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COLD-INDUCED INCREASE OF Δ^9- AND Δ^6-DESATURASE ACTIVITIES IN ENDOPLASMIC MEMBRANES OF CARP LIVER

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Long-term warm-acclimated (30°C) carp were exposed to a sudden decrease in acclimation temperature. Kinetic properties and the time-course of activity of the Δ^9- and Δ^6-desaturases were measured in rough and smooth membranes of endoplasmic reticulum isolated from liver. During early cold exposure of fish, enhancements of desaturase activities are about 30-fold in rough and at least 18-fold in smooth membranes. Enhancements of activity are biphasic in rough endoplasmic reticulum but monophasic in the smooth membranes. They are assumed to be caused mainly by the synthesis of additional desaturase enzyme protein. The significantly higher activities in long-term cold-acclimated (10°C) carp are in accordance with the increased fatty acid unsaturation of their membrane lipids.

Introduction

In a variety of poikilothermic organisms, ranging from bacteria to higher plants and animals, exposure to low temperatures is generally associated with an increased degree of lipid unsaturation, which compensates the cold-induced rigidifying of membranes. This cold-induced lipid-substitution is thought to be controlled by an increased desaturation activity in the cold. Enhancement of activity has been proposed to be achieved by three different mechanisms: (1) increased availability of O_2 at reduced temperatures in yeast and higher plants [1,2]; (2) activation of existing fatty acid desaturase enzyme in protozoa due to reduced membrane fluidity [3,4]; and (3) synthesis of additional desaturase enzyme protein in bacterial systems [5], which is also reported to be involved in dietary induction [6].

Studies on fatty acid unsaturation in thermally acclimated poikilothermic vertebrates are restricted mainly to fish (for a review see Hazel and Prosser [7]). The aspects of evolutionary acclimation to temperature have been discussed by Cossins and Prosser [8]; for the combined effects of temperature and diet, see Ref. 9. All studies indicate a cold-induced increase in membrane lipid unsaturation. In carp, this feature has been documented for whole liver [10], in membranes of muscle and liver mitochondria [11,12] and in endoplasmic reticulum from liver [13].

There is no study on desaturase activity during thermal acclimation of fish, except the work of Brenner and co-workers. Although they found 2-fold higher desaturation activities in liver microsomes of long-term cold-acclimated *Pimelodus maculatus* [14,15], the corresponding fatty acid composition [15] was apparently opposite to the general pattern. As carp respond to changes of environmental temperature in a very pronounced compensation of membrane unsaturation [10–13], they are well suited for study of the relationship between desaturation activity and temperature.

Materials and Methods

Experiments were carried out in late summer with carp, *Cyprinus carpio* L. (500–800 g), pre-acclimated to 30°C and kept at a constant photoperiod (12 h light). Decrease of temperature towards 10°C was conducted in three steps with a cooling rate of 1°C per h. Initially (t_0 = zero time) temperature was reduced from 30°C to 23°C, then (t_1 = 1 day) from 23°C to 14°C, and finally (t_2 = 2 days) from 14°C to 10°C.

Fish were fed ad libitum once a day with Ewos T 52, containing 38% crude protein, 8% fat, 12% ash and 3.5% fibre. Determination of fatty acid composition of the triacylglycerol fraction in the diet fat revealed 31.1, 51.7, 3.7 and 13.7 mol% of saturated, monounsaturated, ($n-6$) and ($n-3$) fatty acids, respectively.

Rough and smooth endoplasmic reticula of carp liver were isolated on a Cs^+-containing discontinuous sucrose gradient according to Dallner [16]. Effective separation of both types of membrane was indicated by the following ratios of membrane-bound RNA to phospholipid (mg/mg): 0.72 and 0.13 (rough and smooth membranes, cold-acclimation); 1.46 and 0.10 (rough and smooth membranes, warm-acclimation). In two-dimensional separations of phospholipids from isolated rough and smooth endoplasmic membranes (not shown) virtually no cardiolipin could be found; thus, any significant contamination by mitochondria can be excluded. In order to estimate plasma-membrane contamination, 5′-nucleotidase has been determined in the homogenate and in the isolated fractions (not shown). Considering that 5% of total liver protein is plasma-membrane protein [17] and that 5′-nucleotidase is approximately equally distributed between plasma and intracellular membranes [18], contamination of the isolated endoplasmic-membrane protein by plasma-membrane protein has been calculated as follows: 1.2% and 5.1% (rough and smooth membranes, cold-acclimation) but 3.4% and 10.4% (rough and smooth membranes, warm-acclimation). Lipid extraction, separation of phospholipids from neutral lipids, and quantitive analysis of fatty acid composition by gas-liquid chromatography were carried out as described for carp liver mitochondria [12].

