IN DROSOPHILA

Leif SØNDERGAARD, N. C. NIELSEN* and R. M. SMILLIE**
Institute of Genetics, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen, Denmark

Received 18 December 1974

1. Introduction

A number of membrane bound enzymes from certain species of plants and animals have been shown to undergo transitions from one Arrhenius activation energy to another as the temperature is varied, whereas these transitions are absent in other species. For example, the Arrhenius activation energy for succinate oxidase in mitochondria isolated from homeothermic animals including rat [1], ox [2], squirrel [3], ground squirrel [4], rabbit [5], and pig [6], characteristically undergoes an abrupt change at a temperature between 17 and 23°C. In contrast, similar changes were not observed in mitochrondria from poikilothermic animals such as rainbow trout [3], catfish [3], or toad [5]. Interestingly, a change in the Arrhenius activation energy of succinate oxidase was also not detected in mitochondria isolated from the liver of a hibernating ground squirrel although mitochondria from non-hibernating animals showed a change around 23°C like other mammalian mitochondria.

Similar observations have been made between different plant species. A marked increase in the Arrhenius activation energy of succinate oxidase activity in mitochondria from tomato [7,8], bean [7]. sweet potato [8], and cucumber [8] occured below about 12°C. These same plants cease to grow below this temperature and with time show irreversible chilling injury. On the other hand, no change in the Arrhenius activation energy of this enzyme was apparent in mitochondria from 'chilling resistant' plants including lettuce [7], pea [7], beet [8], potato [8], and cauliflower [7].

In this report it is shown that a temperature induced transition can be demonstrated around 8°C for succinate–cytochrome *c* reductase activity in mitochondria prepared from wild-type *Drosophila melanogaster*, a poikilothermic organism. There is an additional change in the Arrhenius activation energy of this activity at a considerably higher temperature in mitochondria from the '*Out-cold^{ts}*' (*Ocd^{ts}*) temperature sensitive *Drosophila* mutant [11]. This additional transition takes place at a temperature below which the mutant begins to exhibit paralytic behavior.

2. Materials and methods

The mitochondria used for these studies were isolated from highly inbred stocks of *Drosophila melanogaster* either homozygous for the *Basc* balancer chromosome [12] or carrying this chromosome and an X-chromosome with the *Ocd^{ts}* gene [11]. Experiments were also carried out using either the *Oregon-R* wild type or *Ocd^{ts}* flies backcrossed to *Oregon-R* for 30 generations.

To prepare mitochondria, 1.5 g of whole adult female flies were ground in 30 ml of 0.25 M sucrose containing 1 mM EDTA and 20 mM HEPES (pH 7.5) using a Potter-Elvehjem homogenizer. After filtering

* Present address: Department of Biochemistry and Biophysics, University of California, Davis, California, U.S.A. 95616.
** Visiting professor, University of Copenhagen. Present address: Plant Physiology Unit, CSIRO, Division of Food Research and School of Biological Science, Macquarie University, North Ryde 2113, Sydney, Australia.

the homogenate through cheesecloth, it was centrifuged at 1000 *g* for 10 minutes in a SS-34 Sorvall rotor. The resulting supernatant was centrifuged at 26 000 *g* for 15 min in a 50.1 Spinco rotor. The pellet was washed 3 times in 20 ml of the original medium and finally resuspended in 20 ml of the same medium. Protein was determined using the method of Lowry et al. [14].

The succinate–cytochrome *c* reductase activity was measured as described [13] using a Model 17 Cary spectrophotometer equipped with a cuvette cooling system. The temperature of the assay mixture during the reaction was measured with a calibrated thermocouple inserted directly into the cuvette. At low temperature precooled nitrogen gas was blown over the optical surfaces of the cuvettes to prevent condensation. The succinate–cytochrome *c* reductase activity was determined in triplicate at each temperature, and the activity at 25°C was measured periodically to demonstrate that significant changes in activity of the preparation did not take place during the experiment.

3. Results and discussion

The *Ocd*^{ts} mutant is a cold sensitive paralytic mutant in *D. melanogaster* [11]. When the temperature is lowered from 25°C to temperatures below 18°C the mutant flies become paralysed. Before the flies are paralysed they go through a characteristic sequence of behavioral events with erratic movements of their legs lasting about 10 sec followed by fluttering of their wings. In contrast, wild type flies can withstand temperatures down to about 8°C before they exhibit a similar behavior [11]. While the nature of the lesion resulting in the *Ocd*^{ts} phenotype remains unknown, the erratic leg and wing movements that are observed near 18°C for the mutant and near 8°C for wild type *Drosophila* are reminiscent of limbs whose muscles are fatigued and in tetany.

