Thyroxine Deiodination Associated with NADPH-Dependent (Enzymatic) and Fe^{3+}-ADP-Catalyzed (Nonenzymatic) Reactions in Hepatic Microsomes (37548)

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(Introduced by T. S. Danowski)

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Despite the many reports of thyroid hormone deiodination in various tissues, it has not been clearly established that this is an enzyme-catalyzed reaction (1). Although hepatic microsomes possess high thyroxine deiodinating activity (2–4), the mechanism of this reaction has not been ascertained. With hepatic microsomes and trypsin-treated particles from rat liver, we found a correlation between ferrous ion-induced lipoxygenation and thyroxine deiodination (5). This system, however, requires a high concentration of Fe^{2+} for maximal activity. Recently Poyer and McCay (6) demonstrated that oxidative degradation of phospholipid in hepatic microsomes is produced by at least two different systems. One of these is NADPH-dependent (enzymatic) and the other is ascorbate-dependent (nonenzymatic).

This report describes thyroxine deiodination with and without lipoxygenation.

Materials and Methods. All chemicals were of reagent grade. The rat liver microsomes were prepared by the procedure described by May and McCay (7). Malondialdehyde formation was used to measure lipoxygenation (5). Thyroxine degradation (deiodination) has been estimated from the release of radioactive iodine from ^{131}I-labeled (3′ and 5′) L-thyroxine (5). The composition of the reaction systems and conditions of incubation are described in each figure.

Results. Fe^{3+}-ADP system (S. 1). Hepatic microsomes isolated in Chelex-100 treated phosphate buffer (pH 7.4) manifested a low level of lipoxygenation and thyroxine degradation in the absence of added cofactor (Fig. 1A) or in the presence of just Fe^{3+} or ADP. The addition of Fe^{3+} and NAD increased thyroxine degradation without a significant increase in lipoxygenation. Microsomes heated at 65° for 2 min were as effective as unheated microsomes in thyroxine degradation. With both preparations within the time observed, thyroxine degradation was linear after a short induction period (Fig. 1B).

Fe^{3+}-ADP-Ascorbate system (S. 2). In the presence of added ascorbate-Fe^{3+}-ADP, microsomes promoted both thyroxine degradation and lipoxygenation after a short induction period (Fig. 2A) but the amount of thyroxine degraded in 20 min was only one-half of that produced in system 1. Under the same conditions, prior heating of the microsomes at 65° for 2 min altered the initial rate and the order of reactions (Fig. 2B).

Fe^{3+}-ADP-NADPH system (S. 3). Both lipoxygenation and thyroxine degradation were distinctly increased by the addition of NADPH to system 1 in which no significant increase of lipoxygenation activity had been demonstrated. Both rates were linear after the induction period, until about 50% of added thyroxine was deiodinated (Fig. 3). Heating of the microsomes (65° for 2 min) abolished both activities in this system, suggesting that NADPH inhibits the thyroxine degradation produced by heated microsomes in system 1.

Fe^{3+}-ADP-NADPH generating system. The combination of the NADPH generating system and system 1 duplicated the thyroxine degradation activity in system 1 and promoted lipid peroxidation (Fig. 4). Unexpect-

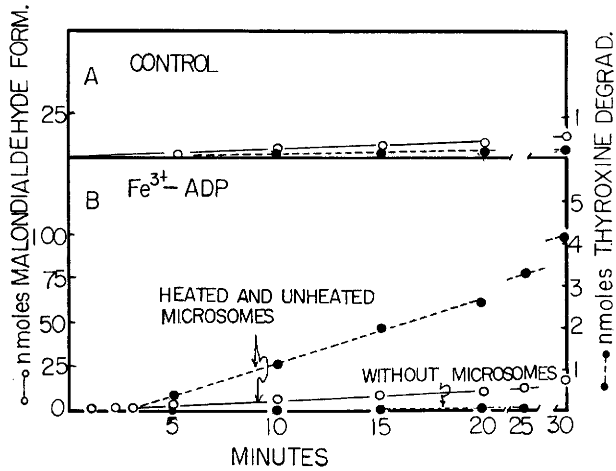

FIG. 1. Thyroxine degradation and malondialdehyde formation in the absence of added cofactor (A) and in system 1 (B) during incubation. The microsomes were isolated in Chelex-100 treated potassium phosphate buffer, pH 7.4. Five milliliters of incubation system contained microsomes (about 1 mg of protein/ml), 4 m*M* ADP, 12 μ*M* Fe^{3+}, 2.5 μ*M* L-thyroxine in all 0.1 *M* Tris–HCl buffer, pH 7.5. All materials, except the microsomes, were mixed just before experiment and incubated for 2 min at 37°. The reaction was initiated by the addition of the microsomes. The thyroxine was omitted in the study of lipoxygenation.

edly, the reaction was of zero order with respect to the malondialdehyde formation and had no induction period.

