FEBS Letters 346 (1994) 295–298

FEBS 14090

Interaction of diiodothyronines with isolated cytochrome *c* oxidase

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Received 11 March 1994; revised version received 18 April 1994

Abstract

Diiodothyronines (3,3′-T_2 and 3,5-T_2) stimulate the activity of isolated cytochrome *c* oxidase (COX) from bovine heart mitochondria. Maximal stimulation of activity (about 50%) is obtained with 3,3′-T_2 at pH 6.4 and with 3,5-T_2 at pH 7.4. In contrast, 3,5,3′-triiodothyronine (T_3) exhibited no or little stimulation of COX activity. Binding of the hormones to COX leads to conformational changes as shown by modified visible spectra of the oxidized enzyme. It is suggested that 'short-term' effects of thyroid hormones on mitochondrial respiration are at least partly due to the allosteric interaction of diiodothyronines with the COX complex.

Key words: Thyroid hormone; Diiodothyronine; Cytochrome *c* oxidase; Conformational change; Mitochondrion; Oxygen uptake

1. Introduction

Thyroid hormones stimulate the metabolism of most animal tissues in two different ways. The 'long-term' effects take days and involve activation of gene expression by binding to specific nuclear c-erb-A related receptor proteins [1], while the 'short-term' effects can be measured within minutes and are assumed to occur by direct interaction of the hormones with mitochondria [2,3].

Short-term effects of thyroid hormones include, among others: (1) an increase of oligomycin-sensitive oxygen consumption after addition of T_3 to the perfusion medium of liver from hypothyroid rats [4]; (2) a fall in the mitochondrial ATP/ADP ratio under the conditions of 1) [5] (3) a rapid stimulation of hepatocyte oxygen consumption in the presence of cycloheximide [6]; (4) a rapid increase of ATP synthesis within minutes [7]; and (5) an increase in the P/O ratio of liver mitochondria isolated 15 min after injection of T_3 into thyroidectomized rats [8].

But, although specific binding sites for T_3 have been reported to occur in isolated mitochondria [9–13], the described short-term effects of thyroid hormones are discussed in a controversial way [2,14]. Recently, however, several authors described 'short-term' effects of diiodothyronines (3,3′-T_2 and 3,5-T_2) on mitochondrial respiration [15–20]. In a previous study we showed a rapid in vitro stimulation of cytochrome *c* oxidase (COX) activity in rat liver mitochondria by 3,3′-T_2 and 3,5-T_2 and suggested that diidothyronines alter the kinetic properties of the COX complex by interaction with nuclear-coded (non-catalytic) subunits of COX [17]. Tissue-specific interactions of adenine nucleotides with the nuclear-coded subunit Vla-H (heart-type) of COX from bovine heart have been described recently [21,22]. Intraliposomal ADP stimulated specifically respiration and respiratory control of reconstituted COX from bovine heart [22].

In this study the effects of thyroid hormones on the activity and spectral properties of isolated COX from bovine heart were investigated. In contrast to T_3, the diiodothyronines (3,3′-T_2 and 3,5-T_2) stimulated the activity and changed the visible spectrum of the isolated oxidized enzyme.

2. Materials and methods

COX was isolated from bovine heart mitochondria as described by Kadenbach et al. [23]. The purity of the preparations, as measured by the absorbance ratio $A_{280\ nm}/A_{420\ nm}$, was 2.4–2.8. COX activity was measured polarographically with a Clark-type electrode [24] in incubation medium (50 mM HEPES/KOH, 10 mM KCl, 1% Tween 20 (w/v) at the indicated pH) containing 9 mM Tris-ascorbate, 50 μM cytochrome *c*, 0.2 μM COX and the indicated thyroid hormone at the given concentration. The thyroid hormones (3,3′-T_2, 3,5-T_2 or T_3) were added to the incubation mixture as concentrated solutions, prepared by dissolving 1 mg of the indicated thyroid hormone in 200 μl of 0.2 N NaOH, followed by immediate dilution to 1 ml with incubation medium without Tween 20.

Difference spectra of isolated oxidized COX were recorded at increasing of the indicated thyroid hormone with a Uvicon 940 spectrophotometer (Kontron). The measurements were performed at 25°C in 10 mM K-HEPES at pH 7.4 or pH 6.4, 0.05% laurylmaltoside and 2 μM COX. The indicated amounts of thyroid hormones were added from concentrated stock solutions, prepared in the same buffer.