A change in the Arrhenius activation energy of succinate–cytochrome *c* reductase activity in mitochondria isolated from both the mutant and wild-type *Drosophila* were detected at the same temperatures as where paralysis took place. As can be seen in fig.1A

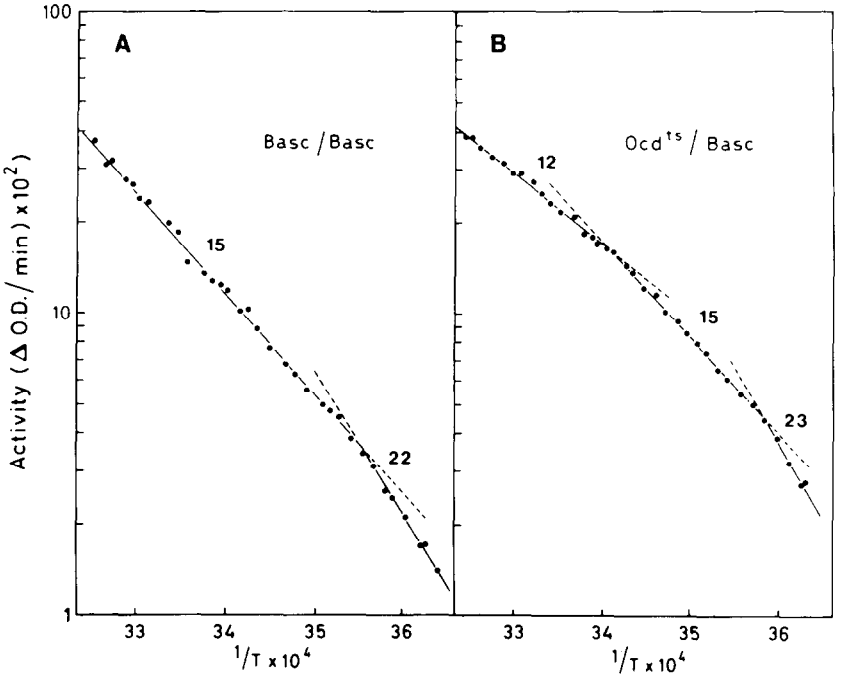

Fig.1. Arrhenius plots of succinate-cytochrome *c* reductase in mitochondria isolated from *Basc/Basc* (A) and heterozygous *Ocd*^{ts}*/Basc* (B) Drosophila. The numbers above the plots indicate the Arrhenius activation energies (Kcal/mol) calculated for each of the different slopes. The assay mixture contained: approximately 150 μg mitochondrial protein, 30 mM phosphate buffer (pH 7.5), 0.6 mM Tris, 0.03 mM EDTA, 0.05% KCN, and 45 mM Na-succinate in a final volume of 3 ml.

Table 1

Genotype of flies	Breakpoint temperatures ± s.d.	Arrhenius activation energies ± s.d. for different temperature ranges	Specific activity at 32°C, μmoles succinate oxidized/min/mg protein ± s.d.
Basc/Basc	7.9 ± 0.8	lower 23.3 ± 2.2 upper 14.9 ± 0.6	0.11 ± 0.03
Ocd^{ts}/Basc	7.0 ± 0.9 18.4 ± 0.9	lower 23.1 ± 0.6 middle 15.8 ± 0.8 upper 12.7 ± 0.6	0.14 ± 0.01

the succinate–cytochrome *c* reductase activity in mitochondria isolated from *Basc/Basc* flies showed a non-linear Arrhenius plot with a transition point at about 8°C. Fig.1B shows that this enzyme is mitochondria from heterozygous *Ocd^{ts}/Basc* flies gave an Arrhenius plot with 2 transitions, one at about 8°C and another at about 18°C. The Arrhenius activation energies of the reaction above and below the transition temperatures for mitochondria from the two types of flies are given in table 1. The Arrhenius activation energies below 8°C and between 8°C and 18°C are similar for the two types of mitochondria. There is, however, a further decrease above 18°C in mitochondria from *Ocd^{ts}* flies which is not found in wild type mitochondria.

Non-linear Arrhenius plots for membrane bound enzymes in both plants and animals [1–8] have frequently been attributed to temperature-induced decreases in the rotational freedom of the membrane lipids, cf [15]. The accompanying reduction in membrane fluidity could be envisioned to induce secondary changes in the catalytic functions by, for example, altering either the interaction between two proteins [16] or the conformation of an individual membrane polypeptide, cf [15]. Alternatively, the changes in the activation energies of these enzymes might be due to temperature-dependent conformational changes intrinsic only to the enzyme. Apparent temperature-dependent conformational changes have been reported for both fumarase [17] and inosine triphosphatase activity in myosin [18] and has been suggested for succinate dehydrogenase [6]. Biphasic Arrhenius plots have also been observed using acetyl-CoA carboxylase purified from both chicken liver [19] and wheat germ (Nielsen, unpublished observations).

The primary lesion responsible for the appearance of the additional transition in the Arrhenius plot for succinate-cytochrome *c* reductase in the *Ocd^{ts}* mutant could therefore reside in either a membrane lipid class or protein.

