CONVERSION OF THYROXINE TO 3,3',5'-TRIIODOTHYRONINE (REVERSE-T_3) BY A SOLUBLE ENZYME SYSTEM OF RAT LIVER

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The reaction by which thyroxine (T_4) undergoes monodeiodination in the nonphenolic ring to form 3,3',5'-triiodothyronine (reverse-T_3) has been studied in rat liver. In whole homogenate the conversion of T_4 to rT_3 is obscured by rapid degradation of the product, whereas in cytosol accumulation of rT_3 is linear for 60 min of incubation. In the cytosol system, the rate of rT_3 formation is maximal at pH 8.2, is thiol-dependent, is inactivated by heat, but is not inhibited by anaerobiosis or absence of light. Propylthiouracil is a potent inhibitor of the reaction. The higher pH-optimum of the rT_3-forming system compared to that of T_4-to-T_3 conversion indicates that the former reaction is mediated by an enzyme which is distinct from that controlling 3,5,3'-triiodothyronine (T_3) formation.

INTRODUCTION

The formation of 3,3',5'-triiodothyronine (reverse-T_3, rT_3) from thyroxine (T_4) by monodeiodination in the nonphenolic ring (5-deiodination) is a major pathway of T_4 metabolism (1-3). This reaction has been demonstrated in perfused dog liver (4) and in cultured monkey hepatocarcinoma cells (5) but has not been convincingly shown in broken-cell preparations. A recent report has suggested that the failure to demonstrate T_4 5-deiodination in homogenates is due to rapid degradation of rT_3 (6). We have succeeded in separating the rT_3-degrading from rT_3-forming activities in rat liver (7). This report describes the properties of a soluble system which converts T_4 to rT_3 and pre-

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sents evidence that it is enzymic in nature and distinct from the T_4 5'-deiodinase which forms 3,5,3'-triiodothyronine (T_3).

MATERIALS AND METHODS

L-T_4 and dithioerythritol (DTE) were obtained from Sigma Chemical Co., St. Louis, U.S.A. Reverse-T_3 was generously provided by Dr. Eugene C. Jorgensen. ^{125}I-rT_3 and ^{125}I-T_3, each labeled in the phenolic ring positions at specific radioactivities of 500-900 μCi/μg, were purchased from Abbott Laboratories, North Chicago, U.S.A. Goat anti-rabbit gamma globulin serum was obtained from Antibodies, Inc., Davis, CA, U.S.A.

Preparation of liver homogenate and cytosol. Sprague-Dawley male rats, weighing 180-220 gm, fed Purina Lab Chow until time of sacrifice, were killed by decapitation and bled. The liver was chilled on ice, weighed and homogenized in 3 volumes ice-cold buffer (0.25 M sucrose - 0.05 M Tris-HCl, pH 7.4) using a Polytron homogenizer for 2-3 seconds. The crude homogenate was centrifuged at 2,000 x g for 10 minutes and the pellet discarded. Part of the supernatant from the initial low-speed spin was used for incubation studies (homogenate) and part for preparation of post-microsomal supernatant (cytosol) by a final centrifugation at 100,000 x g for 1 hour. In most experiments, homogenate and cytosol were dialyzed for 16 hours against two changes of 30 volumes each of either 0.05 M Tris-HCl, pH 7.0-8.5, or 0.1 M sodium acetate-barbital-HCl buffer over a pH range from 6.5 to 8.4. Each of the buffers contained DTE, 5 mM. All operations to this stage were performed at 4°.

Incubation procedure. To 1 ml of homogenate or cytosol was added L-T_4 (1.5 μg) in 10 μl of the same buffer. The mixture was incubated at 37° in glass tubes open to the air. At intervals up to 2 hours, 100 μl samples were removed and added to 0.9 ml ice-cold normal human serum which had been treated with activated charcoal (8) to remove iodothyronine and which contained 1 mM propylthiouracil (PTU). These mixtures of serum and incubation media ("serum extracts") were centrifuged when necessary to remove particulates derived from homogenate and then analyzed for products of T_4 deiodination. Control experiments consisted of (a) incubation of T_4 without tissue fractions, and (b) addition of T_4 to the tissue fraction without incubation (time-zero tubes). Degradation of rT_3 was studied by adding rT_3 (20 ng) to 1 ml homogenate or cytosol (in Tris-HCl, pH 7.2 and 8.2) and incubating as described above.

