Biochimica et Biophysica Acta, 1064 (1991) 343–350

ADONIS 0005273691002085

BBAMEM 75228

Rapid cold-induced changes of membrane order and Δ^9-desaturase activity in endoplasmic reticulum of carp liver: a time-course study of thermal acclimation

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(Received 23 October 1990)

Key words: Temperature adaptation; Homeoviscous adaptation; Desaturase; DPH polarization; Fluorescence

The membrane order of liver endoplasmic reticulum (ER) membranes of 10°C- and 30°C-acclimated carp has been compared using the fluorescence polarization technique with DPH as probe. Membranes from cold-acclimated fish displayed lower polarizations than corresponding membranes from warm-acclimated fish, the difference compensating for 34–50% of the direct effects of temperature upon polarization. The changes in Δ^9-desaturase activity and fluorescence polarization of DPH in ER membranes have been monitored as a function of time during cold acclimation of 30°C-acclimated carp. Cooling was achieved in three stages over 48 h. Desaturase activity in both rough and smooth ER showed a rapid increase in activity for the first three days followed by a decline on day 4 and a second increase up to day 10. Polarization of DPH (measured at 10°C) was rapidly reduced on cooling with no further change after day 4. The halftime for change in polarization and for the first desaturase induction were both approx. 2 days although large changes in polarization were evident within 24 h after the onset of cooling. During the cooling phases the daily changes in DPH polarization were quantitatively related to increments in desaturase capacity. The second desaturase induction had no effect upon membrane structure, at least as indicated by the polarization technique.

Introduction

Poikilothermic animals frequently experience large and rapid changes in body temperature which may cause major physiological problems. However, they are not passive to these problems and, given time, respond by physiological, morphological and biochemical adjustments which offset or compensate for the temperature-induced disturbance [1]. An important cellular response to altered temperature, which may underlie many compensatory responses, is the adjustment in the physical properties of the cellular membranes, a phenomenon termed 'homoeoviscous adaptation' [2].

A number of studies using the fluorescence polarization technique have shown that, with few exceptions, the membranes of cold-acclimated fish are considerably more disordered than those of warm-acclimated fish. The difference between acclimation groups is sufficient to compensate for between 30 and 75% of the ordering effects of cooling (reviewed in Refs. 3 and 4). Whilst these experiments indicate the extent of homoeoviscous adaptation they do not permit any conclusions to be drawn regarding its time-course and its responsiveness to rapid temperature variations. Moreover, the simple comparison of cold- and warm-acclimated fish provide minimal correlative evidence of a relationship of homoeoviscous adaptation with changes in membrane lipid composition, with modifications of lipid biosynthesis or with adaptations of membrane function. A more searching analysis of the relationship between these various aspects of membrane restructuring may be obtained by correlating the time-course of changes immediately after temperature shift.

Cossins et al. [5] have determined the time-course of changes in DPH polarization in brain synaptosomes of goldfish during both cold- and warm-acclimation. The responses were virtually complete in 40 and 10 days, respectively, which corresponded reasonably well with the time-course of changes in lipid composition and in behavioural heat tolerance of the whole animal. It is clear that some aspects of membrane restructuring may occur rapidly upon temperature shift. Hazel and

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Landrey [6,7] have recently determined the time-course during both warm- and cold-acclimation of changes in lipid composition of trout kidney microsomes, though without relating them to changes in membrane hydrocarbon order. They found that the different compositional adjustments varied widely in response times, with rapid adjustments in headgroup composition (8–24 h) and slower changes in the proportion of phosphatides containing polyunsaturated fatty acids (10–20 days).

Homoeoviscous adaptation is thought to be primarily mediated by the alteration of membrane lipid composition and this is dependent upon changes in the activity of lipid metabolising enzymes [2]. In fish, as with other vertebrates, the liver is the principal site of lipid metabolism [8], with acyl-CoA desaturases being predominantly located in the liver endoplasmic reticulum [9]. Schünke and Wodtke [10] have shown that during cold acclimation of carp the activity of Δ^9-desaturase is rapidly and dramatically increased in a complex biphasic manner and the unsaturation indices of endoplasmic reticulum phospholipids were increased. This compositional response was due specifically to elevated proportions of monoene fatty acids with no changes in $(n-6)$ or $(n-3)$ fatty acids. In that the incorporation of an olefinic bond into an otherwise saturated fatty acid produces larger changes in the hydrocarbon order and gel-liquid crystalline transition temperature than addition of olefinic bonds to already unsaturated fatty acids [11,12], these findings suggest a rapid and effective homoeoviscous restructuring in endoplasmic reticulum membranes of carp liver may be mediated through induction of the Δ^9-desaturase.