Multiphasic Arrhenius plots of the type shown by the *Ocd^{ts}/Basc* flies have recently been found for succinate–cytochrome *c* reductase in rat liver mitochondria from rat [9,20] and sheep [20]. On the basis of studies after partial digestion of the membrane phospholipids with phospholipase A, Wilschut and Scherphof [9] concluded that the rate limiting processes giving rise to the two transitions were dependent on phase of different phospholipids distinguishable by melting points and distribution within the membrane. Related studies on microsomal enzymes have been carried out by Eletr et al. [21] who also concluded that the physical state of the lipids at the site of tightly-bound membrane proteins influences their catalytic activity. If interpreted within this framework, the appearance of the additional transition at 18°C in the *Ocd^{ts} Drosophila* mutant could be the consequence of a genetic lesion affecting either the synthesis of a particular class of phospholipids or the binding site for phospholipids on a polypeptide in the mutant mitochondrial membrane.

While it is possible to speculate about the actual mechanism underlying the appearance of bi- or multiphasic Arrhenius plots for enzymes in the different membrane systems which have been examined, comparing the properties of the wild type and *Ocd^{ts}* mitochondrial membranes from *Drosophila* promises to be of special utility in studying these phenomena. The membrane change appears to be a specific effect of the mutation in the *Ocd* gene. Transferring the mutant gene to *Oregon-R* wild type stocks and random-

izing the genetic background by repeated backcrossing of heterozygous *Ocd*^{ts}/+females to *Oregon-R* males for 30 generations did not change the behavior of *Ocd*^{ts} flies or the pattern of transitions in the activation energies observed for different temperature ranges. Experiments with *Oregon-R* flies yielded Arrhenius plots that were similar to those from *Basc/Basc* flies (Søndergaard, unpublished data). Experiments are being designed to pinpoint a chemical difference between the mutant and wild type mitochondrial membranes which could be responsible for the appearance of the transition at 18°C in the *Ocd*^{ts} mutants.

Acknowledgements

This investigation has in part been aided by grant GM 10819 from the National Institutes of Health, United States Public Health Service to Professor D. von Wettstein.

References

[1] Kemp, A. Jr., Groot, G. P. S. and Rutsma, H. J. (1959) Biochim. Biophys. Acta 180, 28–34.
[2] Lenaz, G., Sechi, A. M. Parenti-Castelli, G., Lundi, L. and Bertoli, E. (1972) Biochem. Biophys. Res. Comm. 49, 536–542.
[3] Lyons, J. M. and Raison, J. K. (1970) Comp. Biochem. Physiol. 37, 405–411.
[4] Raison, J. K. and Lyons, J. M. (1971) Proc. Natl. Acad. Sci. U.S. 68, 2092–2094.
[5] McMurchie, E. J., Raison, J. K. and Cairncross, K. D. (1973) Comp. Biochem. Physiol. 44, 1017–1026.
[6] Zeylemaker, W. P., Jansen, H., Veeger, C. and Slater, E. C. (1971) Biochim. Biophys. Acta 242, 14–22.
[7] Shneyour, A., Raison, J. K. and Smillie, R. M. (1973) Biochim. Biophys. Acta 292, 152–161.
[8] Lyons, J. K. and Raison, J. M. (1970) Plant Physiol. 45, 386–389.
[9] Wilschut, J. C. and Scherphof, G. L. (1974) Biochim. Biophys. Acta 356, 91–99.
[10] Raison, J. K. (1973) in: Symposia Soc. Exp. Biol. 27. 485–512.
[11] Søndergaard, L. (1974) Drosophila Inform. Serv. 50, 76.
[12] Lindsley, D. L. and Grell, E. H. (1967). Genetic Variations of Drosophila melanogaster, Carnegie Inst. of Wash. Publ. No. 627.
[13] Fleischer, S. and Fleischer, B. (1967) in: Methods in Enzymology (Estabrook, R. W., Pullman, M. E. eds.), Vol. X, 406–433. Academic Press, New York.
[14] Lowry, O. H., Rosebrough, N. J., Farr, A. K. and Randall, P. J. (1951) J. Biol. Chem. 193, 265–275.
[15] Raison, J. K. (1972) Biochim. Biophys. Acta 275. 559–583.
[16] Vessey, D. A. and Zakim, D. (1971) J. Biol. Chem. 246, 4649–4656.
[17] Massey, V. (1953) Biochem. J. 53, 72–79.
[18] Levy, M. H., Sharon, N., Ryan, E. M. and Koshland Jr., D. E. (1962) Biochim. Biophys. Acta 56, 118–126.
[19] Gregolin, C., Ryder, E. and Lane, M. D. (1968) J. Biol. Chem. 243, 4227–4235.
[20] Raison, J. K. and McMurchie, E. J. (1974) Biochim. Biophys. Acta 363, 135–140.
[21] Eletr, S., Zakim, D. and Vessey, D. A. (1973) J. Mol. Biol. 78, 351–362.