THYROIDAL REGULATION OF SUBSTRATE KINETICS PROPERTIES OF CYTOCHROME OXIDASE IN RAT LIVER MITOCHONDRIA

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ABSTRACT

Effects of thyroidectomy (T_x) and subsequent treatment with 3,5,3'-triiodothyronine (T_3), and combined treatment (T_R) with T_3 + thyroxine (T_4) on substrate kinetics properties of cytochrome oxidase of rat liver mitochondria were examined. T_x resulted in lowering of cytochromes content with decrease in the enzyme activity, and K_m and V_{max} . T_3 and T_R regimens restored the cytochromes contents and the V_{max} values to normal. In control, T_3 and T_R groups the enzyme activity resolved in two kinetic components; in T_x group three kinetic components were evident. The K_m values for all components decreased significantly in the experimental groups with concomitant increase in catalytic efficiency, K_{cat}/K_m . Significant alterations in the contents of total phospholipid and of cholesterol were noted while the changes in the phospholipids composition were only of restricted nature. Regression analysis revealed that total phospholipid, cholesterol and phosphatidylcholine, phosphatidylethanolamine play significant role in fine tuning the enzyme activity.

KEY WORDS

Thyroid hormone, Triiodothyronine, Thyroxine, Liver mitochondria, Cytochrome oxidase, Kinetic properties.

INTRODUCTION

The influence of thyroid hormones on respiratory activity of mitochondria from various tissues is well documented (1,2). Hypothyroidism results in lowering of basal metabolic rate (BMR) and decrease in the respiratory activity of the mitochondria; these changes are reversed by treatment with a single physiologic dose of L- thyroxine (T_4) or 3,5,3'-L-triiodothyronine (T_3). Thyroid-status-dependent changes in mitochondrial lipid/phospholipid profiles have also been reported (3-5).

The enzyme cytochrome oxidase (EC 1.9.3.1) is the terminal sink in electron transport system of the mitochondria (6). The enzyme has known requirement of diphosphatidylglycerol

(DPG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) for its activity (7). Additionally, nitrous oxide (NO) has been shown to be a physiologic modulator of the enzyme (6).

Since thyroid status influences mitochondrial respiratory activity, content of cytochromes and lipid/phospholipid profiles (1-3, 5, 8, 9), it was of interest to examine if the substrate kinetics properties of cytochrome oxidase are influenced by the thyroid status of the organism. In the present studies we examined the effects of thyroid status on the above parameters in rat liver mitochondria. The results are summarized here.

MATERIALS AND METHODS

Chemicals : L-Thyroxine (T_4), 3,5,3'-L-triiodothyronine (T_3), bovine serum albumin (BSA) fraction V and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) were purchased from Sigma Chemical Co. St. Louis, MO, USA. Ascorbic acid was from Sarabhai Chemicals, Vadodara. All the chemicals were of analytical-reagent grade and were purchased locally.

Animals : Weanling (3 week old, 30-35 g body weight) male rats belonging to Charles-Foster strain were surgically

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thyroidectomized as described in details elsewhere (10). The animals were allowed to grow for 8-10 weeks. Only those animals showing 40-50 % reduction in the body weight were used for further studies. The thyroidectomized rats were randomly divided in three groups. One group which did not receive any treatment served as the thyroidectomized control and is designated as T_x . The second group of animals received subcutaneously (s.c.) 25 μg T_3 / 100 g body weight two days prior to sacrifice (10). This group is referred to as T_3 . The third group of animals received a replacement therapy with 0.9 μg T_4 + 0.15 μg T_3 / 100 g body weight s.c. for 18 consecutive days and the animals were sacrificed on the 19th day (11, 12). This group of animals is referred to as T_R . Controls were sham operated and were allowed to grow for 8-10 weeks. These animals which received equivalent amount of saline vehicle (details give below) served as euthyroid controls. Thyroid hormone solutions were prepared fresh daily prior to injection by dissolving the appropriate amount(s) of the hormone(s) in 0.9% saline containing 5 mM NaOH (10).

Isolation of mitochondria : At the end of the experimental/treatment period the animals were killed by decapitation and the liver was quickly excised and kept in beakers containing chilled (0-5°C) isolation medium (0.25 M sucrose, 10 mM Tris-HCl buffer pH 7.4 and 1 mM EDTA containing 250 mg BSA/ml) for isolation of mitochondria according to the procedure described in details elsewhere (12,13).

