Biochimica et Biophysica Acta, 569 (1979) 302—308

BBA 68792

MECHANISM OF ACTION OF IODOTHYRONINE-5′-DEIODINASE

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(Received December 28th, 1978)

Key words: Iodothyronine; Deiodination; Thiouracil; Ping-pong mechanism; Uncompetitive inhibition; Thiol cofactor

Summary

Production of 3,3′-di-iodothyronine (3,3′-T_2) from 3,3′,5′-tri-iodothyronine (reverse T_3, rT_3) as catalysed by rat liver microsomal fraction was measured with a specific radioimmunoassay. The effect of the addition of 2-thiouracil and of varying concentrations of cofactor (dithiothreitol) on the kinetic parameters of this reaction were studied. It was found that thiouracil is an uncompetitive inhibitor with respect to substrate and a competitive inhibitor with respect to cofactor. The effect of a decrease in the concentration of cofactor was similar to the effect of addition of thiouracil, i.e. a proportional decrease in K_m and V. The results strongly suggest that enzymatic 5′-deiodination of iodothyronines follows a ping-pong mechanism, which may be envisaged as a transiodination and the subsequent reduction of the iodo-enzyme complex by cofactor. The intermediate is probably a sulfenyl iodide form of the enzyme, which reacts with thiouracil to yield a mixed disulfide.

Introduction

The observation that 2-thiouracil derivatives inhibit both enzymatic oxidative iodination of thyroglobulin in the thyroid [1] and enzymatic reductive deiodination of thyroid hormones in peripheral tissues [2] is intriguing. It suggests that thiouracil reacts with a similar intermediate in both processes. It has been proposed that in thyroid peroxidase catalysed iodinations the formation of an enzyme-sulfenyl iodide is involved [3—7]. Thioureylenes react selectively with -SI [4,6,7] groups yielding mixed disulfides. It has been shown that thyroid hormone-deiodinating enzymes (iodothyronine-5- and -5′-deiodinase) contain essential sulfhydryl groups [8—10]. Mercapto compounds such as

dithiothreitol, 2-mercaptoethanol and reduced glutathione are cofactors in this deiodination [8] (Eqn. 1, where TI_n and TI_{n-1} are substrate and monodeiodinated iodothyronine, respectively).

$$TI_n + 2\ R\text{-}SH \rightarrow TI_{n-1} + R\text{-}S\text{-}S\text{-}R + HI \quad (1)$$

These findings suggest that a sulfenyl iodide intermediate is involved in both iodination and deiodination of thyroid hormones. To test this hypothesis the kinetics of the conversion of 3,3′,5′-tri-iodothyronine (reverse T_3, rT_3) into 3,3′-di-iodothyronine (3,3′-T_2) (as a model for 5′-deiodinase catalysed deiodinations) were analysed. The results strongly suggest that this reaction follows a ping-pong mechanism. It is shown that thiouracil inhibits the 5′-deiodination of rT_3 uncompetitively with respect to substrate and competitively with respect to cofactor (dithiothreitol). These findings are consistent with the formation of a -SI intermediate.

Materials and Methods

Conversion of rT_3 into 3,3′-T_2 was studied as previously described [11,12]. In short, 0.01—1 μM rT_3 was reacted with rat liver microsomal fraction (3.5—7 μg of protein) in 0.25 ml 0.066 M sodium phosphate, containing 3 mM EDTA and 0.2—10 mM dithiothreitol (pH 6.5 or pH 8.0). In experiments dealing with the effect of thiouracil, this compound was added at a final concentration of 0.5 or 1 μM. After incubation for 15 min at 37° C, the reaction was halted by the addition of 1 ml 1.25% of the detergent Brij-35 in 0.06 M barbital, 0.15 M NaCl, 0.1% bovine serum albumin (pH 8.6) at 0° C. The amount of 3,3′-T_2 produced was determined radioimmunologically in 50 μl of the extract [13]. The sensitivity of this assay is approx. 1 pg (2 fmol) 3,3′-T_2/tube. Cross-reactivities by 3,3′,5-tri-iodothyronine, rT_3, 3,5- and 3′,5′-di-iodothyronine are all less than 0.05%, and by 3- and 3′-iodothyronine approx. 1%. Both incubation and radioimmunoassay were performed in duplicate. The data shown are taken from representative experiments, which were repeated at least once with similar results.

