REGULATION OF PROSTAGLANDIN METABOLISM: INHIBITION OF 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE BY THYROID HORMONES

Hsin-Hsiung Tai, Chen L. Tai and Charles S. Hollander

Endocrine Division, Department of Medicine
New York University School of Medicine
New York, New York 10016

Received January 31,1974

SUMMARY: 15-Hydroxyprostaglandin dehydrogenase has been purified from swine kidney to a specific activity of near 100 miliunits per mg of protein. The purified enzyme was found to be inhibited by thyroid hormone analogues of which triiodothyroacetic acid was the most potent inhibitor. The concentration required for 50% inhibition was 5 μM for triiodothyroacetic acid. The inhibition by thyroid hormones was uncompetitive and non-competitive with regard to NAD^+ and prostaglandin E_1, respectively. The sensitivity of this enzyme to thyroid hormones suggests that these hormones may regulate the metabolism of prostaglandins *in vivo*.

INTRODUCTION

The first step in the metabolism of biological active prostaglandins, the oxidation of the secondary alcohol group at carbon-atom 15 to a ketone (1), is catalyzed by a NAD^+-linked 15-hydroxyprostaglandin dehydrogenase (PGDH). This reaction appears to be a key step in the control of the biological inactivation of prostaglandins since the oxidized prostaglandins possess little biological activity. The possibility that thyroid hormones might have a role in regulating this inactivation reaction was suggested by reports which indicated that thyroid hormones inhibit several zinc-containing NAD^+- linked dehydrogenases including malic dehydrogenase (4), glutamic dehydrogenase (4) and alcohol dehydrogenase (5,6).

In order to examine whether thyroid hormones inhibit PGDH, it was necessary to at least partially purify the enzyme. We selected swine kidney for the initial purification procedure since studies on the distribution of PGDH in swine (2) indicated that kidney cortex is the richest source of this widely distributed enzyme. In this location, the enzyme may function to inactivate prostaglandins synthesized and released from the renal medulla (3). Here we report a partial purification of PGDH from swine kidney, our finding that

thyroid hormones are indeed potent inhibitors of the enzyme and some kinetic studies of the mechanism of this inhibition. A portion of this work has been presented in abstract form (7).

MATERIALS AND METHODS

L-Thyroxine, D-thyroxine, L-3,3',5-triiodothyronine, 3,3',5-triiodothyroacetic acid, L-3,5-diiodothyronine, L-thyronine, L-3,5-diiodotyrosine, dithiothreitol (DTT), NAD^+, DEAE-cellulose, Sephadex G-25 and G-100 were obtained from Sigma Chemical Company. Hydroxylapatite c was purchased from Clarkson Chemical Company. Prostaglandin E_1 (PGE_1) was a kind gift of Dr. John Pike of the Upjohn Company.

Enzyme assay: The standard assay mixture contained 0.1 M potassium phosphate buffer pH 7.5 ($KPB_{7.5}$), EDTA, 1 mM; NAD^+, 0.5 mM; PGE_1, 28 µM and enzyme in a final volume of 1 ml. Reaction was run in air at 37°C. The formation of 15-keto-PGE_1 was measured by the method as described by Anggard et al (2). The formation of NADH was followed spectrofluorometrically by an Eppendorf No. 1101 spectrophotometer with a fluorescence and a recorder attachments, and Hg 313/366 and 400/2000 primary and secondary filters, respectively. One unit of enzyme activity is defined as the amount of enzyme which catalyzes the formation of 1 micromole of product per minute at 37°C under standard assay conditions.

Enzyme preparation: PGDH was purified from swine kidney according to Anggard and Samuelsson (8) with some modifications. 250 gm of swine kidneys, cut in smaller pieces, were homogenized in two volumes of 60 mM of $KPB_{7.5}$ containing 1 mM EDTA with a Waring Blender operated at top speed for 2 minutes. This and all subsequent steps were operated at 0-4°C. The homogenate was centrifuged at 12,000 x g for 15 minutes in a Lourdes refrigerated centrifuge. The supernatant was then centrifuged at 78,000 x g for 30 minutes in a Spinco Model L-2 ultracentrifuge using rotor 30. The clear supernatant was collected and solid ammonium sulfate was then added with stirring to the solution to 30% saturation. The pH was kept near 7.5 by the addition of 3.0 n NH_4OH. The precipitate was removed by centrifugation at 12,000 x g for 15 minutes after stirring for one hour. The clear supernatant was then brought to 55% saturation of ammonium sulfate and the stirring was continued for another hour. The precipitate collected by centrifugation was dissolved in 100 ml of 10 mM $KPB_{7.5}$ containing 1 mM EDTA plus 0.2 mM DTT. The solution was applied on to a Sephadex G-100 column (6 x 100 cm) equilibrated with the above buffer. Elution was carried out with the same buffer. Active fractions were pooled and applied on to a DEAE-Cellulose column (2 x 20 cm) equilibrated with the same buffer. The column was then washed with 20 mM $KPB_{7.5}$ containing 1 mM EDTA plus 0.5 mM DTT until OD_{280} of the eluate was near 0.1. Hereafter, all buffers used for purification always contained 1 mM EDTA plus 0.5 mM DTT. Elution was started with a linear gradient consisting of 800 ml of 20 mM $KPB_{7.5}$ in the mixing chamber and 800 ml of 400 mM $KPB_{7.5}$ in the reservoir chamber. Active fractions were pooled and concentrated with ammonium sulfate precipitation (70% saturation). The precipitate was dissolved in 10 mM $KPB_{7.5}$ and desalted on a Sephadex G-25 column equilibrated with the same buffer. The desalted fraction was absorbed on a hydroxylapatite c column (2 x 15 cm) equilibrated with 10 mM $KPB_{7.5}$. The column was then washed with 20 mM $KPB_{7.5}$ until OD_{280} of the eluate was less than 0.03. The enzyme was then eluted out with 60 mM $KPB_{7.5}$ followed by concentration with ammonium sulfate precipitation. The concentrated enzyme was then desalted with a Sephadex G-25 column equilibrated with 10 mM $KPB_{7.5}$ and stored in small aliquots at -20°C.