Discussion. The NADPH oxidase which appears to be or is involved in both lipid

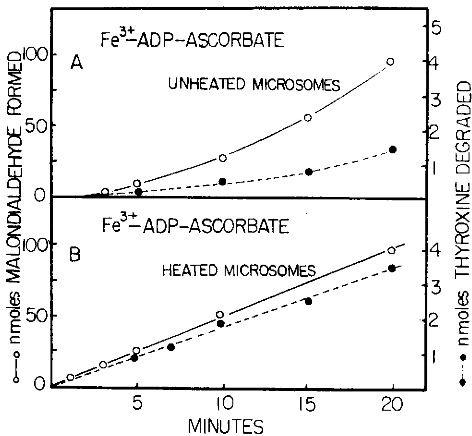

FIG. 2. Thyroxine degradation and malondialdehyde formation in system 2 with unheated (A) and heated microsomes (B). Incubation systems and conditions were the same as in Fig. 1B, except that 1 m*M* ascorbate instead of ADP was added to the components of system 1 before initiation of the reaction.

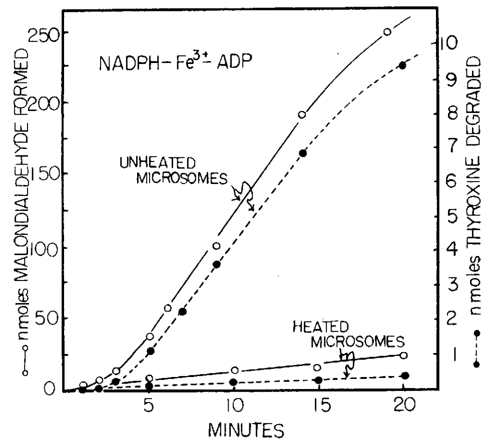

FIG. 3. Thyroxine degradation and malondialdehyde formation in system 3. Incubation systems and conditions were the same as for Fig. 1B, except that 0.3 m*M* NADPH was added before the initiation of the reaction.

peroxidation and drug metabolism is NAD PH-cytochrome *c* reductase (8–11). Recently, a purified preparation of rat liver microsomal NADPH-cytochrome *c* reductase has been shown to catalyze the NADPH-dependent peroxidation of isolated microsomal

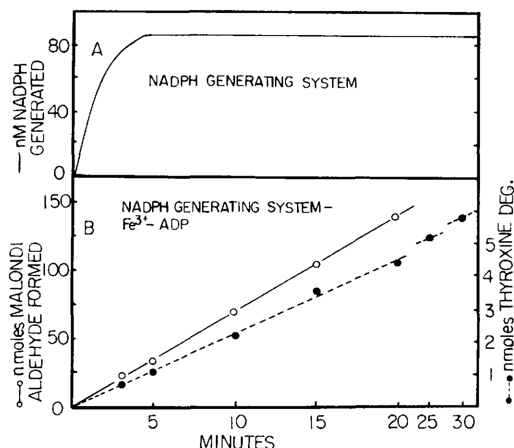

FIG. 4. Thyroxine degradation and malondialdehyde formation in a combination of NADPH generating system and system 1 (B). Incubation systems and conditions were the same as for Fig. 1B, except that the addition of the NADPH generating system was combined. The NADPH generating system contained 50 m*M* nicotinamide, 10 μ*M* $MnCl_2$, 5 m*M* $MgCl_2$, 0.5 m*M* NADP, 5 m*M* DL-isocitrate and 0.1 ml of isocitric dehydrogenase (2 units, Boehringer Mannheim Co.). All materials, except for isocitric dehydrogenase and microsomes, were preincubated for 2 min at 37°. The reaction was initiated by the simultaneous addition of both the enzyme and the microsomes. The time course of NADPH generated at 25° in the absence of microsomes is shown in A.

lipid (12). Even though this enzyme involves both lipoxygenation and drug hydroxylation in hepatic microsomes, the former reaction is distinct from the latter (12). Since cytochrome *p*-450 containing its related system (solubilized from rat liver microsomes) possesses high specific activity in terms of aminopyrine demethylation but no thyroxine degradation activity (13), thyroxine would have to be deiodinated in the main by a lipoxygenation-linked reaction in the presence of molecular oxygen. Figure 5 illustrates these reactions.

