Activation of thyroxine deiodinase by ferrous ions and flavin

It has been shown that incubation of thyroxine and triiodothyronine with skeletal-muscle preparations, unlike most other tissues, led only to their deiodination[1,2]. The absence of complicating side-reactions was the reason for selecting skeletal muscle for quantitative studies on enzymic deiodination. The non-particulate fraction of skeletal muscle, in which the enzyme is principally localized[3], was further fractionated to obtain a highly active thyroxine deiodinase preparation. The aim in presenting here the observation that the activity of thyroxine deiodinase from rabbit skeletal muscle was greatly enhanced in the presence of Fe^{2+} and flavin derivatives is to emphasize that it is different from the thyroidal TPN-dependent iodotyrosine deiodinase described by STANBURY[4,5].

The enzyme was prepared by: (a) reduction in ionic strength of soluble fraction of rabbit skeletal muscle, (b) heat treatment, (c) fractionation with $(NH_4)_2SO_4$, (d) adsorption on and elution from $Ca_3(PO_4)_2$ gel. It was used at a concentration of 3.4 mg protein/ml. Enzyme activity was measured at pH 7.1 as the rate of disappearance of ^{131}I-labelled thyroxine by chromatographic analysis as described previously[1].

In Table I is shown the effect of the presence of Fe^{2+}, flavin derivatives, oxidized and reduced TPN and nicotinamide on the rate of enzymic deiodination of thyroxine.

TABLE I

ACTIVATION OF ENZYMIC DEIODINATION OF ^{131}I-LABELLED L-THYROXINE BY Fe^{2+} AND FLAVIN DERIVATIVES

Each incubation vessel contained 2.5 ml of rabbit skeletal muscle extract to which were added 5.8 mμmoles ^{131}I-labelled L-thyroxine; incubation in air at 37°.

Substance added	*Amount μmoles*	*mμmoles thyroxine deodinated/15 min*	*% Substrate deiodinated*
None	—	0.31	5.3
Fe^{2+}	0.03	1.20	20.1
Fe^{2+}	0.10	2.58	44.5
FMN	0.05	0.57	9.8
FMN	0.15	1.25	21.6
FAD	0.10	1.19	20.0
Riboflavin	0.10	1.07	18.5
FMN + Fe^{2+}	0.05 + 0.10	3.71	64.0
TPN	10.0	0.36	6.2
TPNH	10.0	0.33	5.7
Nicotinamide	20.0	0.28	4.9

Fe^{2+} and either riboflavin, FMN or FAD stimulated deiodination as seen in the first 15 min; this action was additive when Fe^{2+} were added in the presence of flavin. Unlike the thyroidal iodotyrosine-deiodinating enzyme, the rabbit skeletal muscle preparation was unaffected by nicotinamide, TPN and TPNH. Other metal ions and commonly used co-factors also had a negative effect and are not included in Table I. The specificity of activation of thyroxine deiodinase is seen from the inhibition of the enzyme by flavin antagonists and Fe^{2+}-complexing agents (Table II).

Abbreviations: FMN, flavin mononucleotide; FAD, flavin-adenine dinucleotide; TPN, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide.

TABLE II

INHIBITION OF FMN- AND Fe^{2+}-ACTIVATED DEIODINATION OF ^{131}I-LABELLED L-THYROXINE BY FLAVIN ANTAGONISTS AND Fe^{2+}-COMPLEXING AGENTS

Each vessel contained 2.5 ml solution of enzyme to which were added 0.10 μmole FMN, 0.10 μMole Fe^{+2} and 5.3 mμmoles L-thyroxine. EDTA = ethylenediaminetetraacetate.

Inhibitor added	*Amount μmoles*	*mμmoles thyroxine deiodinated/15 min*	*% Inhibition*
None	—	3.18	—
Acriflavine	0.5	2.80	11.9
Acriflavine	1.0	1.95	38.8
Acriflavine	5.0	1.17	63.2
Atebrin	5.0	1.28	59.6
Chlorpromazine	5.0	1.75	45.0
8-Hydroxyquinoline	0.1	2.10	34.0
8-Hydroxyquinoline	1.0	1.30	61.1
8-Hydroxyquinoline	5.0	0.50	84.3
EDTA	5.0	0.69	78.5
α,α′-bipyridyl	2.5	0.41	87.0
Atebrin + 8-Hydroxyquinoline	5.0 + 5.0	0.00	100.0

The major radioactive iodinated product of the reaction was iodide. However about 5–20 % of the ^{131}I liberated from thyroxine was incorporated into protein(s) present in a fully activated enzyme preparation. The incorporation of iodine into proteins was stimulated by flavin derivatives added alone but not by Fe^{2+} without the flavin. This effect resembles the increased protein-bound ^{131}I formed by incubating thyroid homogenates with $^{131}I^-$ in the presence of FMN[6].

Deiodination of thyroxine with other non-thyroidal tissue preparations was found to be catalyzed by an enzyme similar in properties to the one described above. This enzyme has a higher affinity for iodothyronines than for iodotyrosines. Such a difference in substrate specificity is another argument in favour of concluding that the extrathyroidal deiodination of thyroid hormones and the thyroidal deiodination of iodotyrosines are controlled by totally different enzymes. This difference is an important consideration if a possible absence of both thyroidal and extrathyroidal deiodination is to be ascribed to a single gene defect in certain genetically determined disorders of the thyroid.

Details of the purification, properties and physiological significance of the above enzyme will be presented elsewhere.

National Institute for Medical Research, Mill Hill, London (Great Britain) J. R. TATA*

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* Beit Memorial Fellow.