TABLE 1

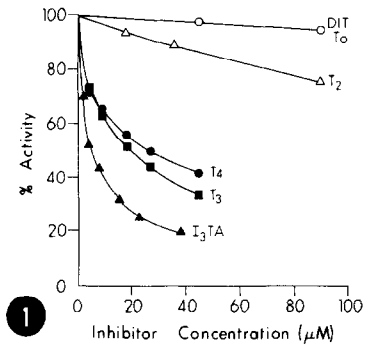
PURIFICATION OF 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE FROM SWINE KIDNEY

Fraction	Activity (units)	Total Protein (mg)	Specific Activity X 10^3 (units/mg protein)
Supernatant (S_{78})	7.67	10,465	0.74
Ammonium Sulfate Precipitate (30-55%)	15.75	5,681	2.77
Sephadex G-100	17.53	1,547	11.33
DEAE-Cellulose	14.36	239	60.10
Hydroxylapatite	6.8	73.3	92.86

RESULTS AND DISCUSSION

A summary of the partial purification of PDGH from swine kidney is shown in Table I. Total activity was found to increase during the early stages of purification. This indicates that some endogenous inhibitors were present in the crude supernatant and were gradually removed during purification. The final hydroxylapatite fraction was free of any inhibitors as evidenced by the linearity of activity and protein relationship (data not shown). The purified preparation has a specific activity of nearly 100 miliunits per mg of protein which is higher than the preparations reported from swine lung (10,11), bovine lung (12) and human placenta (13).

The effects of various thyroid hormone analogues on PGDH activity are shown in Figure 1. All three thyroid hormone analogues which possess metabolic activity inhibited PGDH activity significantly, while diiodothyronine and thyronine showed little effect. Inhibition by thyroid hormones was found to be instantaneous and reversible. Triiodothyroacetic acid was the most potent inhibitor among three thyroid hormones tested. The concentration required for 50% inhibition is about 5 μM for triiodothyroacetic acid (I_3TA) as compared to



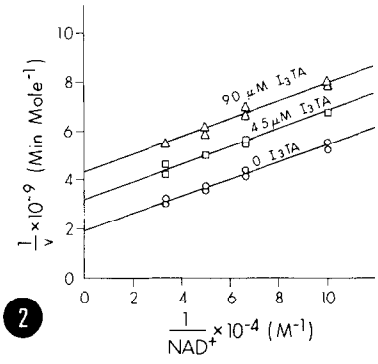


Figure 1-Effect of thyroid hormones and iodinated compounds on PGDH activity. The enzyme was assayed in the presence of indicated compounds under standard conditions. 14.4 μg of purified enzyme was used per assay.
—▲—▲— 3,3',5-Triiodothyroacetic acid (I_3TA)
—■—■— 3,3',5-Triiodo-L-thyronine (T_3)
—●—●— L-Thyroxine (T_4)
—△—△— 3,5-Diiodo-L-thyronine (T_2)
—○—○— L-Thyronine (T_o) and 3,5-Diiodo-L-tyrosine (DIT)

Figure 2-Inhibition of PGDH activity by 3,3',5-triiodothyroacetic acid with NAD^+ as the variable substrate at fixed concentration of PGE_1 (28 μM). 14.9 μg of purified enzyme was used per assay.

19 μM for triiodothyronine (T3) and 25 μM for thyroxine (T4). The inhibitory concentrations were found to be comparable to those found in other dehydrogenase systems (4,5).

