Evidence for a single enzyme in rat liver catalysing the deiodination of the tyrosyl and the phenolic ring of iodothyronines

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The enzymic $5'$-deiodination of $3',5'$-di-iodothyronine and $5$-deiodination of $3,3',5$-tri-iodothyronine by rat liver microsomal fractions were found to be characterized by apparent $K_m$ values of 0.77 and 17.4 $\mu$M respectively. $3',5'$-Di-iodothyronine was a competitive inhibitor of $3,3',5$-tri-iodothyronine $5$-deiodination ($K_i$ 0.65 $\mu$M) and $3,3',5$-tri-iodothyronine was a competitive inhibitor of $3',5'$-di-iodothyronine $5'$-deiodination ($K_i$ 19.6 $\mu$M). In addition, several radiographic contrast agents and iodothyronine analogues inhibited both reactions competitively and with equal potencies ($r = 0.999$). These results strongly suggest the existence of a single hepatic deiodinase acting on both the tyrosyl and phenolic ring of iodothyronines.

The biologically active form of thyroid hormone, $3,3',5$-tri-iodothyronine (tri-iodothyronine), is produced predominantly by enzymic phenolic-ring- or $5'$-deiodination of thyroxine in peripheral tissues like the liver and the kidney, whereas the inactive isomer, $3,3',5'$-tri-iodothyronine (reverse tri-iodothyronine), is formed by tyrosyl-ring- or $5$-deiodination of thyroxine. Both tri-iodothyronines are further degraded by a cascade of deiodinations, with $5'$-deiodination to $3',5'$-di-iodothyronine as the main initial step in the metabolism of reverse tri-iodothyronine (Visser, 1980).

In a variety of clinical settings a concomitant decrease in tri-iodothyronine production and reverse tri-iodothyronine clearance has been observed, presumably owing to a specific decrease in the $5'$-deiodinase activity of the tissues (Braverman and Vagenakis, 1979). This has led to the hypothesis that the sequential deiodination of thyroxine is catalysed by two distinct enzymes, i.e. an iodothyronine $5$-deiodinase and an iodothyronine $5'$-deiodinase (Schimmel and Utiger, 1977; Visser, 1978). However, subcellular fractionation of rat liver homogenate (Fekkes et al., 1979) and partial purification of detergent extracts of microsomal fractions thereof (Fekkes et al., 1980), have not resulted in a separation of these two activities.

In the present paper we describe the effect of various inhibitors on the $5$-deiodination of tri-iodothyronine and the $5'$-deiodination of $3',5'$-di-iodothyronine by rat liver microsomal fractions. Evidence is obtained that a single enzyme is responsible for both deiodination reactions in rat liver.

Materials and methods

Materials
All iodothyronines and thyroxine were from Henning G.m.b.H., Berlin, Germany. Iopanoic acid (Telepaque) and sodium tyropanoate (Bilopaque) were kindly supplied by Sterling-Winthrop Laboratories (Amsterdam, The Netherlands) and iophenoxic acid (Trilombrine) was a gift from the Research Laboratories of Dagra, Diemen, The Netherlands.

Deiodination studies
Rat liver microsomal fraction was prepared as previously described (Fekkes et al., 1979). The protein content of this fraction was measured after solubilization in 0.5 M-NaOH, by a modification of the method of Bradford (1976), with bovine serum albumin as the standard (Spector, 1978). Reaction mixtures contained 0.1 M-sodium phosphate/3 M-EDTA/3 M-dithiothreitol, pH 7.2, 1-10 $\mu$M-tri-iodothyronine or 0.4-3.2 $\mu$M-3',5'-di-iodothyronine, 2-4 $\mu$g of microsomal protein and various inhibitors to be tested, all in a volume of 0.25 ml. The reaction was initiated by addition of microsomal fraction and incubation was carried out for 15 min at 37°C. The reaction was stopped by the addition of 1 ml of 0.06 M-barbitone/0.15 M-NaCl/0.1% (w/v) sodium dodecyl sulphate/0.1% (w/v) bovine serum albumin, pH 8.6. In control experiments, microsomal fraction
was added after the detergent solution. Each experimental point was determined in duplicate. The amounts of 3,3'-di-iodothyronine (5-deiodination product of tri-iodothyronine) and 3'-iodothyronine (5'-deiodination product of 3',5'-di-iodothyronine) were measured in duplicate by specific radio-immunoassays in 50 µl of the extract (Visser et al., 1978; Visser & van Overmeeren-Kaptein, 1980).