Dithiothreitol, Brij-35 and thiouracil were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.; rT_3 and 3,3′-T_2 were purchased from Henning GmbH, Berlin, F.R.G.

Results

Both pH 6.0—6.5 [11] and pH 8.0 [14,15] have been reported to be optimal for the conversion of rT_3 into 3,3′-T_2. These apparently conflicting results are explained by the finding that, in the range 6.5—8.0, K_m as well as V increase with pH; from 0.035 to 0.23 μM and from 0.34 to 0.83 nmol 3,3′-T_2/min/mg protein (37° C), respectively [12]. The present investigations were, therefore, carried out both at pH 6.5 and 8.0. It has been assessed that all substrate added is free to interact with the enzyme by showing a linear relation between conversion rate and microsomal protein concentration [12].

It was found that both at pH 6.5 and 8.0 the degree of inhibition by thiouracil of the conversion of rT_3 into 3,3′-T_2 increased with substrate concen-

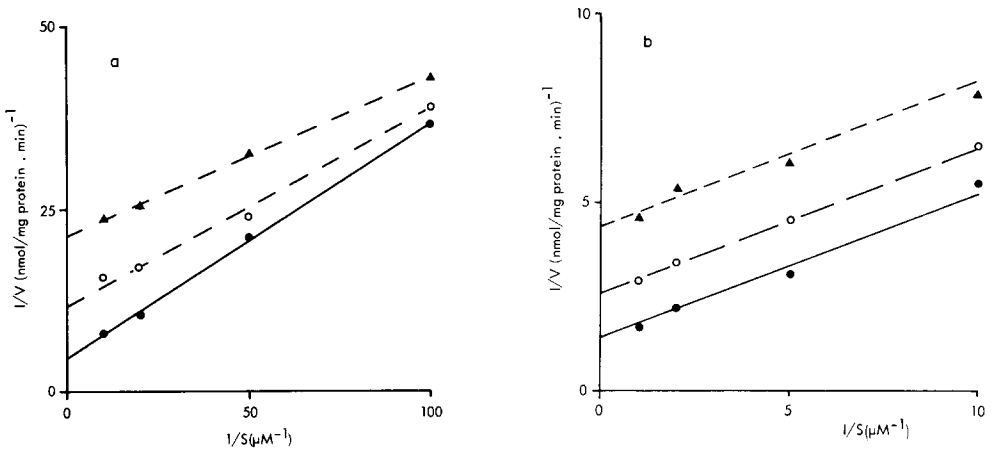


Fig. 1. Lineweaver-Burk plot of the amount of 3,3′-T_2 produced versus initial rT_3 concentration in the absence (●) and the presence of 0.5 (○) and 1 (▲) μM thiouracil at (a) pH 6.5 or (b) pH 8.0. The concentration of dithiothreitol was (a) 1 or (b) 2 mM.

tration. This is indicated by virtually parallel lines in the Lineweaver-Burk plot of the amount of 3,3′-T_2 produced versus initial rT_3 concentratin at different concentrations of thiouracil (Fig. 1), which is characteristic for uncompetitive inhibition [16]. Inhibition by thiouracil was obviated by increasing concentrations of dithiothreitol. Double-reciprocal plots of the amount of 3,3′-T_2 produced versus dithiothreitol concentration at different concentrations of thiouracil demonstrate (Fig. 2) that inhibition by thiouracil is competitive with respect to cofactor both at pH 6.5 and 8.0. Addition of up to 1 mM uracil did