Iodothyronines were gently supplied by MMDRI, Henning Berlin R&D (Berlin, Germany).

3. Results

3.1. Effects of different thyroid hormones on oxygen consumption of isolated COX

The oxygen uptake of isolated COX from bovine heart was measured polarographically at increasing concentra-

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SSDI 0014-5793(94)00476-C

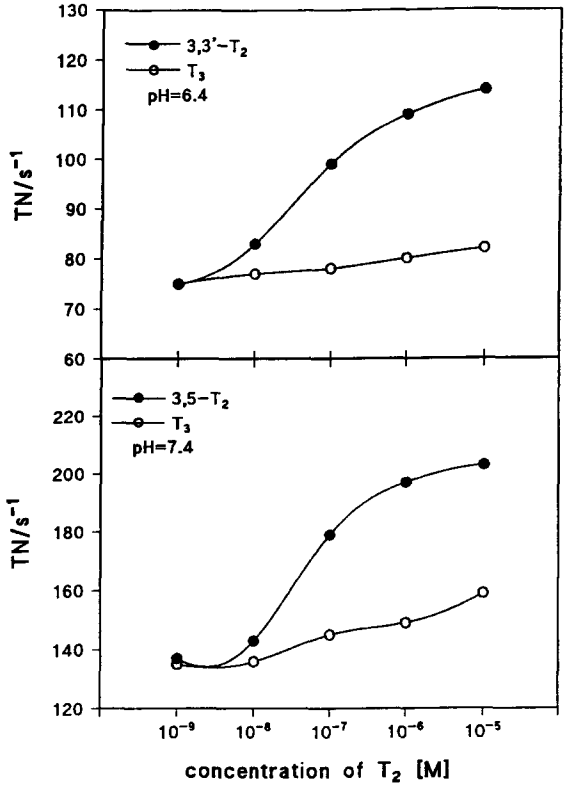

Fig. 1. Influence of thyroid hormones on the activity of isolated COX from bovine heart. The oxygen consumption was measured polarographically at the indicated concentrations of diiodothyronines (closed symbols) and T_3 (open symbols), as described in section 2. The effects of 3,3'-T_2 and T_3 were measured at pH 6.4, the effects of 3,5-T_2 and T_3 at pH 7.4. The activity is given as turnover number (TN, moles electrons (1/4 O_2) × moles heme $aa3^{-1} \times s^{-1}$).

tions of 3,3'-T_2 and T_3 at pH 6.4, and of 3,5-T_2 and T_3 at pH 7.4, as shown in Fig. 1. With both diiodothyronines a saturable increase of activity is obtained at concentrations between 10^{-7} and 10^{-5} M. In both cases about 50% maximal stimulation of activity is obtained. In contrast, very little increase of oxygen uptake is found with T_3, the most effective thyroid hormone in 'long-term' effects.

The two diiodothyronines showed a marked difference in their stimulatory activity depending on the pH (Fig. 2). While 3,3'-T_2 showed its maximal stimulatory activity at pH 6.4, the 3,5-T_2 had its maximal effect at pH 7.4. Very little or no stimulation of respiration was obtained at the extreme pHs of 5.5 and 8.0 with both diiodothyronines.

3.2. *Effect of thyroid hormones on the visible spectrum of oxidized COX*

Fig. 3 presents the difference spectrum of isolated COX in the Soret region in the presence and absence of 1 μM 3,3'-T_2 or 3,5-T_2, measured in 10 mM K-HEPES either at pH 6.4 or pH 7.4. The absolute spectrum of oxidized COX is also presented at a 20 times reduced scale of absorbance. With both diiodothyronines a minimum of the difference spectrum was obtained at 413 nm when measured at pH 6.4, and at 427 nm when measured at pH 7.4. No clear spectral changes could be measured in the region of the α-band (not shown).

The concentration dependences of various thyroid hormones on the absorbance differences of isolated COX, measured at pH 6.4 and 7.4, are summarized in Fig. 4. At pH 7.4 the highest change of absorbance difference (550–427 nm) is obtained with 3,5-T_2. Smaller changes are found with 3,3'-T_2 and with 3'-monoiodothyronine. No change of absorbance difference is seen with T_3. At pH 6.4 all four thyroid hormones lead to changes of the absorbance difference (550–413 nm), but again T_3 had the smallest effect.