The activities of Δ^9- and Δ^6-desaturase systems were estimated spectrophotometrically by monitoring substrate-stimulated reoxidation of cytochrome b_5 in a Shimadzu dual-wavelength spectrophotometer at 424 and 409 nm (ϵ = 185 $mM^{-1} \cdot cm^{-1}$) according to Oshino and Sato [19]: membranes (approx. 0.3 nmol cytochrome b_5, equivalent to 2–4 mg protein) were preincubated in 100 mM Tris-acetate buffer (pH 7.2) for 5 min at 30°C. Cytochrome b_5 redox cycle was initiated with 1–4 μM NADH in the presence and in the absence of acyl-CoA derivates (test volume, 2.5 ml). Activities of the desaturases are given as rate constants $k = k^+ - k^-$; k^+ and k^- are the cytochrome b_5 reoxidation rate constants in the presence (+) and absence (−) of substrate. Stearyl-CoA (S-5626) and linoleoyl-CoA, lithium salt (L-9754), were purchased from Sigma GmbH (Munich), NADH, Grade I, from Boehringer (Mannheim), all other chemicals were of highest available purity.

Student's t-test was employed to test for significant differences between the means of data.

Results

Fatty acid composition of rough and smooth membranes of the endoplasmic reticulum

Table I provides information about the phospholipid fatty acids of rough and smooth membranes of the endoplasmic reticulum isolated from the livers of long-term warm- (30°C) and cold- (10°C) acclimated carp. During cold-acclimation in both membranes the saturated fatty acids are markedly decreased, whereas the proportion of the monounsaturated species is significantly increased. Similarly the ($n-6$) family is elevated; its fatty acid pattern (not shown) reveals a temperature-independent proportion of linoleic acid (18 : 2, $n-6$), but an approx. 50% increase of arachidonic acid (20 : 4, $n-6$) during cold-acclimation. A comparable result has been obtained with liver mitochondria of thermally acclimated carp and has been discussed with respect to an increase of Δ^6-desaturation in the cold environment [12]. In addition, the amount of ($n-3$) fatty acids is not affected by acclimation temperature. The cold-induced increase in general unsaturation is reflected by the higher unsaturation indices (number

TABLE I

THE INFLUENCE OF ACCLIMATION TEMPERATURE ON THE MOLAR ACYL CHAIN COMPOSITION OF ENDOPLASMIC RETICULUM (ER) MEMBRANE PHOSPHOLIPIDS FROM CARP LIVER

Membrane lipids were extracted and phospholipids were separated from neutral lipids by one-dimensional thin-layer chromatography, eluted with chloroform, transmethylated and subjected to gas-liquid chromatography for determination of fatty acid compostion as described in Ref. [12]. Mol% of the individual fatty acids were summarized as fatty acid families; P values indicate level of significance (10°C vs. 30°C); n.s., not significant. Data are given as mean of three preparations ± S.D.

Σmol% fatty acids	Rough ER membranes			Smooth ER membranes		
	10°C	30°C	P	10°C	30°C	P
Saturated	36.4 ± 1.8	44.3 ± 0.6	< 0.002	37.6 ± 1.0	48.8 ± 1.2	< 0.001
Mono-unsaturated	33.2 ± 1.3	27.6 ± 1.9	< 0.020	31.7 ± 1.2	26.1 ± 2.3	< 0.020
($n-6$)	12.7 ± 0.4	9.5 ± 0.8	< 0.010	12.2 ± 0.9	8.1 ± 0.7	< 0.010
($n-3$)	17.9 ± 0.7	18.6 ± 2.3	n.s.	17.9 ± 0.6	17.0 ± 2.3	n.s.

of olefinic bonds per 100 fatty acids): 178.1 ± 5.5 (rough membranes, 10°C-acclimated carp) and 176.3 ± 2.8 (smooth membranes, 10°C-acclimated carp) compared with 163.1 ± 10.4 rough membranes, 30°C-acclimated carp) and 149.8 ± 9.8 (smooth membranes, 30°C-acclimated carp).

