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Influence of thyroid status on the membranes of rat liver mitochondria

Unique localization of L-glycerol-3-phosphate dehydrogenase

Z. Beleznai, E. Amler*, H. Rauchová*, Z. Drahota* and V. Jancsik

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary and **Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia*

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The effect of thyroid status on the physical properties of rat liver mitochondrial membranes and on the lipid microenvironment of proteins was investigated. The steady-state fluorescence anisotropy of diphenyl-1,3,5-triene and 1-[4-{trimethylaminophenyl}phenyl]-6-phenylhexa-1,3,5-triene revealed an increase of the order of the membranes with the increase of hormone level. Protein arrangement in the inner mitochondrial membrane altered with the thyroid status, which was reflected by digitonin subfractionation of mitochondria. The microenvironment of FAD-linked L-glycerol-3-phosphate dehydrogenase was dramatically influenced by thyroxine.

Thyroid hormone; Steady-state fluorescence anisotropy; Digitonin subfractionation; FAD-linked L-glycerol-3-phosphate dehydrogenase; (Rat liver mitochondria, Mitoplast)

1. INTRODUCTION

The influence of the thyroid status on the composition of liver mitochondrial membranes [1], as well as on mitochondrial metabolism [2], is widely documented. These two groups of phenomena are interrelated as membrane fluidity and lipid composition play important roles in the functioning of proteins and enzymes associated to the membrane [3] and vice versa [4,5]. In the lipid pattern, the cholesterol content, phospholipid composition and the degree of fatty acid saturation change most significantly with hormone level [1,6]. The question arose as to which way different hormone levels would affect the physical properties of membranes, lipid-protein interactions and consequently the membrane-bound enzyme activities.

In the present article the properties of mitochondrial membranes, as derived from the steady-state fluorescence anisotropy values of fluorescent probes DPH and TMA-DPH, were investigated in hyper-, eu- and hypothyroid states and compared with the stability of lipid-protein interactions, as estimated from digitonin solubilization studies.

Correspondence address: Zsuzsa Beleznai, Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, H-1502 Budapest, PO Box 7, Hungary

Abbreviations: DPH, diphenylhexa-1,3,5-triene; GDH, L-glycerol-3-phosphate dehydrogenase; GluDH, glutamate dehydrogenase; MDH, malate dehydrogenase; RINCR, rotenone insensitive NADH-cytochrome-*c* oxidoreductase; SDH, succinate dehydrogenase; TMA-DPH, 1-[4-{trimethylaminophenyl}phenyl]-6-phenylhexa-1,3,5-triene

2. MATERIALS AND METHODS

TMA-DPH and DPH were obtained from Molecular Probes, Inc. (USA), cytochrome *c* from Boehringer (FRG) and other chemicals from Sigma (USA). All other chemicals were analytical grade. Male CFY rats of 200–250 g were used throughout. Hypo- and hyperthyroid states were induced according to Nelson et al. [7].

Liver mitochondria were prepared according to Johnson and Lardy [8].

Subfractionation of mitochondria by digitonin was performed according to Schnaitman et al. [9]. The mitochondrial protein concentration was 5 mg/ml in the reaction mixture.

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Mitoplasts were prepared by the same method using 1, 1.5 and 1.5 mg/ml digitonin concentration for mitochondria prepared from hypo-, eu- and hyperthyroid animals, respectively. The intactness of the inner membrane was checked by measuring the activity of the marker enzymes.

Marker enzyme activities were determined as follows: cytochrome-c oxidase (EC 1.9.3.1) according to [10], SDH (EC 1.4.1.3) according to [11], RINCR (EC 1.6.2.2) according to [12], MDH (EC 1.1.1.37) and GluDH (EC 1.4.1.3) by the methods of [13] and [14], respectively. Mitochondrial GDH (EC 1.1.99.5) activity was determined according to Dawson and Thorne [15] and by the method of Estabrook [16] using a Clark-type oxygen electrode at 20°C to control the three hormonal states. The enzyme activities were 2.8 ± 1.2, 4.1 ± 0.4 and 35 ± 11 ngatom O_2/min per mg protein in hypo-, eu- and hyperthyroid states, respectively. In the mitoplasts these values were: 2.9 ± 0.7, 5.6 ± 1.2 and 41 ± 18 ngatom O_2/min per mg protein.