**Data analysis**

The amount of product formed was corrected for non-enzymic deiodination by subtraction of the respective control value, which was always less than 10% of enzymic deiodination. Regression lines of the double-reciprocal plots were calculated by unweighted least-squares analysis. $K_i$ values for competitive inhibitors were estimated by using the equation:

$$K'_m = K_m (1 + [I]K_i)$$

where $K_m$ and $K'_m$ are $-1/\text{intercept on the abscissa in the absence or presence of inhibitor respectively, and}$ $[I]$ is the concentration of inhibitor.

**Results**

The enzymic 5-deiodination of tri-iodothyronine was characterized by a mean apparent $K_m$ value of 17.4 µM, and the 5'-deiodination of 3',5'-di-iodothyronine by a mean apparent $K_m$ value of 0.77 µM (Table 1). Tri-iodothyronine was a competitive inhibitor of the 5'-deiodination of 3',5'-di-iodothyronine (Fig. 1), with a mean apparent $K_i$ of 19.6 µM (Table 1). Similarly, 3',5'-di-iodothyronine was a competitive inhibitor of tri-iodothyronine 5-deiodination (Fig. 2) with a mean apparent $K_i$ of 0.65 µM (Table 1).

As potential inhibitors of enzymic 5- and 5'-deiodination were tested 3.5-di-iodothyronine, thyroxine and the radiographic contrast agents iopanoic acid, iophenoxic acid, and tyropanoic acid. All these compounds inhibited both tri-iodothyronine 5-deiodination and 3',5'-di-iodothyronine 5'-deiodination competitively with respect to the substrates (see tyropanoic acid as an example in Figs. 1 and 2). Spanning over two orders of magnitude, inhibitor constants of the different compounds were very similar for the two types of deiodination (Table 1). Among the inhibitors, the 2,4,6-tri-iodophenol derivatives, iophenoxic acid, and the

![Fig. 1. Lineweaver–Burk plot of the 5'-deiodination of 3',5'-di-iodothyronine to 3'-mono-iodothyronine in the absence (○) or presence of 20 µM-tri-iodothyronine (●) or 30 µM-tyropanoic acid (△).](image)

For experimental details, see the text. Results shown are means of two closely agreeing values obtained from experiments performed in duplicate.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tri-iodothyronine 5-deiodination</th>
<th>3',5'-Di-iodothyronine 5'-deiodination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tri-iodothyronine</td>
<td>17.4 ± 4.2*</td>
<td>19.6 ± 6.1</td>
</tr>
<tr>
<td>3',5'-Di-iodothyronine</td>
<td>0.65 ± 0.16</td>
<td>0.77 ± 0.41*</td>
</tr>
<tr>
<td>3.5-Di-iodothyronine</td>
<td>11.5 ± 3.1</td>
<td>10.1 ± 2.3</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>125 ± 29</td>
<td>90 ± 16</td>
</tr>
<tr>
<td>Iopanoic acid</td>
<td>1.9 ± 0.6</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Iophenoxic acid</td>
<td>1.9 ± 0.8</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>Tyropanoic acid</td>
<td>27.2 ± 11.6</td>
<td>21.5 ± 14.2</td>
</tr>
</tbody>
</table>
Fig. 2. Lineweaver–Burk plot of the 5-deiodination of tri-iodothyronine to 3,3′-di-iodothyronine in the absence (O) or presence of 0.5 μM-3′,5′-di-iodothyronine (●) or 20 μM-tyropanoic acid (△)

For experimental details, see the text. Results are means for three experiments performed in duplicate.