Kinetic studies of Δ^9- and Δ^6-desaturases

Fig. 1 presents the relationships between reactions velocity, expressed as rate constant, k, and substrate concentration for both types of desaturase in rough and smooth endoplasmic membranes of cold-acclimated carp. In both membrane preparations the Δ^6-desaturase shows typical hyperbolic saturation curves, whereas the Δ^9-desaturase is inhibited at high substrate concentrations. Inhibition is more pronounced in smooth membranes at 30°C and rough membranes at 10°C, but less distinct in rough membranes at 30°C.

The V_{max} of Δ^6-desaturase is approximated by the maximum rate constants obtained at 40 μM linoleoyl-CoA, whereas Δ^9-desaturase exhibits maximum k values at concentrations of about 10 μM stearyl-CoA. As judged from substrate concentrations at half-maximum desaturation rates (approx. 20 μM for linoleoyl-CoA/4 μM for stearyl-CoA), substrate affinity of Δ^9-desaturase is approx. 5-fold higher as compared with the Δ^6-desaturation reaction. From the data for rough mem-

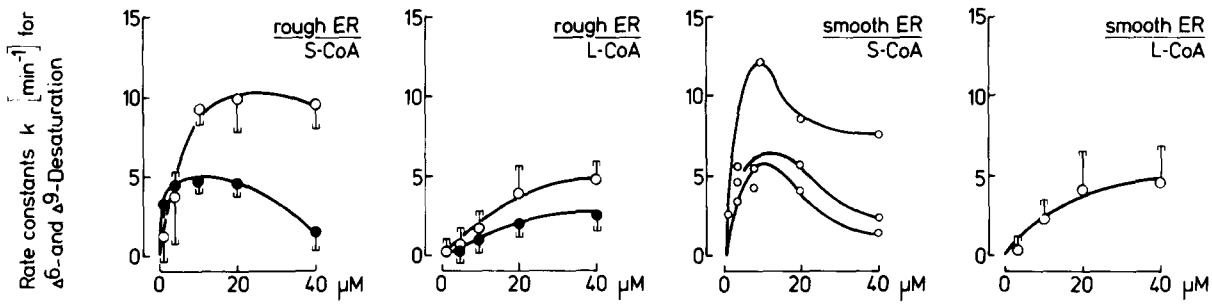

Fig. 1. Influence of acyl-CoA-concentrations on the rate constants of Δ^9- and Δ^6-desaturation in rough and smooth membranes of the endoplasmic reticulum from 10°C-acclimated carp. ER, endoplasmic reticulum; S-CoA and L-CoA, stearyl- and linoleoyl-CoA. The rate constants for reoxidation of NADH-reduced cytochrome b_5 were measured with added acyl-CoA derivates (k^+) and without acyl-CoA derivates (k^-); rate constants for Δ^9- and Δ^6-desaturation are given as $k = k^+ - k^-$. k^--rate constants are as follows (experimental temperature in brackets): rough ER 1.66 ± 0.09 (10°C) and 7.56 ± 1.04 (30°C), smooth ER 6.19 ± 1.40 (30°C). Saturation curves represent the mean of three preparations (bars indicate S.D.), except smooth ER/S-CoA (three individual preparations). Experimental temperatures: 10°C (●) and 30°C (○).

TABLE II

INFLUENCE OF ACYL-CoA CONCENTRATIONS ON THE RATE CONSTANTS OF CYTOCHROME b_5-REOXIDATION IN ROUGH AND SMOOTH MEMBRANES OF THE ENDOPLASMIC RETICULUM (ER) FROM 30°C-ACCLIMATED CARP

Rate constants for the cytochrome b_5-reoxidation are given as k^- (without acyl-CoA) and as k^+ (in the presence of acyl-CoA); P values indicate the level of significance (k^+ versus k^-); significant higher k^+ values as compared with k^- values indicate acyl-CoA desaturation. For the ($k = k^+ - k^-$) values, see text. Data are given as mean of three preparations ± S.D.. Individual preparations are generally assayed in triplicate.