In order to investigate this hypothesis we have determined the time-course of changes in DPH polarization and of Δ^9-desaturase during the acclimation to cold of warm-acclimated carp. The results indicate that liver ER membranes rapidly become more disordered than those of warm-acclimated carp and that the time-course of changes in membrane order correlates closely with the early phase of the desaturase induction but does not mirror a second peak in desaturase activity. Preliminary accounts of this work have been presented [13,14].

Materials and Methods

Materials

1,3-Diphenyl-1,3,5-hexatriene (DPH, 'puriss' grade) was obtained from Aldrich Chemical Co. Stearoyl-CoA (S-5625) and bovine serum albumin (BSA, A-7030) were purchased from Sigma GmbH (Munich), and NADH (grade 1) from Boehringer (Mannheim). All other chemicals were of the highest available grade.

Animals

Carp (*Cyprinus carpio* L., 0.6–1.0 kg) were obtained from the Bundesforschungsanstalt für Fischerei (D-2070 Ahrensburg, F.R.G.) in late summer and kept in aerated tap water for several months at $21 \pm 2°C$. Animals were acclimated to 30°C for approximately 50 days or to 10°C for approximately 120 days, both with 12 h daylength and fed ad libitum with Ewos T52 food (38% crude protein, 12% ash, 8% fat and 3.5% fibre, fatty acid composition as reported in Ref. 10). For the time-course experiment, water temperature was reduced from 30°C to 10°C in three stages with a cooling rate of 1 C°/h. At time zero temperature was reduced from 30 to 23°C, then at 24 h temperature was reduced from 23 to 14°C and at 48 h from 14 to 10°C. Animals were killed, membrane fractions prepared and desaturase activity determined during the months of February and March.

Membrane fractionation

Rough and smooth membranes from the endoplasmic reticulum of carp liver were prepared as described previously [10]. Liver homogenates (30 ml containing 7.5 g liver in 250 mM sucrose, Teflon-glass homogeniser rotating at 500 rpm, 5 passes) were centrifuged at $10000 \times g$ for 30 min. Aliquots (5 ml) of the supernatant were layered in 10 ml tubes over 2 ml 0.6 M sucrose containing 15 mmol CsCl/l, which in turn was layered over 3 ml 1.3 M sucrose containing 15 mmol CsCl/l. Centrifugation for 80 min at $250000 \times g$ (60000 rpm) was performed using a titanium rotor (Rotor 43.14-125) in a MSE (Crawley, U.K.) Superspeed 65 Centrifuge. The pelleted rough membranes were resuspended in 250 mM sucrose. The lower part of the upper sucrose layer was pelleted by centrifugation at $250000 \times g$ for 40 min and the pellet was resuspended to give the smooth membrane fraction. A portion of the suspension was reserved for desaturase assay and aliquots (0.5 ml) of the remainder were immediately frozen and stored in liquid nitrogen. The purity of the rough and smooth fractions have been described previously [10].

Steady-state DPH polarization measurements

The polarization of DPH under steady illumination was measured on the analogue T-format fluorimeter described previously [15–17]. Cuvette contents were continuously mixed with a magnetic follower driven by an electromagnet (Rank Bros, Bottisham, Cambridge, U.K.) positioned beneath the cuvette. The temperature of the cuvette contents were monitored by a linear thermistor to an accuracy of ± 0.1 C°. Cuvette temperature was maintained to within 0.1 C° of the desired value using a Julabo (Seelbach, Germany) refrigerated thermocirculator. Polarization values were determined with samples equilibrated at eight temperatures between 4 and 37°C. With some preparations the DPH values at exactly 10 and 30°C were obtained by interpolation from the nearest polarization/temperature values.