Protein concentrations were determined by the method of Peterson [17] with bovine serum albumin as standard.

Steady-state fluorescence anisotropy measurements were performed in a Perkin-Elmer MPF-3 spectrofluorometer equipped with polarizers and a Kiethley 614 electrometer as described in [18] using DPH and TMA-DPH for labelling.

3. RESULTS AND DISCUSSION

Altered phospholipid composition, changes in cholesterol and cardiolipin content, degree of fatty acid saturation and lipid to protein ratio of the mitochondrial membranes are known to be associated with changes in thyroid hormone level. Therefore, first we tried to answer the question of how these changes in lipid composition are reflected in the physical properties of the membranes.

For the steady-state fluorescence anisotropy measurements DPH and TMA-DPH fluorophores were used. The probes applied allowed us to determine the physical properties at different depths in the lipid bilayer because DPH serves as a probe of the hydrophobic core [19], whereas TMA-DPH is anchored at the polar head groups by its cationic part [20], thus providing information about the membrane-water interface. Steady-state fluorescence anisotropy measurements in mitochondria can give only an average picture about the outer and the inner mitochondrial membranes. To reduce this problem mitoplasts were studied as well, where only the inner membrane is present.

Steady-state fluorescence anisotropy (r_S) data of TMA-DPH and DPH labelled mitochondria and mitoplast preparations are shown in table 1. The trend of the changes with thyroid status is the same

Table 1

The steady-state fluorescence anisotropy of mitochondria and mitoplasts isolated from livers of rats in different thyroid states

Thyroid status	Mitochondria		Mitoplasts	
	TMA-DPH	DPH	TMA-DPH	DPH
Hypo-	0.247	0.171	0.235	0.170
Eu-	0.250	0.183	0.243	0.177
Hyper-	0.251	0.184	0.248	0.182

All data represent the mean of 3–5 independent measurements. Errors were less than 0.4%

with both labels in either mitochondria or mitoplasts. Our results suggest membrane 'rigidification' (higher r_S) in hyperthyroid state and 'fluidization' (lower r_S) in hypothyroid state. This is in good agreement with earlier observation of phase transition shift to higher temperatures due to enhanced thyroid level [21].

The steady-state fluorescence anisotropy of the labels, either TMA-DPH or DPH, was significantly higher in intact mitochondria than in the mitoplasts in each thyroid case (table 1). For the interpretation of the data we refer to the differences between the structure of membranes in the mitochondria and mitoplasts [22,23]. The inner and outer mitochondrial membranes are connected via contact points, which are dynamic structures composed of parts of the outer plus the inner boundary membranes [23–25]. In mitoplasts part of the outer membrane is still attached to the whole inner membrane via these contact points. This remaining part of the outer membrane lowers the order in the lipid structure. The lower steady-state anisotropy of mitoplasts compared to mitochondria comes mainly from the changed structure of the inner membrane. In mitoplasts, due to the removal of the outer mitochondrial membrane, the lateral pressure of the inner membrane decreases because the membrane of cristae becomes smoother.

In the hyperthyroid state the highest saturation of fatty acids (especially in cardiolipin) causes the highest order (r_S) in mitochondria and mitoplasts. In whole mitochondria (as detected by TMA-DPH as label) this effect can be reduced by cholesterol, which can rigidify the membranes, but its concentration in the mitochondrial membrane is the lowest in the hyperthyroid case.

To characterize the membrane surrounding of some individual inner membrane enzymes we performed subfractionation of the mitochondria by digitonin. This method, developed originally to determine submitochondrial localization of enzymes [9], gave useful indications in the study of some aspects of membrane topology as well. It provided basic informations about the contact points between the two mitochondrial membranes [23–25], and allowed us to propose the existence of outer membrane domains differing in sterol and protein composition [25]. This suggestion was recently supported by ultrastructural studies [26]. We studied the effect of the thyroid hormone level of the animal on the release by digitonin of three marker enzymes of the inner membrane (SDH, GDH and cytochrome-*c* oxidase), two markers of the matrix space (MDH, GluDH) and RINCR as outer membrane marker.