Conditions	Cytochrome b_5-reoxidation rate constants (min^{-1})							
	Rough ER membranes ($k^- = 2.30 \pm 0.24$)				Smooth ER membranes ($k^- = 2.46 \pm 0.35$)			
Acyl-CoA (μM)	Stearyl-CoA		Linoleoyl-CoA		Stearyl-CoA		Linoleoyl-CoA	
	k^+	P	k^+	P	k^+	P	k^+	P
1	2.31 ± 0.04	n.s.	2.04 ± 0.22	–	2.82 ± 0.43	n.s.	2.46 ± 0.01	n.s.
2	2.54 ± 0.50	n.s.	2.10 ± 0.06	–	2.84 ± 0.24	n.s.	2.56 ± 0.19	n.s.
4	2.98 ± 0.04	< 0.01	2.16 ± 0.28	–	2.74 ± 0.29	n.s.	2.74 ± 0.13	n.s.
10	2.94 ± 0.18	< 0.05	2.82 ± 0.33	n.s.	2.36 ± 0.43	–	3.28 ± 0.23	< 0.05
20	2.70 ± 0.16	n.s.	3.08 ± 0.50	n.s.	2.04 ± 0.36	–	3.64 ± 0.43	< 0.05
40	1.68 ± 0.47	–	3.21 ± 0.39	< 0.05	1.44 ± 0.22	–	3.74 ± 0.76	(< 0.10)

branes/stearyl-CoA, it would appear that decreasing the experimental temperature increases substrate affinity.

In contrast to the description of desaturase kinetics for the rough and smooth endoplasmic membranes of 10°C-acclimated carp in Fig. 1, Table II contains the cytochrome b_5-reoxidation rate constant, k^+ and k^-, for the membranes of 30°C-acclimated carp. Except for smooth endoplasmic reticulum/stearyl-CoA, k^+ values slightly but significantly exceed the corresponding k^- values – and thus reflect some desaturation – at those substrate concentrations which produce maximum activity in the membranes of 10°C-acclimated carp. Evaluation of the data yields the following desaturase rate constants for the $\Delta^9(\Delta^6)$-desaturases in the membranes of 30°C-acclimated carp (substrate concentration, 10 (40) μM; experimental temperature, 30°C):

$k_{rough,\Delta^9} = 0.65 \pm 0.20\ min^{-1}$

$k_{rough,\Delta^6} = 0.91 \pm 0.62\ min^{-1}$

$k_{smooth,\Delta^6} = 1.28 \pm 1.08\ min^{-1}$

There is virtually no measurable activity in smooth endoplasmic membranes of warm-acclimated carp.

Suprisingly, the cytochrome b_5-reoxidation rates in the presence of increasing concentrations of stearyl-CoA are decreased to values below k^- as particularly pronounced in smooth membranes. Similar observations in rat microsomes have been attributed to a detergent-like action of stearyl-CoA on endoplasmic membranes [20].

Induction of desaturase activity

As shown in Fig. 2, a decrease in environmental temperature results in an instant and dramatic increase of desaturation activities, which, however, are reduced in long-term cold-acclimated carp to values considerably above the initial activities. Desaturase activities in rough membranes are characterized by two hyperinduction-attenuation phases around days 3 and 10, enclosing a minimum below the final activity level, whereas smooth membranes exhibit a single activity peak at day 3 only. Within the first 3 days after onset of temperature decrease desaturation activities in rough endoplasmic reticulum increase 34-fold (stearyl-CoA) and 22-fold (linoleoyl-CoA). In smooth membranes, rate constants are elevated for Δ^9-desaturase from zero level to $k_{3\,days} = 34\ min^{-1}$ and for Δ^6-desaturase from $k_0 = 1.3\ min^{-1}$ to $k_{3\,days} = 18\ min^{-1}$ by a factor of 14.