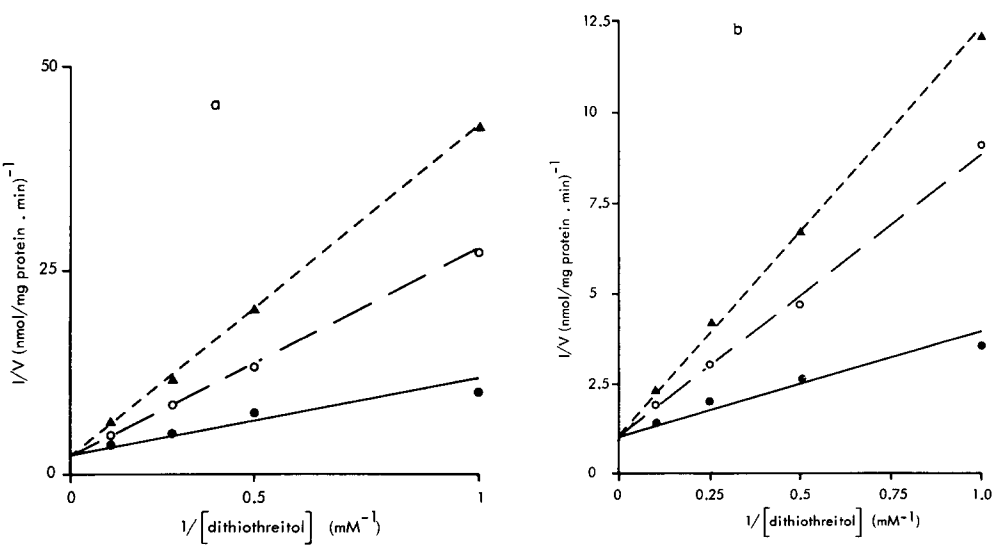


Fig. 2. Lineweaver-Burk plot of the amount of 3,3′-T_2 produced versus dithiothreitol concentration in the absence (●) and the presence of 0.5 (○) and 1 (▲) μM thiouracil at (a) pH 6.5 and (b) pH 8.0. The concentration of rT_3 was (a) 0.1 or (b) 1 μM.

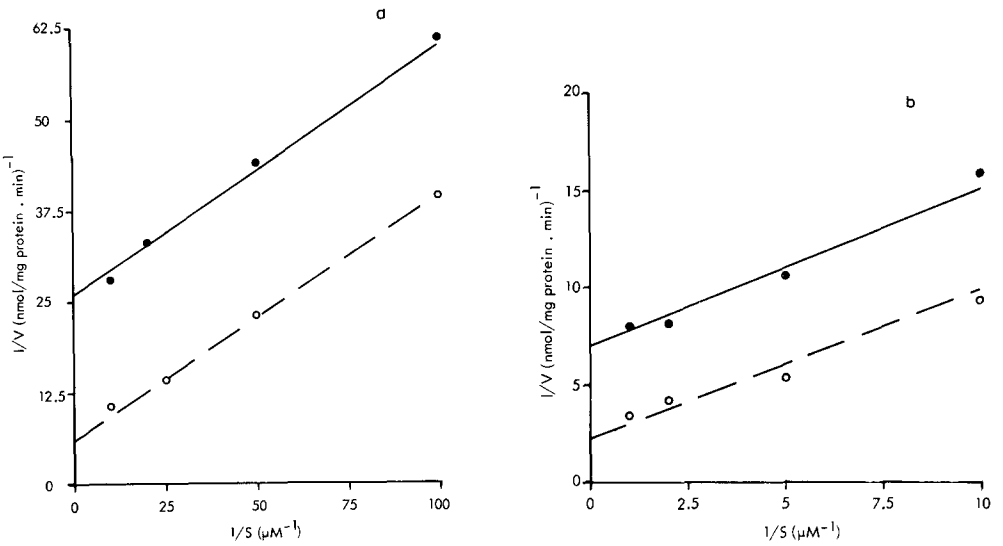


Fig. 3. Lineweaver-Burk plot of the amount of 3,3′-T_2 produced versus initial rT_3 concentration at (a) pH 6.5 or (b) pH 8.0. The concentration of dithiothreitol was 0.2 (●) or 1 (○) mM.