Linearization of the early time-course of changes in polarization at 10°C were obtained by plotting $t \cdot (\Delta P_t/\Delta P_0)^{-1}$ as a function of t where t is the time after onset of temperature decrease, ΔP_t is the mean polarization at 10°C of long-term warm-acclimated carp at day 0 minus the mean polarization of membranes at time t, and ΔP_0 is the mean polarization of long-term cold-acclimated carp minus the mean polarization of long-term warm-acclimated carp at day 0.

Δ^9-Desaturase assay

Cytochrome b_5 concentrations and Δ^9-desaturation were measured spectrophotometrically by monitoring NADH-induced reduction and stearoyl-CoA stimulated reoxidation of cytochrome b_5 in a Shimadzu (Kyoto, Japan) UV-300 spectrophotometer operated in the dual wavelength mode at 424 and 409 nm ($\epsilon = 185$ mmol l^{-1} cm^{-1}). Freshly prepared membranes were used. The activity of the Δ^9-desaturase, v, was calculated from the stearoyl-CoA stimulated cytochrome b_5 reoxidation rate constant, k (min^{-1}), determined as described by Oshino and Sato [18] and Schünke and Wodtke [10], and from the cytochrome b_5 concentration, c, (μmoles (mg protein)$^{-1}$) according to $v = 0.5 \cdot k \cdot c$ as previously described [14].

Protein assay

Protein was determined by a modified Lowry procedure according to Wang and Smith [19] using BSA as standard.

Results

Fig. 1 compares the DPH polarization for membranes of long-term cold- and warm-acclimated carp over the temperature range 4–37°C. DPH polarization for rough ER was lower than for smooth ER. The semilog plots were linear over the entire temperature range. Regression analysis for rough ER from individual carp yields slopes (Δ(log polarization)/C°) of -0.0083 ± 0.0002 (30°C-acclimated, mean ± S.D., $r^2 = 0.997$–1.000) and -0.0086 ± 0.0002 (10°C-acclimated) which are not significantly different ($P > 0.05$). Equivalent values for smooth ER (-0.0065 ± 0.0003 and -0.0073 ± 0.0004 for 30°C- and 10°C-acclimated fish, respectively) were significantly different ($P < 0.05$).

For both rough and smooth ER fractions the membranes from cold-acclimated fish displayed lower DPH polarization than membranes of warm-acclimated fish, indicating a more disordered membrane interior. The extent of the difference between acclimation groups can be characterised by the 'homoeoviscous efficacy' (Ref. 20, HE) though its calculation depends upon the two curves being parallel. Because for smooth ER the graphs for the two acclimation groups were not quite parallel, a different approach is required to calculate a value determining the extent to which the direct ordering effects of cooling on 30°C-acclimated membranes was offset by the acclimation-dependent decrease in order. This value refers specifically to the adaptive change in order which occurs during cold-acclimation and to distinguish this quantity from HE it has been termed 'homoeoviscous response' (%HR); it was calculated according to

$$\%HR = 100 \cdot \Delta(\log P) \cdot (\Delta T)^{-1} [-\Delta(\log P)/C°]^{-1}$$

where Δ(log P) is the difference in log polarization observed at 10°C for the 30°C- and 10°C-ER membranes, ΔT is the change in acclimation temperature in °C, and Δ(log P)/C° is the slope of the linear regression of log P against temperature for the 10°C-

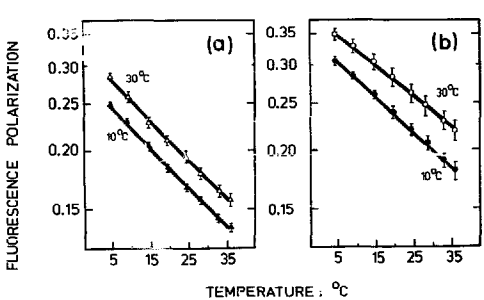

Fig. 1. Comparison of DPH polarization in rough (a) and smooth (b) endoplasmic reticulum membrane fractions from the liver of 10°C- and 30°C-acclimated carp. Values represent means ± S.D. of four preparations with each one carp. Acclimation temperature-induced differences are highly significant ($P < 0.001$, Student's t-test) at all temperatures tested.