Analytical methods. Measurements of rT_3 and T_3 in "serum extracts" were made by specific radioimmunoassays previously described (3). In each assay the sensitivity was 30 pg per ml serum (300 pg per ml of incubation mixture). The presence of liver fractions in the serum, in the proportions used, had no significant effect either on the recovery of rT_3 and of T_3 or on the sensitivity of these assays. There was negligible cross-reactivity in the rT_3 assay by T_4, T_3, the acetic acid derivatives of T_4 and T_3, and by glucuronide and sulfo-conjugates of rT_3. In control incubations (tubes containing T_4 in tissue-free buffer and unincubated, time-zero tubes) the rT_3 content of the incubation mixture was less than 1 ng per ml and was subtracted from the measured rT_3 values in the experimental tubes.

The protein concentration of incubation mixtures was determined by the method of Lowry et al (9).

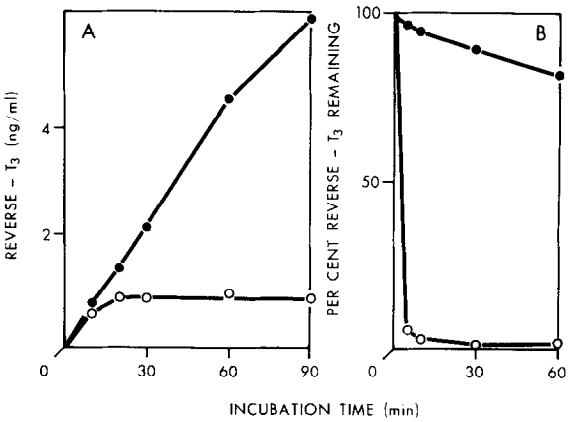

FIGURE 1. THE GENERATION (A) AND DEGRADATION (B) OF REVERSE-T_3 IN LIVER HOMOGENATE (OPEN CIRCLES) AND CYTOSOL (CLOSED CIRCLES). A. Incubation mixtures contained 2 μM T_4, 5 mM dithioerythritol (DTE), in 0.05 M Tris-HCl, pH 8.2. The rT_3 content of unincubated tubes (time-zero) was less than 0.8 ng/ml and has been subtracted from the results. B. Reverse-T_3 (20 ng/ml) was added at time zero to homogenate or cytosol in 0.05 M Tris-HCl, pH 8.2, containing 5 mM DTE. In both A and B, each point is the average of duplicate tubes, which varied by less than 10 per cent.

RESULTS AND DISCUSSION

During incubation of liver homogenate with T_4 at pH 8.2, rT_3 accumulated only during the initial 10-20 minutes and then remained constant. In contrast, cytosol showed a linear rate of rT_3 generation for 60 minutes (Fig. 1A). This difference in the time-course of rT_3 accumulation between the two preparations was explained by the finding of a 20-fold faster rate of rT_3 degradation in homogenate than in cytosol (Fig. 1B). In separate experiments it was determined that rT_3 degradation in cytosol was only slightly faster at pH 7.2 than at pH 8.2. Preliminary studies have localized the rT_3-degrading activity of liver to the microsomal fraction.

The properties of the cytosol system of T_4 5-deiodinase were investigated. The rate of conversion of T_4 to rT_3 was directly proportional to cytosol protein concentration in the range 0.2 to 10 mg/ml, at a T_4 concentration of 8 μM. The activity of cytosol did not vary significantly when homogenization

Table 1. Effects of possible cofactors and inhibitors on conversion of T_4 to rT_3 by liver cytosol.