Cytochrome oxidase activity : Measurements of cytochrome oxidase activity were carried out polarographically using a Clarke-type oxygen electrode. The assay medium (1.6 ml) contained 50 mM potassium phosphate buffer pH 7.4, 0.4 mM AlCl_3 and 0.4 mM CaCl_2 (14). Concentration of TMPD was varied from 3- 1000 μM . Saturating amount (10 mM) of sodium ascorbate was used as the electron donor system. All the measurements were carried out at 25°C. The cytochrome oxidase activity is expressed as nmole O_2 /min/mg of protein.

Determination of mitochondrial cytochromes content :

The contents of cytochromes were quantified by recording difference spectra of dithionite reduced samples against ferricyanide oxidized samples (10,12). The contents of cytochromes aa_3 , b and $\text{c}+\text{c}_1$ were calculated using the wavelength pairs 604–624, 559–580 and 535–552 nm and millimolar extinction coefficients 24, 23.4 and 18.7, respectively (15).

Analysis of substrate kinetics data : The data on increase in the cytochrome oxidase activity with increasing concentration of TMPD were analyzed by Lineweaver-Burk, Eadie-Hofstee, and Eisenthal and Cornish-Bowden plots for calculating the K_m and V_{max} values as described in details (13,14,16). The values obtained by the three methods were in close agreement and were averaged for the final presentation of the data.

The values of K_{cat} (number of substrate molecules transformed/sec by the enzyme) were calculated from the corresponding values of V_{max} and cytochrome aa_3 content. (Tables 1 and 2). The values of K_{cat}/K_m ($\text{M}^{-1} \text{sec}^{-1}$) were derived by dividing K_{cat} values by K_m (mole/L) (14,17). Hill plot analysis was carried out to find out the number of TMPD molecules bound by the enzyme (16).

Lipid extraction and analysis : The extraction of lipids/phospholipids with freshly prepared chloroform: methanol (2:1 vol/vol) was according to the procedures described previously (14,18,19). Aliquots of the samples containing 8-10 μg of phospholipids phosphorous were spotted on TLC plate. The solvent system used for separation of phospholipid classes was consisted of chloroform: methanol: acetic acid: water (25:15:4:2 v/v) (20). After the run was completed, the solvent was allowed to evaporate completely and the plates were briefly exposed to iodine vapor to visualize the individual phospholipid spots and the spots were marked. Iodine was

Table 1. Thyroid-status-dependent changes in cytochrome oxidase activity and cytochrome content in rat liver mitochondria

Animals (n)	Activity	Cytochromes content (pmoles/mg protein)		
		aa_3	b	$\text{c}+\text{c}_1$
Control (8)	29.3 $\pm$ 0.71	142.6 $\pm$ 5.21	233.7 $\pm$ 11.60	268.6 $\pm$ 9.77
T_x (4)	18.1 $\pm$ 0.43 ^c	114.1 $\pm$ 8.66 ^b	196.7 $\pm$ 11.03	^a 199.1 $\pm$ 1.33 ^c
T_3 (4)	27.4 $\pm$ 3.10 [*]	130.7 $\pm$ 5.07	220.2 $\pm$ 2.07	272.0 $\pm$ 1.85 ^{***}
T_R (4)	27.3 $\pm$ 2.25 ^{**}	155.2 $\pm$ 8.16 ^{**}	259.2 $\pm$ 8.47 ^{**}	316.6 $\pm$ 27.36 ^{**}

The cytochrome oxidase activity (nmole O_2 /min/mg of protein) was determined at 25°C in the presence of 0.1 mM TMPD. The results are given as mean $\pm$ SEM of the number of independent observations indicated in the parentheses.

a, $p < 0.05$; b, $p < 0.02$ and c, $p < 0.001$ compared to euthyroid control; ^{*} $p < 0.02$; ^{**} $p < 0.01$, ^{***} $p < 0.001$ compared to T_x

Table 2 : Thyroid-status-dependent changes in substrate kinetics properties of cytochrome oxidase from rat liver mitochondria.