No significant change was detected with the hormonal level in the digitonin concentration required for the release of the outer membrane marker RINCR (fig.1a).

The digitonin concentration needed to release both matrix enzymes shifted towards higher values with increasing hormone level (fig.1b; demonstrated by GluDH). Matrix enzymes are released when the inner membrane is ruptured, thus, at high thyroxin level large digitonin-sensitive patches (relatively rich in cholesterol) are probably less abundant in the inner membrane. This agrees with earlier findings that cholesterol content decreases with increasing hormone level.

The inner membrane domains containing SDH and cytochrome-*c* oxidase are influenced only slightly by the thyroid hormone. The release curves of these two enzymes were influenced to the same relatively small extent by the thyroid status of the animal (fig.1c; demonstrated with cytochrome-*c* oxidase).

The release of the FAD-linked L-glycerol-3-phosphate dehydrogenase is affected most significantly by the hormonal level (fig.1d). In hypothyroid state it was solubilized by surprisingly low digitonin concentrations while in hyperthyroid state GDH resided in a membrane region particularly insensitive to digitonin. Thus the localization of GDH within the inner membrane of liver mitochondria is particularly sensitive to the thyroid hormone level of the animal. Among the mitochondrial enzymes investigated GDH activity depends most profoundly on the thyroid status. Enhanced enzyme synthesis was shown to be predominantly responsible for the elevated activity in hyperthyroid state [27]. Nevertheless, additive factors, like rigidification of the membrane surroundings of GDH might influence the activity of GDH as well. This suggestion is in line with our

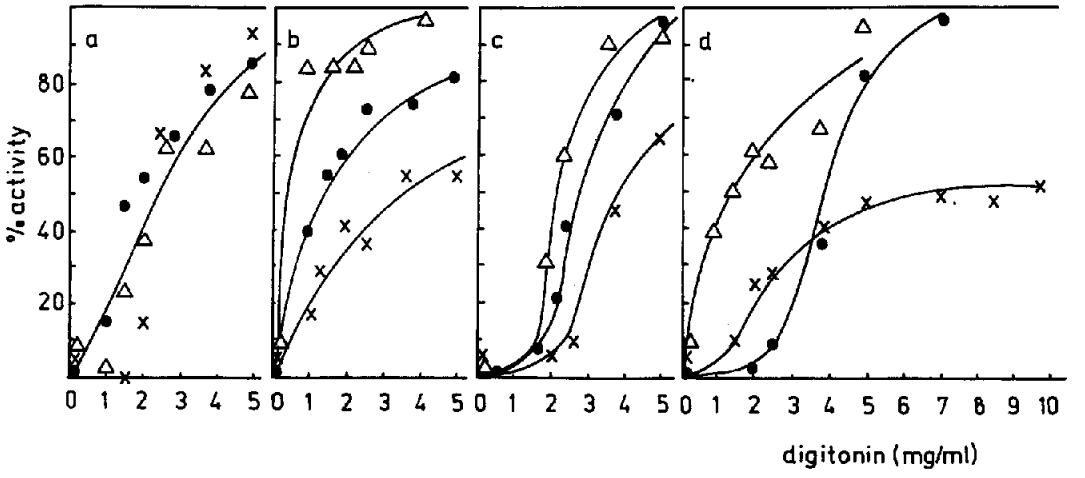

Fig.1. Digitonin subfractionation of mitochondria isolated from livers of hypo-, eu- and hyperthyroid rats. After subfractionation the enzyme activities were determined in the pellet and supernatant; % activity represents enzyme activity in the supernatant in percent of total activity. Results of a representative experiment. Release by digitonin of (a) RINCR, (b) GluDH, (c) cytochrome-*c* oxidase and (d) GDH from mitochondria isolated from hypo- (△), eu- (●) and hyperthyroid (×) rats.

finding [18] that in brown adipose tissue mitochondria inhibition of GDH by free fatty acids correlates with lower membrane microviscosity.

We conclude that several alterations in the physical characteristics of mitochondrial membranes occur as a result of changes in the thyroid hormone level. A considerable rearrangement of the proteins within the inner membrane also occurs due to the action of thyroid hormone, which probably causes changes in protein-lipid and protein-protein interactions.

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