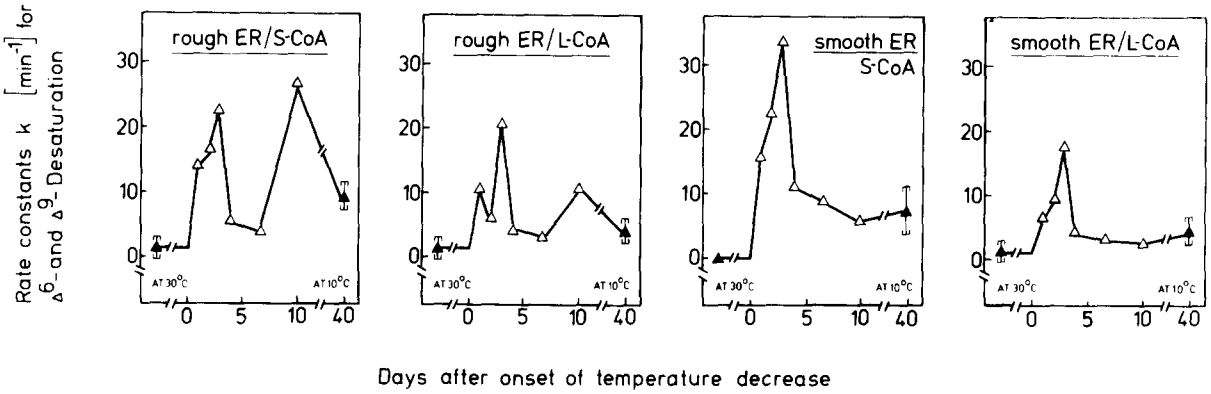

Fig. 2. Time-course of Δ^9- and Δ^6-desaturation activity in response to a decrease in environmental temperature. Abbreviations as per Fig. 1. The desaturation activity is expressed as rate constant, k (see legend to Fig. 1). Steady states of long-term warm- (30°C) and long-term cold- (10°C) acclimated carp (▲) are given as means of three preparations ± S.D. During the time-course, each datum point represents one individual preparation, generally mean of three determinations (△). Substrate concentrations were 10 μM (stearyl-CoA) and 40 μM (linoleoyl-CoA). AT, acclimation temperature of carp in thermal steady-state (long-term acclimated fish). Experimental temperature was 30°C.

Discussion

As expected, membrane lipids from warm-acclimated carp are more saturated as compared with those obtained from carp after a 40-day period of cold acclimation (Table I). Thus, following a sudden downward shift in temperature a considerably rapid membrane restructuring would be favourable. Indeed, this is most efficiently initiated by the pronounced enhancement of desaturation activities immediately after the onset of temperature decrease, as shown in Fig. 2. Such a phenomenon is similar to the hyperinduction of desaturase activity found in *Bacillus megaterium* [21] and *Tetrahymena pyriformis* [22] after cold exposure. In both organisms, enhanced activity is due to increased synthesis of enzyme protein. It is likewise assumed here that the time-course of desaturase activities in carp endoplasmic membranes following cold exposure of fish reflect controlled levels of enzyme protein.

In addition to the cold-induced first peak of desaturase activity in rough membranes, the occurrence of a second activity peak under isothermal conditions would support the notion that desaturase levels are controlled by a modulator system which directly senses fluidity and consequently responds to temperature changes.

Oshino and Sato [23] have proposed that desaturase protein is synthesized on polysomes attached to rough endoplasmic membranes and is then transferred to the smooth membranes. In contrast to the initial enhancement of activity, there is obviously no elevated transfer of enzyme protein to the smooth membranes during the second activity peak (Fig. 2).

The hypothesis of a cold-induced enhancement of desaturase activity via decrease of membrane fluidity (self-regulation), as proposed by Kates and Pugh [24], is not in accordance with Q_{10} values of 1.43 and 1.46, as calculated from Fig. 1 (data for rough endoplasmic reticulum with stearyl-CoA (10 μM) and linoleoyl-CoA (40 μM).

From the rate constants in Fig. 2, determined at 30°C, enzyme levels in rough membranes from long-term cold-acclimated carp would appear to be increased approx. 15-fold for the Δ^9-desaturase but only 5-fold for the Δ^6-desaturase compared with long-term warm-acclimated fish. Considering the actual (in vivo) temperatures in cold- (10°C) and warm- (30°C) acclimated carp, maximum desaturation capacities in vivo of the Δ^9- and Δ^6-systems, however, are enhanced only 6.9-fold and 2.5-fold for cold-acclimated carp.

The very low desaturation activities in the endoplasmic membranes of 30°C-acclimated carp suggest that during warm acclimation the requirements of carp for unsaturated fatty acids are

covered mainly by dietary lipids. The more pronounced increase in Δ^9-activity as compared with Δ^6-activity might be caused by a significantly restricted availability of saturated and monounsaturated fatty acids from the diet at 10°C, which is compensated by an increased de novo synthesis of saturated fatty acids (see Ref. 25), which then are to be Δ^9-desaturated.

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