4. Discussion

Several reports have demonstrated that diiodothyronines (3,3'-T_2 and 3,5-T_2), under particular conditions, are the only iodothyronines which rapidly stimulate in vivo and in vitro mitochondrial respiration or COX activity [15,20]. The physiological significance of these results, however, was not clear and no insight into the mechanism, by which diiodothyronines exert their action, was available.

In this study we demonstrate that diiodothyronines directly and rapidly interact with the isolated COX complex. The interaction leads to conformational changes of the complex, as shown by spectral changes, and to stimulation of enzymatic activity. In contrast, T_3 which is most effective in 'long-term' effects, has little or no influence on the activity or spectrum of COX. Both, the effects of diiodothyronines on COX activity and on the spectra, are observed at hormone concentrations be-

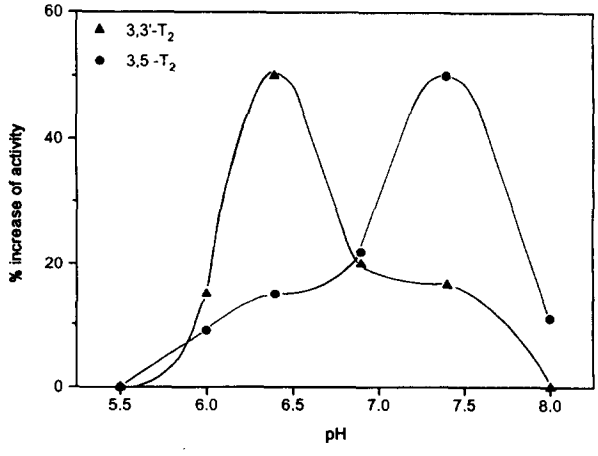

Fig. 2. Stimulation of COX activity by diiodothyronines at different pH values. COX activity was measured polarographically at the indicated pH values before and after addition of the hormones, as described in section 2. 3,3'-T_2 (triangles) and 3,5-T_2 (circles) were added at 10^{-6} M final concentrations.

tween 10^{-8} and 10^{-7} M and show a saturation at concentrations between 10^{-6} and 10^{-5} M.

The difference in the optimal pH for the effects of 3,3′-T_2 and 3,5-T_2 could be explained by the different outer ring iodination of the diiodothyronines. In fact, the more iodinated the outer ring, the lower the optimal pH. At pH 6.4, 3,3′-T_2 is most effective in inducing spectral changes, followed by 3′-T_1 and 2,5-T_2. At pH 7.4, on the other hand, 3,5-T_2 showed the greatest effect, followed by 3,3′-T_2 and 3′-T_1.

Our data confirm previous results, showing diiodothyronines as the most effective thyroid hormone analogues that stimulate mitochondrial respiration in vivo and in vitro [15,17]. In addition, they support the hypothesis that the COX complex is the interaction site for diiodothyronines. This contrasts with previous studies ascribing essentially no physiological potency to diiodothyronines [25,26]. However, in these old studies classical parameters, such as anti-goitrogenic activity, growth promotion or stimulation of basal metabolic rate were considered, which are useful to evaluate the general thyroid hormone potency. Diiodothyronines either could have different effects, or could not reach their intracellular targets when injected into animals. As previously hypothesized [15,16], T_3 could be responsible for long-lasting action of thyroid hormones, whereas diiodothyronines could be involved in rapid energetic adjustment to an altered physiological state, such as cold exposure, overfeeding etc.