Animals	Component I		Component II		Component III	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Control (8)	16.64 ± 2.01	18.19 ± 1.42	112.53 ± 5.00	44.52 ± 2.53	—	—
T _x (4)	1.73 ± 0.12 ^a	10.17 ± 0.30 ^a	43.30 ± 2.08 ^a	26.14 ± 0.51 ^a	134.18 ± 6.74	44.56 ± 2.46
T ₃ (4)	1.77 ± 0.14 ^a	18.82 ± 2.65 ^{**}	59.75 ± 5.19 ^{a,***}	44.65 ± 4.80 [*]	—	—
T _R (4)	3.37 ± 0.47 ^a	18.39 ± 1.27 ^{***}	45.07 ± 6.64 ^a	39.23 ± 3.28 [*]	—	—

The K_m (μ M) and V_{max} (n mole O₂/min/mg protein) values were calculated by three different methods as described in the text and were averaged. The results are given as mean ± SEM of independent experiments indicated in the parentheses.

a, $p < 0.001$ compared to euthyroid control; * $p < 0.01$; ** $p < 0.002$ and *** $p < 0.001$ compared to T_x

allowed to sublime off after which the areas corresponding to marked spots of individual phospholipids class were carefully scraped and the silica gel was transferred to clean test tubes. To each tube 0.5 ml of 10 N H₂SO₄ was added and the samples were heated on a sand bath for 8-10 h. The tubes were allowed to cool after which a drop of 70 % perchloric acid was added. The tubes were again heated for 3-4 hours till the solution in the tubes was clear and smell of chlorine was undetectable (19). An equal aliquots of the samples (containing 8-10 μ g of phospholipids phosphorous) was subjected to similar treatment. The estimation of phospholipid phosphorus content was according to the procedure of Bartlett (21). Recovery of applied phospholipids phosphorous ranged from 98-105%. The phospholipids composition was derived from the % distribution of the phospholipids phosphorous in the individual spots. The content of TPL was derived by multiplying the phospholipids phosphorous content by 25.

The contents of individual phospholipid classes were calculated by multiplying the values of total phospholipid (TPL) with % composition of the individual phospholipid classes (19). Estimations of cholesterol (CHOL) was carried out as described by Zlatkis et al. (22). Protein estimation was according to the method of Lowry et al. (22) using bovine serum albumin as the standard.

Statistical evaluation of the data was done by student's 't' test. Regression analysis across the groups was carried out using Jandel Sigmastat Statistical Software, version 2.0, Jandel Corporation, San Rafael, CA, USA.

RESULTS

The data in Table 1 show that the cytochrome oxidase activity decreased by 32% in the T_x animals and in the T₃ as well. T_R groups the activity was normalized. Thyroidectomy also resulted in 16-26% decrease in the contents of all the cytochrome classes viz. aa₃, b and c+c₁ and both the

treatments (T₃ and T_R) were able to normalize the contents of the three classes of cytochrome. In fact, with T_R regimen the contents of the three cytochrome classes increased from 9-18% over the euthyroid values with maximum increase being seen for the content of cytochrome c+c₁.

In the next set of experiments we evaluated the effect of thyroid status on substrate kinetics properties of the enzyme cytochrome oxidase by three different methods (13, 14,16) as outlined in Materials and Method Section. However, for sake of brevity, only typical Eadie-Hofstee plots are shown in Fig 1. As is evident, in the euthyroid controls the enzyme activity resolved in two kinetic components which is consistent with our earlier report (14). In the T_x animals the activity resolved in three kinetic component whereas in the T₃ and T_R groups once again only two kinetic components were evident. The analysis of K_m and V_{max} values revealed that in the euthyroid control group the K_m values of the two components were 17 and 113 μ M and the corresponding V_{max} values were 18.2 and 44.5 nmole O₂/min/mg protein (Table 2). These values agree well with our previously reported observations (13). T_x resulted in a substantial decrease in K_m of component I (1.7 μ M against 17 μ M); for component II the K_m decreased from 113 μ M to 43 μ M. Under these conditions the V_{max} values of the two components decreased by 44 and 41%. The values of the K_m and V_{max} of component III were comparable to those of component II of euthyroid animals. T₃ treatment normalized the V_{max} values of both the components. However, the K_m values were still comparable to those of T_x animals. More or less similar trend was seen even for the T_R group although the value of K_m of component I doubled as compared to the T_x group (Table 2).