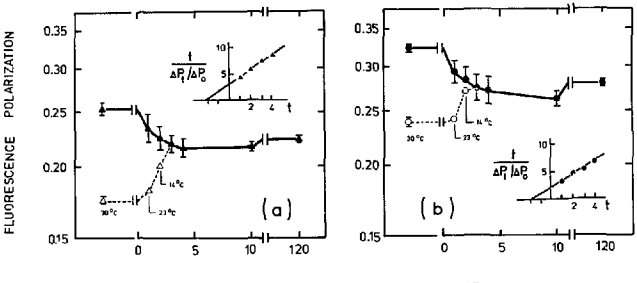

Fig. 2. The time-course of changes in DPH polarization in rough (a) and smooth (b) endoplasmic reticulum membrane fractions of carp liver. Values are shown for membranes from 30°C-acclimated carp and from 30°C-acclimated carp exposed for varying periods of time to a decrease in environmental temperature to 10°C. Closed symbols: polarization at 10°C. Open symbols: polarization at temperature experienced by carp during the cooling procedure. Values represent mean ± S.D. of four preparations with each one carp. Values for rough and smooth membranes obtained throughout the entire time course are statistically different from the controls (not cold-exposed): $P < 0.01$ or better, except for day-1- rough ER with $P < 0.05$ (Student's t-test). The insets present a linearization of the early time-course of changes in polarization calculated as described in Methods. Linear regression provided the plotted lines: $y = 1.387x + 3.000$ ($r^2 = 0.996$) for rough ER and $y = 1.146x + 2.200$ ($r^2 = 0.989$) for smooth ER. Calculated halftimes for the change in polarization were 2.16 and 1.92 days for rough and smooth ER, respectively.

acclimated membrane. Values of %HR obtained by this calculation were 33.9% for rough and 49.8% for smooth ER.

Fig. 2 shows the change with time of DPH polarization at 10°C (solid symbols) during cold-acclimation of 30°C-acclimated carp. Open symbols show the polarization values at the water temperatures experienced by the animals. For both membrane fractions there was a rapid decrease in polarization which became stable between 4 and 10 days but which increased only slightly thereafter. The apparent half-time of the response was calculated as described in Methods and in the legend to Fig. 2 to give values of 1.92 days for smooth ER and 2.16 for rough ER. No significant changes were observed in the

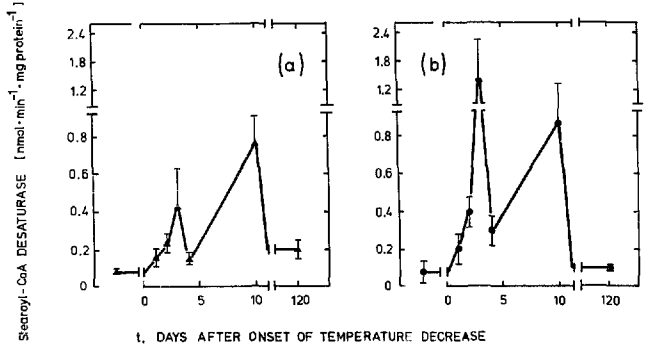

Fig. 3. The time-course of changes in the activity of Δ^9-desaturase from rough (a) and smooth (b) ER fractions. Samples were from the same preparations used for measurement of DPH polarization. Values are shown for membranes from 30°C-acclimated carp and from 30°C-acclimated carp exposed for varying periods of time to a decrease in environmental temperature to 10°C. Symbols represent mean ± S.E. of four preparations with each one carp. Enzyme activity was determined at 30°C. ER-membrane activities during cold-exposure significantly exceed the controls (not cold-exposed) in rough ER on days 2, 4, and 10 and in smooth ER on days 2 and 10 ($P < 0.05$ or better, Student's t-test).

temperature dependence of polarisation in membranes prepared during the full time course of cold-acclimation (data not shown).