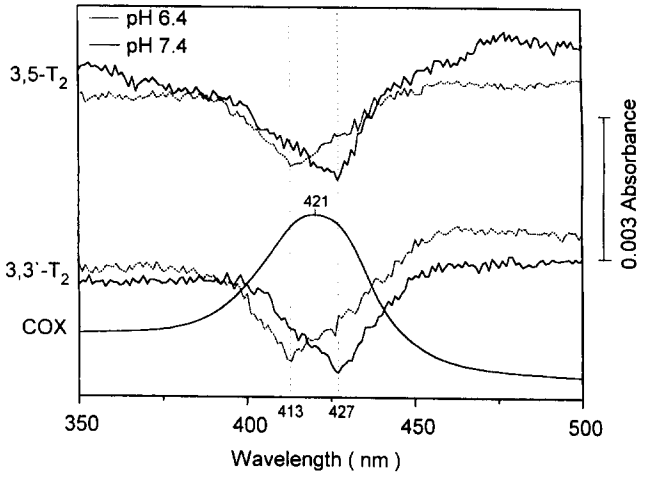

Fig. 3. Difference spectra of isolated oxidized COX in the absence and presence of diiodothyronines. The measurements were performed in tandem cuvettes, containing COX and the hormone either in the same (sample) or in separate compartments (reference). The two compartments of the cuvettes contained 10 mM K-HEPES, pH 6.4 or 7.6, 0.05% laurylmaltoside, and one compartment of each cuvette contained 2 μM COX. Volume changes were corrected by buffer additions. The effects of 3,5-T_2 (upper curves) and 3,3′-T_2 (lower curves) were measured at pH 6.4 (dotted lines) and pH 7.4 (solid lines) at final concentrations of 10^{-6} M. The absolute spectrum of oxidized COX at a 20 times reduced scale of absorbance, measured at pH 6.4 and pH 7.4 were found in the same line.

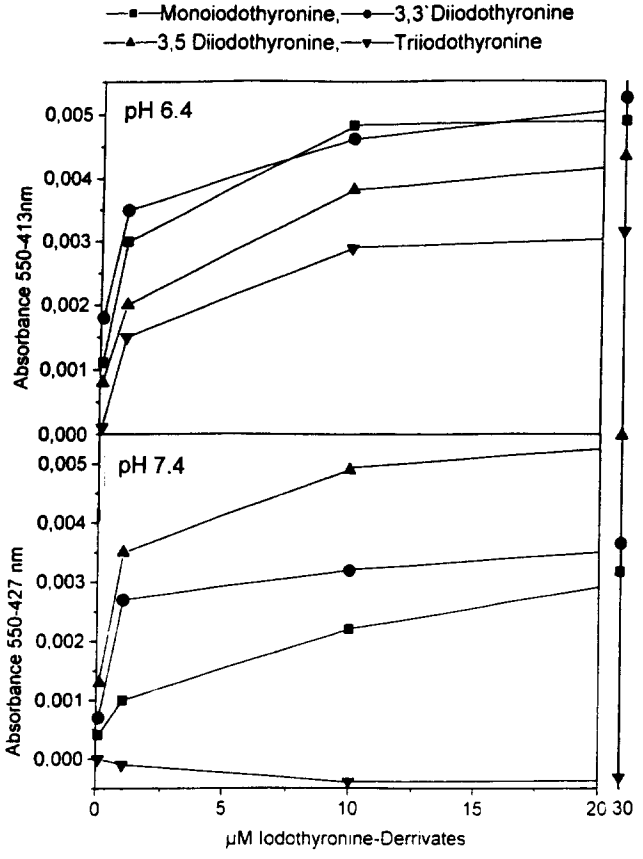

Fig. 4. Concentration dependence of the influence of thyroid hormones on the absorbance difference of oxidized COX from bovine heart. The absorbance differences at 550–413 nm were taken from spectra recorded at pH 6.4, those at 550–427 nm from spectra recorded at pH 7.4. Spectra were taken at concentrations of 0.1, 1, 10, and 30 μM of monoiodothyronine (squares), 3,3′-T_2 (circles), 3,5-T_2 (triangles, pointing up) and T_3 (triangles, pointing down).

Concerning the lack of diiodothyronines effects on basal metabolic rate, after their administration in vivo [15,25], it is possible that plasma membranes are not permeable to diiodothyronines and that they are only formed within cells from T_3. Only after chronic treatment of hypothyroid animals, it is possible to show an effect of diiodothyronines in some tissues such as the liver, which could be more permeable to diiodothyronines [12,13], but this has only little effect on basal metabolic rate (≅ 10–20% stimulation) [15,16].

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft (Ka 192/28-1) and Fonds der Chemischen Industrie and by grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (40% and 60%) and Centro Nazionale delle Ricerche (91.00412.CT04 and 92.00699.CT04).

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