These significant changes especially in K_m values prompted us to evaluate the catalytic efficiency of the enzyme in terms of the K_{cat}/K_m values. These data are given in Table 3 which show that the K_{cat}/K_m value of component I in the T_x group was about 7 times higher; in T₃ and T_R groups these values

Figure 1 : Typical Eadie-Hofstee plots for cytochrome oxidase in rat liver mitochondria. The enzyme activity v (n mole O_2 /min/mg protein) on abscissa is plotted versus $v/[S]$ on ordinate where $[S]$ represents the concentration of TMPD in μM . The plots are typical of 4-8 experiments as indicated in the text.

were about 10 and 5 times higher. For component II the value of K_{cat}/K_m was about 2 times higher in all the experimental groups.

Table 3 : Thyroid-status-dependent changes in the K_{cat}/K_m values of kinetic components of cytochrome oxidase from rat liver mitochondria

Animals (n)	$K_{cat}/K_m (M^{-1} sec^{-1}) \times 10^6$		
	Component I	Component II	Component III
Control (8)	0.264 ± 0.016	0.094 ± 0.001	—
T_x (4)	1.780 ± 0.200^b	0.181 ± 0.017^b	0.099 ± 0.001
T_3 (4)	2.630 ± 0.357^b	0.193 ± 0.019^a	—
T_R (4)	1.240 ± 0.160^b	0.195 ± 0.024^a	—

The results are given as mean $\pm$ SEM of the number of independent experiments indicated in the parentheses.

a, $p < 0.01$ and b, $p < 0.001$ compared to euthyroid control.

Hill plot analysis indicated that in the euthyroid control animals, up to 30 μM concentration 1 molecule of TMPD was bound beyond which two molecules were bound. As against this in the T_x and T_3 animals up to 41-49 μM concentrations hardly any TMPD was bound. However, beyond this concentration range, one molecule of TMPD was bound. In the T_R animals the pattern of binding of TMPD became comparable to the euthyroid controls (Table 4).

The enhanced catalytic efficiency especially of component I in all experimental groups prompted us to find out if the

Table 4 : Thyroid-status-dependent changes on Hill plot analysis on rat liver mitochondrial cytochrome oxidase

Animals (n)	Hill coefficient		Transition concentration (μM)
	n_1	n_2	
Control (8)	0.75 ± 0.02	1.20 ± 0.05	30.3 ± 2.36
T_x (6)	0.27 ± 0.02	1.18 ± 0.13	41.2 ± 1.88
T_3 (6)	0.28 ± 0.02	0.96 ± 0.27	49.4 ± 5.57
T_R (4)	0.49 ± 0.03	1.26 ± 0.04	49.1 ± 2.91

The results are given as mean $\pm$ SEM of the number of independent experiments indicated in the parentheses.

changes in membrane lipid/phospholipid content and/or composition had any modulatory role. The data on TPL and CHOL as influenced by thyroid status are given in Table 5. Thus the TPL and CHOL contents decreased by 35 and 54% respectively in the T_x animals. T_3 treatment resulted in raising the TPL content closer to the euthyroid level although the value was still 20% lower. The CHOL content was normalized under this condition. In the T_R group the TPL content was still 7% lower compared to the euthyroid animals but the CHOL content increased by 19% over the euthyroid control value. These changes resulted in differentially affecting the TPL/CHOL (mole:mole) ratios (Table 5).

Table 5 : Thyroid-status-dependent changes in the TPL and CHOL content, and in the TPL/CHOL (mole:mole) ratio in rat liver mitochondria

Animals	TPL (μg /mg protein)	CHOL (μg /mg protein)	TPL/CHOL (mole:mole)
Control (8)	175.3 ± 4.77	45.9 ± 1.32	1.92 ± 0.09
T_x (4)	114.7 ± 4.07^d	21.1 ± 0.81^d	2.73 ± 0.15^d
T_3 (4)	$140.9 \pm 1.91^{d,*}$	$50.8 \pm 3.09^*$	$1.27 \pm 0.06^{d,*}$
T_R (4)	$162.7 \pm 2.37^{a,*}$	$54.8 \pm 2.65^{b,*}$	$1.49 \pm 0.09^{c,*}$

The results are given as mean $\pm$ SEM of the number of independent observations indicated in parentheses.

a, $p < 0.05$; b, $p < 0.02$; c, $p < 0.01$ and d, $p < 0.001$ compared to euthyroid control; *, $p < 0.001$ compared to T_x

The phospholipids composition was unchanged in the T_x animals. However, the T_3 group was characterized by lowering of lysophospholipids (Lyso) and about 2 fold increase in phosphatidylinositol (PI) and PS components. In the T_R group the proportion of PC increased significantly with a reciprocal decrease in PE component. Thus, apparently, the compositional changes seemed to be only of restricted nature (Table 6). However, since the contents of TPL changed the thyroid hormone status of the animals, it was important to find out how the content of individual phospholipid class was affected under different experimental condition. These data are given in Table 7.