Fig. 3. Correlation between the negative logarithms of mean apparent \( K_m \) and \( K_i \) values for substrates and competitive inhibitors of tri-iodothyronine 5-deiodination and 3′,5′-di-iodothyronine 5′-deiodination

For details, see Table 1.

Discussion

Investigations in vitro involving rat liver preparations have revealed striking similarities between enzymic 5- and 5′-deiodination of iodothyronines.

(1) Both 5- and 5′-deiodinase activities have been localized in the endoplasmic reticulum (Fekkes et al., 1979; Auf dem Brinke et al., 1979, 1980).

(2) Both types of deiodination are reductive processes, and are driven by thiols like dithiothreitol (Visser et al., 1978).

(3) Enzymic 5- as well as 5′-deiodinations are inhibited by derivatives of 2-thiouracil (Visser et al., 1978), and in both cases this inhibition is uncompetitive with the iodothyronine substrate (Chopra et al., 1978). Thiouracil is a mechanism-based inhibitor, since it reacts, under formation of a mixed disulphide, with an enzyme–sulphenyl iodide (′E–SI′) complex that is an intermediate in the deiodination process (Leonard & Rosenberg, 1978; Visser, 1979). These findings therefore indicate that 5- and 5′-deiodination follow the same reaction mechanism.

(4) Reaction of rat liver microsomal fractions with 3,5-di-iodothyronine, a substrate of which only the tyrosyl ring can be deiodinated, yields the sulphenyl iodide complex of the enzyme catalysing the phenolic-ring deiodination of other iodothyronines as evidenced by the persistent inactivation of 5′-deiodinase activity in the presence of thiouracil (Visser & Van Overmeeren-Kaptein, 1981).

(5) Iodothyronine 5- and 5′-deiodinase activities in detergent-treated rat liver microsomal fractions have very similar chromatographic and sedimentation properties, although, admittedly, none of the methods used has resulted in a considerable increase in specific enzyme activity (Fekkes et al., 1980).

(6) However, a major improvement in enzyme purification has recently been achieved applying the technique of isoelectric focusing to W-1 ether [a 1:1.78 (v/v) mixture of cetyl 10 ether (Brij 56) and cetyl 20 ether (Brij 58)] extracts of rat liver
microsomal fractions. Both before and after removal of phospholipids, 5- and 5'-deiodinase activities were found to co-migrate, indicating identical isoelectric points (Fekkes & Visser, 1981).

(7) We demonstrate here that rat liver 5- and 5'-deiodinase activities are equally affected by several competitive substances covering a wide range of inhibitory potencies. Furthermore, the $K_i$ value for tri-iodothyronine as a competitive inhibitor of 3',5'-di-iodothyronine 5'-deiodination is equal to its $K_m$ value in the 5-deiodination to 3,3'-di-iodothyronine. Conversely, the $K_i$ value for 3',5'-di-iodothyronine as a competitive inhibitor of the 5-deiodination of tri-iodothyronine is equal to the apparent $K_m$ for 3',5'-di-iodothyronine in its 5'-deiodination to 3'-iodothyronine.

In conclusion, the present study in conjunction with previously presented findings seriously challenges the generalization of two specific enzymes, i.e. iodothyronine 5- and 5'-deiodinase, being involved in the sequential deiodination of thyroxine in peripheral tissues (Schimmel & Utiger, 1977; Visser, 1978). Evidence is now compelling that, at least in rat liver, a single enzyme is capable of carrying out both phenolic- and tyrosyl-ring deiodination. If the same holds true for human liver and if this organ plays an important role in the extra-thyroidal conversion of thyroid hormone, then our results imply causes other than changes in deiodinase activity for the regulation of this process.

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References

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