not affect 3,3′-T_2 production rate (Visser, T.J., unpublished observations). It was observed that decreasing the concentration of dithiothreitol resulted not only in decreased V values but also was there a proportional decrease in K_m. This is shown by the parallel lines in the Lineweaver-Burk plot of the amount of 3,3′-T_2 produced versus initial rT_3 concentration at different concentrations of dithiothreitol both at pH 6.5 and 8.0 (Fig. 3), which strongly suggests a ping-pong mechanism [16] for this reaction.

Discussion

The finding that inhibition by thiouracil of the 5′-deiodination of rT_3 is uncompetitive with respect to substrate (Fig. 1) is in accordance with data published by Chopra et al. [14] on the effect of 6-*n*-propyl-2-thiouracil. Chopra also found that conversion of thyroxine (T_4) into 3,3′-5-tri-iodothyronine (T_3) by rat liver homogenate is inhibited uncompetitively by propylthiouracil [9]. These observations indicate that thiouracil derivatives react with an intermediate in the enzymatic deiodination of iodothyronines [16]. The finding that the inhibition by thiouracil is competitive with respect to cofactor (Fig. 2) is in line with a report by Leonard and Rosenberg in abstract form [17] on the effect of propylthiouracil on the 5′-deiodination of T_4 in rat kidney tissue preparations. It is, however, in conflict with a previous communication from this laboratory [11], where it was noted that the degree of inhibition by propylthiouracil of the deiodination of several iodothyronines in rat liver homogenate was similar whether or not incubations were carried out in the presence of exogenous cofactor (dithiothreitol). The reason for this apparent contradiction remains unclear but, obviously, the degree of inhibition by thiouracil derivatives will depend on the reaction conditions such as the

type and concentration of cofactor and the concentration of substrate.

The effects of thiouracil on the kinetic parameters of the 5′-deiodination of rT_3 (Fig. 1) are similar to the effects of a decrease in the concentration of dithiothreitol (Fig. 3), i.e. a proportional decrease in K_m and V.

It is concluded that conversion of rT_3 into 3,3′-T_2 follows a ping-pong mechanism involving the formation of an intermediate which is reduced by dithiothreitol to the native enzyme. The intermediate may also react with thiouracil yielding an inactive complex. The importance of the sulfur in thiouracil is illustrated by the finding that uracil is devoid of inhibitory activity. It has been suggested that, beside the well-documented reaction of thiourea and thiouracil with sulfenyl iodides [4,6], thiourea may also react with -S-S- groups [3]. However, it has been shown that the reaction rate of thiouracil with disulfides is negligible compared with its reactivity towards sulfenyl iodides [4,6]. Reaction of thiouracil with sulfenyl iodides is much faster than the reaction of thiols with -SI groups [4]. In our study a significant effect of 0.5 μM thiouracil was observed in the presence of over 1 mM dithiothreitol. Therefore, our results strongly suggest that an enzyme sulfenyl (E-S^+) group — probably a sulfenyl iodide — is formed during 5′-deiodination of iodothyronines.

Because of the similarity in enzymatic 5- and 5′-deiodination — both are stimulated by mercapto compounds and inhibited by thiouracil derivatives — a general reaction pathway is proposed (Eqns. 2 and 3).

$$TI_n + \text{E-SH} \rightarrow TI_n \cdot \text{E-SH} \rightarrow \rightarrow \rightarrow \text{E-SI} + TI_{n-1} \quad (2)$$

$$\text{E-SI} + 2\ \text{R-SH} \rightarrow \text{E-SH} + \text{R-S-S-R} + \text{HI} \quad (3)$$