Table 6 : Thyroid-status-dependent changes in the phospholipid composition in rat liver mitochondria.

Phospholipid Class	Composition (% of Total)			
	Control (8)	T _x (4)	T ₃ (4)	T _R (4)
Lysophospholipid	1.47 ± 0.05	1.15 ± 0.13 ^a	0.99 ± 0.01 ^c	1.35 ± 0.13
Sphingomyelin	2.95 ± 0.27	4.46 ± 0.82	2.98 ± 0.06	3.83 ± 0.36
Phosphatidylcholine	43.14 ± 1.22	44.90 ± 2.07	41.46 ± 0.57	52.64 ± 2.77 ^b
Phosphatidylinositol	1.57 ± 0.11	1.93 ± 0.15	3.23 ± 0.14 ^{d, **}	1.34 ± 0.23 [*]
Phosphatidylserine	1.65 ± 0.09	1.45 ± 0.28	3.25 ± 0.13 ^{d, *}	1.39 ± 0.20
Phosphatidylethanolamine	38.11 ± 1.20	34.42 ± 1.36	36.54 ± 0.74	28.53 ± 2.51 ^{c, *}
Diphosphatidylglycerol	11.11 ± 0.45	11.69 ± 1.66	11.55 ± 0.25	10.91 ± 0.35

The results are given as mean ± SEM of the number of independent experiments indicated in the parentheses.

a, p< 0.05; b, p< 0.02; c, p<0.01 and d, p<0.001 compared to euthyroid control; *, p< 0.05 and **, p<0.002; compared to T_x

Thus in the T_x animals the content of all phospholipid classes decreased from 17-50%. T₃ treatment caused a significant 60% increase in the contents of PI and PS whereas the contents of sphingomyelin (SPM), PE and DPG were still lower by 17-23%. In the T_R group the contents of SPM and PC increased by 11 and 16% respectively, whereas the contents of PS, PI and DPG were lower by 9-30% with the maximum effect being seen for PE component (Table 7).

In view of the significant changes in the cytochrome oxidase activity, content of cytochromes, and values of K_m, V_{max} and K_{cat}/K_m, and lipid/phospholipid profiles and contents (Tables 1-7) it was of interest to seek correlation amongst the observed changes. This was achieved by regression analysis. Since the changes in the phospholipids composition were of restricted nature, correlation studies were carried out with the contents of individual phospholipids classes. These data are given in Table 8.

Regression analysis revealed that the enzyme activity (Table 1) correlated positively with the contents of cytochrome aa₃ and TPL, CHOL and PC, with the values of correlation coefficient r, being in the range from r = + 0.585 to +0.681. It may be mentioned here that the activities given in Table 1 were determined in the presence of 100 μM TMPD. Therefore the net activity measurement represent a composite picture of the contribution by components I, II and III as the case may be.

TPL was a common positive modulator of K_{m1} and K_{m2}. PE and CHOL appeared to be the positive modulator of K_{m1} and K_{m2}. The V_{max1} and V_{max2} values were influenced by CHOL content. V_{max1} was also dependent on contents of cytochromes aa₃ and c+c₁ as well as TPL. The (K_{cat}/K_m) I value correlated negatively with contents of TPL, PC and PE which is consistent with the correlation data on activity and K_{m1} value. (K_{cat}/K_m)II correlated negatively with PE. The

Table 7. Thyroid-status-dependent changes in the phospholipids content in rat liver mitochondria