Judging from the effect of Fe^{3+}-ADP complex on NADPH linked peroxidation in microsomes (14), the induction period observed in our experiments may represent the time required for the formation of the Fe^{3+}-ADP complex (catalyst). Probably Fe^{2+} reacts promptly to produce the iron-ADP complex.

If the Fe^{3+}-ADP complex is not converted to the Fe^{2+}-ADP complex directly by a reducing agent (ascorbate) or indirectly through NADPH-cytochrome *c* reductase from NADPH (or also from ascorbate), oxygenation of endogenous lipid present in microsomes does not occur. The inactivation of the NADPH-dependent enzyme by heating probably blocks electron transfer to the Fe^{3+}-ADP complex and isolates the nonenzymatic systems: one for Fe^{3+}-ADP-dependent thyroxine degradation and the other for ascorbate-Fe^{3+}-ADP-dependent lipoxygenation with thyroxine degradation. In unheated microsomes, ascorbate may act mainly as a donor of electrons to the Fe^{3+}-ADP complex through the cytochrome reductase, as well as NADPH. However, ascorbate may also convert Fe^{3+} to Fe^{2+} with a rapid for-

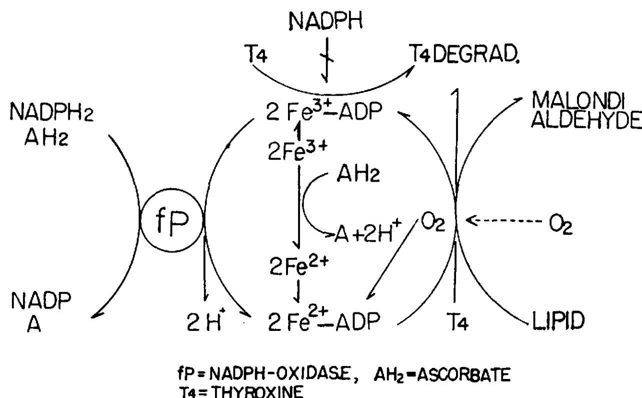

FIGURE 5.

mation of the Fe-catalyst and thereby promote nonenzymatic lipoxygenation and thyroxine degradation by heated microsomes without an induction period. These two different actions of ascorbate on hepatic microsomal lipoxygenation have already been cited by Poyer and McCay (6). The inhibition of thyroxine degradation by NADPH in the Fe^{3+}ADP system could result from a reduction of the thyroxine radical (intermediate of thyroxine degradation) back to thyroxine by $NADPH_2$ or $NADPH^{\cdot}$ (15). However, we do not know why lipoxygenation and thyroxine degradation produced by enzymatically generated NADPH (instead of the direct addition of NADPH) have no induction period.

Even though all of the steps in the degradation of thyroxine in hepatic microsomes are not known, the NADPH-linked reaction herein proposed should prove to be physiologically important. There two reasons for this suggestion: hepatic microsomes from phenobarbital-treated rats manifest enhanced thyroxine degrading activity (16) and the activity of hepatic NADPH-cytochrome *c* reductase and of NADPH-generating enzyme (glucose-6-phosphate dehydrogenase) increases in thyrotoxicosis (17, 18).

Summary. Oxidative degradation of phospholipid in rat liver microsomes accompanied by degradation of added thyroxine can be effected in at least two different systems. One of these is NADPH-dependent (enzymatic) and the other is Fe^{3+}-ascorbate-dependent (nonenzymatic). Ascorbate can replace the NADPH as the substrate in the enzyme-catalyzed reaction.

In addition, the ferric iron–adenosine diphosphate complex, which serves as a catalyst in the NADPH-dependent lipid peroxidation, can also in the presence of rat liver microsomes catalyze thyroxine degradation without lipid peroxidation.

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