Fig. 3 shows the changes in activity at 30°C of the Δ^9-desaturase in the same ER membrane preparations during cold-acclimation. Values were determined at 30°C, since, at least with membranes low in specific activity, the precision of the enzyme assay is better at 30°C as compared with 10°C. Activity in warm-acclimated specimens was very low but rapidly increased over the first 3 days followed by an equally rapid fall to day 4. This was followed by a second increase to day 10. Long-time cold-acclimated carp (120 days) showed only slightly greater activities than those recorded in long-time warm-acclimated carp and both were much smaller than those recorded during the 10 days after transfer from warm to the cold.

Discussion

Δ^9-Desaturase activity was very low in warm-acclimated carp and was only slightly greater for cold-acclimated animals. Schünke and Wodtke [10] have previously shown pronounced fluctuations in desaturase activity during the first 10 days after transfer of warm-acclimated animals to 10°C, with a biphasic time-course in rough ER and a uniphasic induction in smooth ER. In both fractions the early phase was especially rapid, peaking at the 3rd day. In the present study, a similar time-course of induction was observed, except that the smooth ER also displayed a biphasic induction with a pronounced initial peak. In both smooth and rough ER the initial increase peaked at day 3 and declined at day 4. The halftime of increase for this initial peak was approx. 2 days for both smooth and rough ER.

With regard to the differences between acclimation groups in membrane order it is clear that both ER fractions of carp liver displays sizeable homoeoviscous responses. The smooth ER showed somewhat greater responses (as indicated by %HR) than rough ER. These adaptive adjustments are similar to those recorded in a wide variety of membrane types from fish tissues, ranging from brain synaptic membranes [21] to the basolateral membranes of intestinal mucosa [17].

In a previous study the time-course of homoeoviscous adaptation in goldfish brain membranes during cold-acclimation was found to be relatively slow, with a halftime of approx. 20–30 days [5]. By contrast, a half-time of 4.3 days was reported for the shift of the discontinuities in the Arrhenius plots of a hepatic mitochondrial membrane-bound enzyme during cold-acclimation of carp, a response which has been tentatively attributed to a lipid adaptation [22]. In the present studies the changes in liver ER membranes during cold-acclimation were even more rapid, with halftimes of approximately 2 days in both rough and smooth ER.

The differences between the speed of response of the mitochondrial and ER membranes in the two studies may be due to differences between the two studies in the temperatures experienced during the early phases of the cold transfer procedure. The average temperature over the initial 2 days after cold transfer in the present study was approximately 18.5°C (23 and 14°C) whilst the average temperature over the initial 4 days in the mitochondrial study was 11.5°C [22]. The responses recorded in ER membranes therefore occurred at significantly higher temperatures than for mitochondrial membranes and given the expected high temperature dependence of the biochemical reactions which underlie these responses (Q_{10} of 2–3) it is perhaps not surprising that they occurred more rapidly.

It is worth emphasizing at this point that the apparent halftimes estimated from the present experiments are not true halftimes for a simple one-step change in temperature. In order to prevent undue cold-shock to the carp, the overall 20°C decrease in water temperature was imposed as a series of three cooling episodes at intervals of 24 h. If the membrane responses were rapid (i.e. < 24 h) then successive cooling episodes would lead to successive homoeoviscous responses and to an artefactually long halftime for the adaptive response. It is clear from the time-course graphs that a particularly large change in polarization was evident within 24 h of the onset of the first temperature shift and only 17 h after it was complete.

Some idea of the extent of this short-term response can be gained by the comparison of %HR over the first 24 h (Table I) with that calculated for long-term acclimated carp. Thus following the onset and completion of the first cooling episode of 7°C particularly large %HR values were recorded in both smooth and rough ER, values which exceeded, by a large margin, the corresponding values for long-term acclimated carp. The values for the second day indicate reduced %HR, though this was for a particularly large drop in water temperature (9 C°). For the third day the %HR was increased to values which were similar to those of long-term acclimated carp, though this was for a small reduction in temperature. These high values of %HR recorded over the short-term indicate that homoeoviscous adaptation occurs rapidly. A more precise idea of the true speed of homoeoviscous responses in carp liver must await more detailed time-course studies, but it is clear that the actual halftime is considerably less than 24 h.