The formation of a non-covalent, iodothyronine-enzyme complex ($TI_n \cdot$ E-SH) is followed by a displacement reaction, which may include generation of one or more distinct intermediates. Monodeiodinated iodothyronine (TI_{n-1}) is released and an enzyme-sulfenyl iodide (E-SI) is formed (Eqn. 2). The latter is reduced to the native state by cofactor (Eqn. 3). E-SI may also react with thiouracil (X-SH) yielding a mixed disulfide (E-S-S-X) (Eqn. 4).

$$\text{E-SI} + \text{X-SH} \rightarrow \text{E-S-S-X} + \text{HI} \quad (4)$$

In both latter reactions the second product (I^-) is released. Free enzyme is obtained from E-S-S-X by reaction with cofactor [6] (Eqn. 5).

$$\text{E-S-S-X} + 2\ \text{R-SH} \rightarrow \text{E-SH} + \text{R-S-S-R} + \text{X-SH} \quad (5)$$

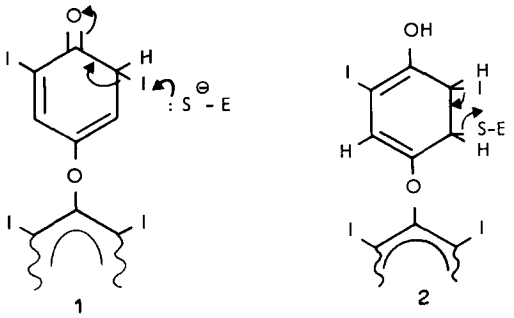
It should be emphasized that reactions 3 and 5 involve two thiol groups of the cofactor. In our case these are supplied by one molecule dithiothreitol.

A corollary of the present study is that the substrate is not directly reduced by the cofactor. Rather, the catalytic process is characterized by two reactions: transiodination (Eqn. 2) and subsequent reduction of the iodo-enzyme complex by cofactor (Eqn. 3).

Reminiscent of the work of Hartman et al. on non-enzymatic deiodination of di-iodotyrosine by cysteine [18—20], enzymatic 5′-deiodination may proceed by addition of a proton to $C^{5'}$ yielding (1). This is facilitated by the electron-donating effect of a dissociated phenolic hydroxyl group. An enzymic

sulfhydryl group may then assist the elimination of I^+ by forming a sulfenyl iodide. This model, however, does not account for the finding that rT_3 is a much better substrate for the 5′-deiodinase than T_4, the K_m being 40-fold lower and the V being 20-fold higher at pH 7.2 and 37°C [12].

Another possible mechanism of action of iodothyronine-5′-deiodinase is related to that of thymidylate synthetase in the dehalogenation of 5-bromo- and 5-iodo-2′-deoxyuridylate (IdUMP) (Ref. 21, see also Refs. 10, 11). This implies a primary attack of an enzymic sulfhydryl group to $C^{6'}$ yielding a covalent enzyme-5′-6′-dihydrosubstrate complex (2). The elimination of I^+ may



then be accomplished in a concerted mechanism as shown or with the aid of a second sulfhydryl group yielding an E-SI complex. Steric hindrance by bulky substituents on both C^3 and C^5 in T_4 may interfere with the approach of the enzymic sulfhydryl group to $C^{6'}$. Release of steric hindrance by deletion of one of these iodine atoms on the tyrosyl ring may account for the preferred reaction with rT_3. Also one can envisage 5-deiodination to occur by this mechanism and not by the former because of the absence of the activating hydroxyl group on the tyrosyl ring.

Because of the similarities between the structures of IdUMP, iodothyronines and 5-*n*-propyl-2-thiouracil — another strong inhibitor of the deiodination of thyroxine [2,22] — it was previously hypothesized [10,11] that the latter might inhibit the conversions competitively with respect to substrate. This appears to be ruled out by the present study.

Acknowledgements

The technical assistance of Mrs. L.M. Krieger and Mrs. E. van Overmeeren and the secretarial assistance of Mrs. C. Boot is gratefully appreciated.

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