Phospholipid Class	Content (μg /mg of mitochondrial protein)			
	Control (8)	T _x (4)	T ₃ (4)	T _R (4)
Lysophospholipid	2.6 ± 0.08	1.3 ± 0.17 ^c	1.4 ± 0.03 ^c	2.2 ± 0.18 ^{**}
Sphingomyelin	5.2 ± 0.60	5.2 ± 1.08	4.2 ± 0.07	6.2 ± 0.63
Phosphatidylcholine	75.6 ± 2.86	51.5 ± 2.74 ^c	58.4 ± 0.70 ^c	85.7 ± 4.71 ^{***}
Phosphatidylinositol	2.8 ± 0.26	2.2 ± 0.18	4.5 ± 0.15 ^{c, ***}	2.2 ± 0.38
Phosphatidylserine	2.9 ± 0.22	1.7 ± 0.36 ^c	4.6 ± 0.15 ^{c, ***}	2.3 ± 0.31
Phosphatidylethanolamine	66.7 ± 2.38	39.5 ± 2.24 ^c	51.5 ± 1.67 ^{c, **}	46.4 ± 3.92 ^c
Diphosphatidylglycerol	19.6 ± 1.15	13.4 ± 1.86 ^b	16.3 ± 0.40 ^a	17.8 ± 0.80

The results are given as mean ± SEM of the number of independent experiments indicated in the parentheses.

a, p< 0.05; b, p< 0.02; and c, p<0.001 compared to euthyroid control; *, p<0.02; **, 0.002; and ***, p<0.001 compared to T_x

Table 8 : Correlation of kinetic parameters of cytochrome oxidase with cytochrome and membrane lipid/phospholipid content

Parameter	Correlation with	
	Positive	Negative
Activity	Cytochrome aa ₃ (+ 0.566), TPL (+ 0.681), CHOL (+0.598), PC (+0.585)	—
K _{m1}	TPL (+ 0.650), PE (+ 0.805)	—
K _{m2}	TPL (+ 0.578), CHOL (+ 0.708)	—
V _{max1}	Cytochrome aa ₃ (+0.640), Cytochrome c+c ₁ (+0.558) TPL (+ 0.836), CHOL (+0.708)	—
V _{max}	2 CHOL (+0.634)	—
(K _{cat} /K _m) I	—	TPL (-0.598), PC (-0.556), PE (-0.510)
(K _{cat} /K _m) II	—	PE (-0.826)

The experimental details are given in the text. Values in the parentheses indicate the regression coefficient *r*, which is based on 4-8 independent experiments.

important observation emerging from regression analysis is that the lowered contents of TPL and PE were responsible for lowering the values of K_m of component I and K_m of component II thereby increasing the catalytic efficiency of the enzyme which is reflected in terms of increased values of K_{cat}/K_m especially of component I (Table 3).

DISCUSSION

If one assumes that at the given concentration of 100 μM TMPD the contribution to the measured activity (Table 1) with component I correspond with V_{max} value, the relative contribution to the measured activity by component II would be about 14-25%. It may hence be suggested that the low K_m high affinity component I may be physiologically more relevant. Such an assumption is consistent with the observation on the catalytic efficiency of enzyme which increased significantly in spite of the fact that content of cytochrome aa₃ had decreased significantly in T_x and T₃ animals. In other words under the experimental conditions the lowering of TPL and PE content served as fine tuning control for enhancing the catalytic efficiency of enzyme.

The fact that the K_m values especially of component I decreased significantly in the T₃ and T_x animals and to somewhat lesser extent in the T_R group deserves some comment. The data on Hill plot analysis show that in the two former experimental groups hardly any TMPD was bound especially in the lower concentration range. This would suggest that TMPD was directly interacting with the active site of the

enzyme without binding which is not the case for the euthyroid group. The T_R group represents a mixed type of picture which is consistent with the intermediate value of K_{cat}/K_m of component I (Table 3). In the high concentration range, the differences abolish which would lend credence to such an assumption as above. It is possible that the decreased lipid bulk in the membrane in the experimental groups (Tables 5 and 7) might have rendered the active site directly accessible to TMPD without binding.

It has been reported that the activity of cytochrome oxidase is dependent on PC, PE and DPG (7). Surprisingly, however, we did not get any correlation between any of the parameter examined with DPG. It may hence be suggested under all experimental conditions the content of DPG is adequate for supporting the enzyme activity. Our regression analysis data suggest that PE may also play a role in fine tuning of the catalytic activity of the enzyme.

In the present studies we employed two treatment regimens with thyroid hormone. As pointed out earlier treatment with physiologic dose of T₃ (10) did restore the activity as well as cytochrome content. Similar results were obtained with replacement therapy (T_R) which has been shown to normalize tissue thyroid hormones contents (11). However, results of our present studies point out that despite this apparent normalization the lipid/phospholipid profiles and contents, and the fine tuning mechanisms do not return to normal by either of the treatment.

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