It is evident from this work and from the recent compositional analyses by Hazel and Landrey [6,7] and Carey and Hazel [23] that, in particular membrane-types, the processes which contribute to membrane restructuring may occur with unsuspected speed. Moreover, the rapid responses in the cold must be appreciated within the context of a large reduction in the rates of biochem-

TABLE I

Short-term homoeoviscous responses, increments in desaturase activities and desaturase-normalized homoeoviscous responses in rough and smooth ER during cold-acclimation of 30°C-acclimated carp

Membrane fraction	Experimental conditions		Membrane responses			
	Time (days)	Final temp. (°C)	Homoeoviscous response (%HR) [a] (%)	Daily increment in desaturase activity		Homoeoviscous response (%HR), normalized [c] (%)
				actual [b] (nmol min^{-1} $(mg\ protein)^{-1}$)	fractional [b]	
rough ER	1st	23	60.9	0.077	1.00	60.9
	2nd	14	20.0	0.077	1.00	20.0
	3rd	10	33.7	0.195	2.53	13.3
smooth ER	1st	23	92.5	0.125	2.21	41.9
	2nd	14	24.2	0.194	3.43	7.06
	3rd	10	57.0	1.013	17.9	3.19

[a] Homoeoviscous responses. %HR was calculated from the values of DPH polarization, P, at 10°C using the equation: $\%HR = 100 \cdot \Delta(\log P) \cdot (\Delta T)^{-1} \cdot [-\Delta(\log P)/C^{\circ}]^{-1}$, where $\Delta(\log P)$ is the change in log polarization during a 24 h interval of acclimation, ΔT is the change in acclimation temperature over the same interval and $\Delta(\log P)/C^{\circ}$ is the slope of the linear regression of log P against temperature determined experimentally for the ER membranes in question. Means of the slopes obtained at different times during the acclimation regime were not significantly different to those of 30°C-acclimated fish (data not shown).

[b] Desaturase activity. 'Actual' values are the increments of desaturase specific activity (measured at 30°C) over successive 24 h intervals after the onset of cold-acclimation ('actual Δ(desaturase)'). 'Fractional' values were calculated according to: Fractional Δ(desaturase) = actual Δ(desaturase)$\cdot F \cdot$[actual Δ(desaturase) rough ER for first 24 h period]$^{-1}$, where $F = 1$ (rough ER) or 1.36 (smooth ER) accounts for the differences in lipid-to-protein ratios in carp ER-membranes (Schünke and Wodtke, unpublished data).

[c] Normalized homoeoviscous responses. %HR was normalized for variations in daily changes in desaturase activity according to Normalized $\%HR = \%HR \cdot [\text{fractional } \Delta(\text{desaturase})]^{-1}$.

ical processes caused by the sudden reduction in temperature. Thus, at least in this specific respect, cellular biochemistry is responsive to changes in temperature which occur over 24 h and therefore may show responses to diurnal temperature variations. In some animals membrane responses may be even more rapid.

Perhaps the most interesting question is how closely the time-course of changes in DPH polarization correlate with the time-course for induction of the Δ^9-desaturase activity since this may provide evidence of a causal link between the two. The rapid changes in DPH polarization during the first three days correspond with corresponding increases in desaturase activity, the apparent halftimes for the changes being approx. 2 days in each case. However, there was no further change in polarization between day 4 and 10 during the second peak of desaturase activity and there was only a small increase in polarization between day 10 and the long term cold-acclimated carp. This strongly suggests that only the first, rapid induction of desaturase is linked to any change in membrane physical structure, the second desaturase induction having no effect detectable by the fluorescence polarization technique.

The large changes in DPH polarization during the first 24 h were associated with comparatively small increments in desaturase activity. By contrast, the large increase in desaturase activity on day 3 was associated with a comparatively small change in DPH polarization. This suggests that the changes in homoeoviscous response in ER membranes and its relationship to desaturase activity is influenced not only by the desaturase capacity but also by reductions in temperature. In order to estimate the effect of temperature alone upon %HR we have corrected the daily %HR values for the corresponding changes in desaturase activity to provide a 'normalized' %HR. This reflects the effect of a constant increment of desaturase activity upon %HR at the different temperature on days 1–3. The calculation also takes into account the reasonable expectation that %HR reflects changes in the lipid moiety of the membranes and that membrane lipid-protein ratio is 1.36-fold greater in smooth ER compared to rough ER (Schünke and Wodtke, unpublished data) so that normalized %HR for the two membrane fractions can be compared.