The nature of this inhibition appears to be quite complicated. With NAD^+ as the variable substrate, triiodothyroacetic acid gave an uncompetitive inhibition pattern in the double reciprocal plot (Figure 2). When PGE_1 was used as the variable substrate, triiodothyroacetic acid exhibited a non-intersecting non-competitive inhibition pattern (Figure 3). Similar inhibition patterns with respect to NAD^+ and PGE_1 were also observed with triiodothyronine and thyroxine. The uncompetitive inhibition with respect to NAD^+ suggests that thyroid hormones and NAD^+ interact with different enzyme forms. If the kinetic mechanism of PGDH is similar to that of a variety of dehydrogenases (14) in which NAD^+ binds to the enzyme first followed by the other substrate, thyroid hormone would be expected to bind to the enzyme after NAD^+ has been added and thereby interfere with the binding of the second substrate

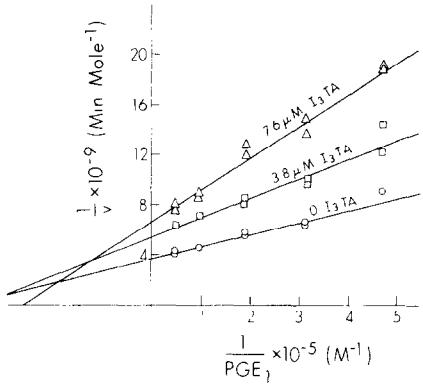


Figure 3-Inhibition of PGDH activity by 3,3',5-triiodothyroacetic acid with PGE_1 as the variable substrate at fixed concentration of NAD^+ (0.5 mM). 11.4 μg of purified enzyme was used per assay.

PGE_1. The non-intersecting non-competitive inhibition pattern with respect to PGE_1 as well as the concave upward curve of slope replot (data not shown) raise the possibility that thyroid hormones may interact with the enzyme at more than one site. Reports on the mechanism of thyroid hormone inhibition of other dehydrogenases have also been complex. Gilleland and Shore (6) concluded that inhibition of horse liver alcohol dehydrogenase was competitive with regard to coenzyme. However, McCarthy and co-workers (5) studied the same enzyme and in their hands T4 and T3 were uncompetitive inhibitors of horse liver alcohol dehydrogenase and the dose response curves for these two compounds were similar to those we have observed with the PGDH system. They also found that thyroid hormones showed no competition for the coenzyme binding site by equilibrium dialysis studies but quenched the enhanced fluorescence of bound coenzyme. This intricate situation was appreciated by a two-step coenzyme binding mechanism proposed by Theorell et al (15). In Theorell's model, the initial binding of ADP-ribose moiety is believed to induce conformational changes within the enzyme, which then allow the binding of the nicotinamide moiety at a different site. This process is accompanied by an enhancement of the fluorescence of the nicotinamide moiety of the bound NADH. This model led McCarthy and co-workers to propose that thyroid hormones inhibit the enzyme by blocking the second step of coenzyme

binding. Whether thyroid hormones inhibit PGDH by a similar mechanism remains to be determined. A homogeneous preparation of PGDH will certainly be a prerequisite to study the detailed mechanism of its inhibition by thyroid hormones.

The physiological significance of our observations remained to be seen. In view of the comparative potency of D-thyroxine (Ki=20 μM), inhibition of PGDH is not likely the primary mode of action of thyroid hormones. However, thyroid hormones may otherwise exert some of their action by elevating cellular level of prostaglandins, a family of lipids with potent biological effects, through blocking the initial step of their catabolism.

Acknowledgments: We are indebted to Dr. John Pike of the Upjohn Company for the supply of prostaglandins. This study was supported by grants from USPHS (5 RO1-AM14314-04) and the New York Heart Association. H.H. Tai is a recipient of Senior Investigatorship of the New York Heart Association.
Present Address: The Isaac Gordon Center of Gastroenterology, The Genesee Hospital, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14607. Reprint requests should be addressed to H.H. Tai.

References:

1. Anggard, E. and Larsson, C., Eur. J. Pharmacol., 14: 66 (1971).
2. Anggard, E., Larsson, C. and Samuelsson, B., Acta Physiol. Scand., 81: 396 (1971).
3. Lee, J.B. In the Prostaglandins Vol. L. (Ramwell, P.W., Ed.) Plenum Press, New York (1973) p. 133.
4. Wolff, J. and Wolff, E.C. Biochem. Biophys. Acta, 26: 387 (1957).
5. McCarthy, L., Lovenberg, W. and Sjoerdsma, A.J., Biol. Chem., 243: 2754 (1968).
6. Gilleland, M.J. and Shore, J.D., J. Biol. Chem., 244: 5357 (1969).
7. Tai, H.H., Tai, C.L. and Hollander, C.S., 9th International Congress of Biochemistry abstract p. 405 (1973).
8. Anggard, E. and Samuelsson, B., Ark. Kemi., 25: 293 (1966).
9. Nakano, J., Anggard, E. and Samuelsson, B., Eur. J. Biochem., 11: 386 (1969).
10. Anggard, E. and Samuelsson, B. In Methods of Enzymatic Analysis (Bergmeyer, H.U., Ed.) Verlag Chemie (1970) p. 1814.
11. Marrazzi, M.A. and Matschinsky, F.M. Prostaglandins, 1: 373 (1972).
12. Saeed, S.A. and Roy, A.C. Biochem. Biophys. Res. Commun., 47: 96 (1972).
13. Jarabak, J. Proc. Nat. Acad. Sci., U.S.A. 69: 533 (1972).
14. Wratten, C.C. and Cleland, W.W. Biochemistry, 2: 935 (1963).
15. Theorell, H., Ehrenberg, A. and deZalenski, C. Biochem. Biophys. Res. Commun., 27: 309 (1967).