	Condition or Agent		Reverse-T_3 Formed (ng/ml/hr)†
A.	Basal System*		1.10
	plus β-mercaptoethanol,	5 mM	3.15
	" dithioerythritol,	5 mM	5.01
	" $HgCl_2$,	5 mM	0.07
B.	Complete System**		6.56
	Nitrogen Atmosphere		8.33
	Dark		6.61
	Heat (60°/30 min)		0.22
	EDTA	5 mM	5.90
C.	Complete System**		5.51
	Propylthiouracil 5 μM		4.85
	50 μM		2.80
	500 μM		0.05
	Methimazole 100 μM		5.46
	3,5,3'-triiodothyronine	1 μM	5.60
		5 μM	5.96

* Basal System: Cytosol in 0.05 M Tris pH 8.2, 2 μM T_4.
** Complete System: Basal System plus dithioerthyritol, 5 mM.
† Results shown are averages of duplicate experiments which varied by less than 10 per cent.
Cytosol protein concentration in these incubation mixtures ranged from 6 to 8 mg/ml.

of liver was performed at different rates or by different methods. The reaction rate revealed Michaelis-Menten kinetics; a double reciprocal plot of rT_3 formation against T_4 concentration was linear, with an apparent K_m of 5×10^{-6} M.

Dialysis of cytosol for periods less than 24 hours had no significant effect on rates of rT_3 formation. More prolonged storage, however, at 4° or -20°, caused progressive loss of activity. Fifty per cent loss occurred after one week at -20°. Other properties of the system are given in Table 1. A requirement for thiol compounds is evident. Activity was not dependent on oxygen or light, indicating that a non-enzymic, light-dependent deiodination (10)

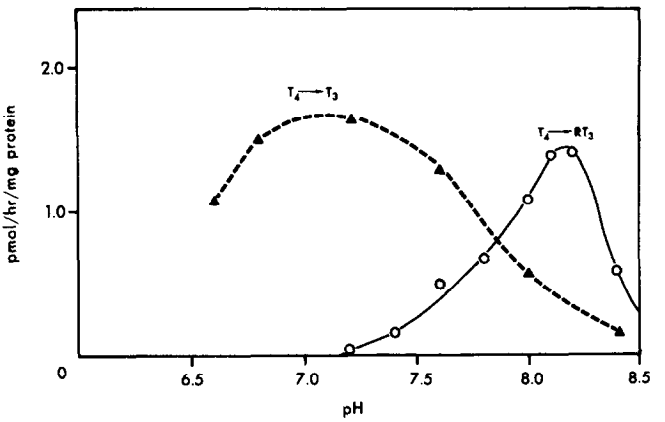

FIGURE 2. EFFECT ON pH ON GENERATION OF T_3 FROM T_4 BY LIVER HOMOGENATE (BROKEN LINE) AND OF RT_3 FROM T_4 BY CYTOSOL (CONTINUOUS LINE). Incubation mixtures contained 2 μM T_4 and 5 mM DTE in 0.1 M sodium acetate-barbital-HCl. Each point is the average of duplicates, which varied by less than 10 per cent.

is not involved. The system is heat-labile; short periods of heating to 60° led to irreversible inactivation. Propylthiouracil (PTU) but not methimazole inhibited T_4 to rT_3 formation. (PTU did not increase rT_3 degradation in this system.) The finding that T_3 did not inhibit T_4-to-rT_3 conversion in the concentration tested is of interest in view of Chopra's report that rT_3 competitively inhibits the formation of T_3 from T_4 in liver homogenates (11).

The pH-dependency of the 5-deiodinase activity in cytosol is shown in Fig. 2 and compared with the pH-curve of the T_4-to-T_3 reaction in homogenate. Almost identical results were obtained in Tris buffer.

The 5-deiodinase activity of cytosol is nearly equal to the 5'-deiodinase activity of homogenate when each was assayed at its optimum pH and expressed in terms of protein concentration. T_4-to-T_3 converting activity was also present in cytosol (pH optimum approximately 7.0) but at a lower level than in homogenate. To date, we have been unable to separate the two deiodinases by subcellular fractionation. Isolated liver microsomes, which contain most of the 5'-deiodinase activity of homogenate (12), have been observed also to convert T_4 to rT_3, but at a low rate (13).

The major findings in the present study are (a) T_4 5-deiodinase activity is present in the soluble fraction of liver and appears to be enzymic in nature, and (b) the pH optimum of the 5-deiodinase is higher than that of the 5'-deiodinase. In several respects, however, the two enzymes are similar. Thus, T_4 5'-deiodinase activity in liver is thiol-sensitive (12), heat labile (11), and inhibited by PTU but not by methimazole (11,14) or anaerobiosis (15). The difference in pH optima, demonstrated in the present study, provides evidence that the alternative pathways of T_4 deiodination are mediated by different enzymes.

Acknowledgements

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