Table I shows that despite the fluctuating absolute %HR over the first 3 days, the normalized %HR declined progressively with time and with reducing temperature. Fig. 4a shows this as Arrhenius plots of the normalized %HR which for both ER fractions was impressively linear. It is clear that normalized %HR increases with increasing temperature and extrapolation of the Arrhenius plots to higher temperatures produces a perfect homoeoviscous response (i.e., log normalized %HR = 2) at 27.5°C for both ER fractions. The apparent activation energies indicate a strong temperature dependence of normalized %HR. For rough ER this was equivalent to a Q_{10} (10–30°C) of 3.19. Δ^9-Desaturase activity, by contrast, exhibits a Q_{10} of 1.43 over the

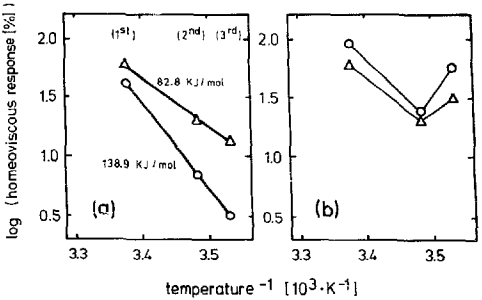

Fig. 4. Arrhenius plots of short-term (24 h) homoeoviscous responses. %HR's, in carp liver ER fractions. 'Normalized' homoeoviscous responses (a) as well as the basic %HR-values (b) were calculated as described in Table I for the three daily intervals after the start of cold-acclimation. Values were transformed to log %HR and plotted against the reciprocal temperature. Triangles, rough ER; circles, smooth ER. 1st, 2nd and 3rd denote the sequence of sampling intervals (days) after the start of cold-acclimation. In (a) the linear regression for rough ER was $y = -4.31x + 16.34$ ($r^2 = 0.997$) and for smooth ER was $y = -7.23x + 26.03$ ($r^2 = 0.999$). The Arrhenius activation energies calculated from these regressions are shown next to each graph.

same temperature interval [10]. This disparity indicates that homoeoviscous adaptation and Δ^9-desaturase activity are not directly coupled, but are linked, both, kinetically by de- and reacylating activities, and at the substrate level by the desaturation of the acyl-CoA pool.

The linearity of the Arrhenius plots in Fig. 4a suggests that homoeoviscous response was rapid and largely completed within the isothermal phase of each of the first three days. If there were any significant homoeoviscous responses occuring after this time the Arrhenius graph would be expected to curve upwards with decreasing temperature. In contrast to the linear plots, Fig. 4b shows distinctly non-linear Arrhenius plots for the absolute %HR due to an enhanced response on day 3. This is clearly brought about by the massive induction of desaturase activity after cooling of carp to 10°C during day 3.

Wodtke [13] has shown a rapid decrease in the proportion of saturated fatty acids of ER membranes immediately following cold exposure. The time-course of the changes in saturation in rough ER correlated well with the biphasic induction of desaturase in that a rapid decrease in saturation occurred between day 0 and day 3 with little further change up to day 7. This was followed by a second large decrease in unsaturation between day 7 and day 10. This, however, was transient in that the saturation of ER membranes from long-time acclimated carp was similar to that observed after the first phase of desaturation. Thus whilst the second phase of desaturation was associated with a transient shift in the composition of phospholipid acyl groups [13] we show here that it was not associated with any effect upon DPH polarization. It is suggested, therefore, that the second phase of desaturation as elicited under isothermal conditions is not associated with homoeoviscous adaptation per se but with some other membrane restructuring, the nature or significance of which is not clear.

One possibility is that the second phase of desaturation is necessary to offset other compositional changes which occur during cold-acclimation. Although the membrane cholesterol-to-phospholipid ratio is generally lower in long term cold-acclimated carp [13,24–26], it might peak transiently around day 10 as a consequence of the substantially elevated capacity for hepatic cholesterol synthesis in cold-acclimated carp [27]. Since elevated membrane cholesterol increases membrane order [28,29], the second phase of desaturation might effectively 'titrate' the cholesterol effect, giving no change in DPH polarization.

