Discontinuities in the Arrhenius Plots of Mitochondrial Membrane-Bound Enzyme Systems from a Poikilotherm: Acclimation Temperature of Carp Affects Transition Temperatures*

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Summary. 1. Evidence is presented for ‘breaks’ in the Arrhenius plots of succinate and cytochrome oxidase systems of mitochondria from carp liver and red epaxial muscle.

2. Arrhenius activation energies (E_a) of succinate and cytochrome oxidase systems above and below the transition temperatures (T*) are about half those reported for homiotherms (Table 3). Succinate oxidase and cytochrome oxidase obey Michaelis-Menten kinetics. The K_m-values for succinate and tetramethyl-p-phenylenediamine (TMPD) (25 °C) are similar to those in homiotherms; they are not affected by acclimation temperature (Table 2).

3. The change in E_a of carp liver mitochondria (C.L.M.) occurs at low (high) experimental temperatures if carps are acclimated to low (high) ambient temperatures for at least 4 (3) weeks prior to the experiments (Fig. 1).

4. The T* shift in Arrhenius plots following a change in acclimation temperature has been studied for succinate oxidation by carp liver mitochondria (Fig. 2). The time constant of T* change was calculated as t_accl.1/2 = 4.3 days (Fig. 3). Based on the concept of lipid protein interaction the acclimation temperature dependency of T* is discussed as a possible consequence of a lipid adaptation.

5. Carp acclimated to 26 °C and 10 °C do not differ significantly with respect to specific activity of liver succinate oxidase determined at 25 °C (Table 1). The same is true for the liver-somatic index and the amount of mitochondrial protein per gram of liver (Table 1). Changes, however, are observed in these parameters if carp are in a transitory state of temperature acclimation (early after changing from 26 °C to 10 °C; Table 1).

6. A bimodal action of temperature change during the process of acclimation is discussed combining the results in a model of succinate oxidase system of carp liver (Fig. 4).

* Dedicated to Professor Dr. H. Precht at the occasion of his 65th birthday

Abbreviations: AT = acclimation temperature, TMPD = tetramethyl-p-phenylenediamine
Introduction

Apparent activation energies of membrane bound enzymes often change at defined temperatures as indicated by distinct breaks in the slopes of Arrhenius plots. A great number of studies (cited below) document this feature as a characteristic of certain membrane bound enzymes irrespective of their phylogenetic origin. This assumption, however, has been contradicted by Lyons and Raison (1970), who pointed out differences between cold and warm blooded animals and interpreted their studies as “...demonstrating that the discontinuity and change in activation energy is a unique property of succinate oxidation in homeotherms not observed in poikilotherms” (compare McMurchie et al., 1973).

Transition temperatures in the Arrhenius plots of mammalian enzymes have been demonstrated for succinate oxidation catalyzed by different types of mitochondrial preparations (Cerletti et al., 1968; Lyons and Raison, 1970; Zeylemaker et al., 1971; Raison et al., 1971b; Lenaz et al., 1972; Smith, 1973), for ouabain-sensitive (Na⁺-K⁺)-ATPase (Charnock et al., 1971, 1973), for oligomycin-sensitive Mg²⁺-ATPase (Lenaz et al., 1972). A break in the Arrhenius plot of mammalian cytochrome oxidase has been documented by Smith and Newton (1968) and stated by Raison et al. (1971b) but was not confirmed in a recent study (Lenaz et al., 1972). In poikilothermic vertebrates, transition temperatures have been shown in Arrhenius plots for oxidation rates in coupled mitochondria from Rana pipiens (Pye, 1973) as well as in mitochondrial oxidase systems from eels (Wodtke, 1972). A comparable change in Arrhenius activation energy has been demonstrated with Mg²⁺-ATPase (Watson et al., 1973; Janki et al., 1974), succinate dehydrogenase (Watson et al., 1973) and cytochrome oxidase (Ainsworth et al., 1972) in yeast mitochondria, and with Mg²⁺-ATPase from Mycoplasma spec. (Rottem et al., 1973) and Acholeplasma laidlawii (De Kruyff et al., 1973), as well as with Ca²⁺-ATPase from E. coli (Sipheriz et al., 1973).

The present study provides evidence for breaks in the Arrhenius plots of succinate oxidase and cytochrome oxidase in mitochondrial preparations from carp liver and carp red muscle. It has been proposed (Kumamoto et al., 1971; Raison et al., 1971a; Overath and Träuble, 1973) that breaks in Arrhenius plots of membrane bound enzymes reflect phase changes in the lipid constituents of membranes (concept of lipid-protein interaction). Effects of environmental temperature on various functions of biomembranes are known to be compensated for by lipid adaptation (reviewed by Love, 1970; Hazel, 1973; see also Njus et al., 1974).

The Arrhenius function of the succinate oxidase system is studied following a change in acclimation temperature (AT) of carp: an AT-dependency of the Arrhenius plots and the time-course during a change might indicate adaptive changes in the composition of membrane phospholipids.

Materials and Methods

Carp, Cyprinus carpio L. (400–600 g) obtained from a hatchery near Kiel were maintained in aerated tap water at 18–20 °C. During acclimation to different temperatures a constant photoperiod (15 h light) was maintained and food was given ad libitum. Three groups of carp were studied:

Group I: Fish were acclimated to 10 °C for at least 28 days prior to experiments (longtime cold acclimated carps).

Group II: Fish were acclimated to 26 ± 1 °C for at least 21 days before experiments (longtime warm acclimated carps).