Acknowledgement

E.W. thanks the Deutsche Forschungsgemeinschaft (Wo242/5-4) for research and travel funds.

References

1 Cossins, A.R. and Bowler, K. (1987) Temperature Biology of Animals, Chapman and Hall, London.
2 Hazel, J.R. (1988) in Advances in Membrane Fluidity (Aloia, R.C., ed.), pp. 149–188, Alan R. Liss, New York.
3 Cossins, A.R. and Raynard, R.S. (1988) in Temperature and Animal Cells (Bowler, K. and Fullen, B.J., eds.), pp. 95–111, Society for Experimental Biology Symposium Series, Cambridge University Press, Cambridge.
4 Cossins, A.R. and Macdonald, A.G. (1989) J. Bioeng. Biomembr. 21, 115–135.

5 Cossins, A.R., Friedlander, M.J. and Prosser, C.L. (1977) J. Comp. Physiol. 120, 109–121.
6 Hazel, J.R. and Landrey, S.R. (1988) Am. J. Physiol. 255, R622–R627.
7 Hazel, J.R. and Landrey, S.R. (1988) Am. J. Physiol. 255, R628–R634.
8 Green, D.H.S. and Selivonchick, D.P. (1987) Prog. Lipid Res. 26, 53–85.
9 Ninno, R.E., DeTorrengo, M.P., Castuma, J.C. and Brenner, R.R. (1974) Biochim. Biophys. Acta 360, 124–133.
10 Schünke, M. and Wodtke, E. (1983) Biochim. Biophys. Acta 734, 70–75.
11 Coolbear, K.P., Berde, C.B. and Keogh, K.M.W. (1983) Biochemistry 22, 1466–1473.
12 Stubbs, C.B. and Smith, A.D. (1984) Biochim. Biophys. Acta 779, 89–137.
13 Wodtke, E. (1986) in Biona Report 4 (Laudien, H., ed.; Nachtigall, W., Ser. ed.), pp. 129–138, Gustav Fischer, Stuttgart.
14 Wodtke, E., Teichert, T. and König, A. (1986) in Living in the Cold (Heller, H.C., Musacchia, X.J. and Wang, L.C.H., eds.), pp. 35–42, Elsevier, New York.
15 Cossins, A.R. (1977) Biochim. Biophys. Acta 470, 395–411.
16 Cossins, A.R. and Macdonald, A.G. (1984) Biochim. Biophys. Acta 776, 144–150.
17 Lee, J.A.C. and Cossins, A.R. (1990) Biochim. Biophys. Acta 1026, 195–203.
18 Oshino, N. and Sato, R. (1972) Arch. Biochem. Biophys. 149, 369–377.
19 Wang, C.-S. and Smith, R.L. (1975) Anal. Biochem. 63, 414–417.
20 Cossins, A.R. (1983) in Cellular Acclimatisation to Environmental Change (Cossins, A.R. and Sheterline, P., eds.), pp. 3–32, Cambridge University Press, Cambridge.
21 Cossins, A.R. and Prosser, C.L. (1982) Biochim. Biophys. Acta 687, 303–309.
22 Wodtke, E. (1976) J. Comp. Physiol. 110, 145–157.
23 Carey, C. and Hazel, J.R. (1989) J. Exp. Biol. 147, 375–391.
24 Wodtke, E. (1978) Biochim. Biophys. Acta 529, 280–291.
25 Wodtke, E. (1983) J. Therm. Biol. 8, 416–420.
26 Wodtke, E. and Schünke, M. (1983) J. Therm. Biol. 8, 421–423.
27 Teichert, T. and Wodtke, E. (1987) Biochim. Biophys. Acta 920, 161–170.
28 Yguerabide, J. and Foster, M.C. (1981) in Molecular Biology, Biochemistry and Biophysics, Vol. 31 (Grell, E., ed.), pp. 199–269, Springer Verlag, Heidelberg.
29 Presti, F.T. (1985) in Membrane Fluidity in Biology, Vol. 4 (Aloia, R.C. and Boggs, J.M., eds.), pp. 97–146, Academic Press, Orlando.