HIGH AFFINITY THYROID HORMONE BINDING SITES ON PURIFIED RAT LIVER PLASMA MEMBRANES

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SUMMARY: Two orders of saturable binding sites for L-T_3 were detected on purified rat liver plasma membranes--a high affinity, low capacity binding site with a Kd of 3.2 + 0.5 nM, and a lower affinity, higher capacity site with a Kd of 220 + 50 nM. Competition-inhibition studies revealed that both D-T_3 and L-T_4 (two compounds with lower biological potencies than L-T_3) were also less potent than L-T_3 in competing for these binding sites. The present studies demonstrate, therefore, the presence of specific thyroid hormone binding sites on rat liver plasma membranes. In addition, they suggest that these sites may have a role both in mediating the known effects of thyroid hormones on membrane functions, and in regulating the entry of thyroid hormones into target cells.

INTRODUCTION

Thyroid hormones are potent substances that regulate the metabolism of most tissues. The effects of thyroid hormones range from rapid actions on plasma membrane transport to delayed effects on nuclear RNA synthesis (1). The exact mechanism(s), however, through which thyroid hormones carry out these diverse effects are unknown.

It is becoming clear that certain effects of thyroid hormones are the result of the entry of these substances into target tissues and their subsequent binding to intracellular organelles (1). From the studies of Tata et al. and others, it is evident that a number of effects of thyroid hormones are the result of their interactions with specific components of target cell nuclei (2). In concert with these observations, specific high affinity binding sites for thyroid hormones have been identified in the nuclei of several tissues (3-6). Further, thyroid hormones are known to regulate mitochondrial functions,

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and Sterling and Milch have identified specific high affinity binding sites for thyroid hormones in soluble extracts of inner mitochondrial membranes (7). In addition to acting in the cell interior, thyroid hormones have been demonstrated to directly regulate amino acid and glucose uptake at the cell surface (8-13). Although the presence of plasma membrane binding sites for thyroid hormones has been suggested (14,15), the exact nature of these binding sites has not been investigated. The purposes of the present study were to establish the presence of specific binding sites for thyroid hormones on highly purified plasma membranes from rat liver, and to investigate the characteristics of these binding sites.

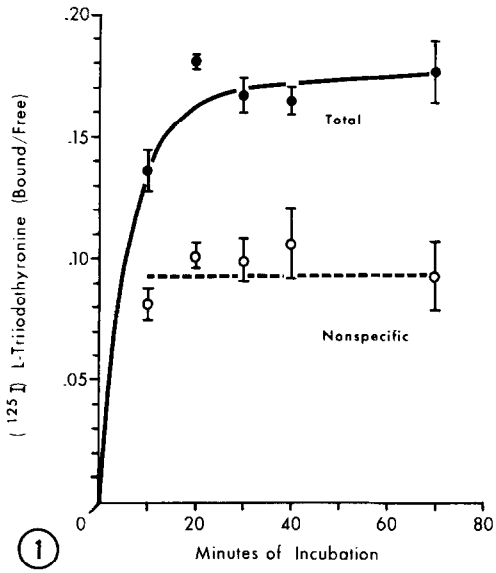
MATERIALS AND METHODS

Female Sprague Dawley rats, 100-200 g were fed ad libitum and killed by decapitation between 8:00 and 10:00 a.m. Purified rat liver membranes were then prepared according to the method of Ray (16). Electron micrographs of the plasma membranes revealed that there was no contamination with either mitochondria or nuclei. [^{125}I]L-triiodothyronine was purchased from Abbott Laboratories (400-550 μCi/mg) and unlabeled L-triiodothyronine (L-T_3), D-triiodothyronine (D-T_3), and D-thyroxine (D-T_4) were purchased from Sigma Chemical Company. The buffer employed in these studies was that used by Spindler and co-workers to examine the binding of L-T_3 to purified rat liver nuclei (6). It contained 0.25 M sucrose, 20 mM Tris, 1 mM $MgCl_2$, 2 mM EDTA, 50 mM NaCl, 0.1 mM DTT, and 5% glycerol, pH 7.6.

Binding Studies. To measure total hormone binding, rat liver plasma membranes (50-100 μg protein/ml) were incubated with [^{125}I]L-triiodothyronine (5×10^{-11} M) in 1.0 ml of buffer at 23°C. After incubation, the samples were chilled on ice and centrifuged at 1200 x *g* for 10 min at 4°. The pelleted membranes were then washed once in 1 ml of iced buffer and recentrifuged. The washed plasma membrane pellets containing the bound hormone were then counted in a well-type gamma scintillation counter. To determine nonspecific binding, the same amount of labeled L-T_3 was incubated with an excess of unlabeled L-T_3 (10^{-5} M). Specific binding was calculated by subtracting nonspecific from total binding. Specific binding of L-T_3 was expressed as the ratio of bound over free hormone per 100 μg of membrane protein.

RESULTS

L-T_3 bound rapidly to rat liver plasma membranes. Total hormone binding was one-half maximal by 5 min, and maximal within 15 min; a binding plateau was maintained for up to 1 hour or more (Fig. 1). Nonspecific binding was approximately 50% of total and did not change appreciably during the incubation period (Fig. 1). Further washings of the membrane pellet did not reduce



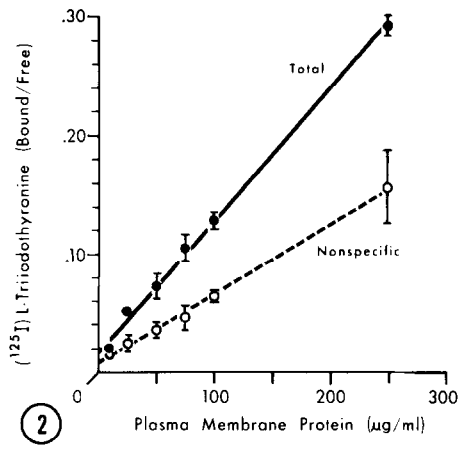


Figure 1. Time course of L-T_3 binding to purified rat liver plasma membranes. Total binding is that obtained with 5 x 10^{-11} M [^{125}I]L-triiodothyronine alone. Nonspecific binding is that value obtained with [^{125}I]L-triiodothyronine plus 10^{-5} M unlabeled L-triiodothyronine. Each value is the mean ± standard deviation for triplicate determinations.

Figure 2. The effect of increasing membrane protein concentrations on L-T_3 binding. Total and nonspecific binding were determined as in Fig. 1. The duration of incubation was 30 min. Each value represents the mean ± standard deviation for triplicate determinations.

the ratio of total to nonspecific binding. Increasing or decreasing the incubation temperatures increased and decreased the rate of binding respectively, but did not appreciably change the amount of hormone bound at steady state. Both total and nonspecific binding increased linearly with increasing protein concentrations up to 250 µg protein/ml (Fig. 2).

The specific binding of labeled hormone was decreased by increasing concentrations of unlabeled L-T_3. An inhibitory effect was consistently detected at an unlabeled L-T_3 concentration of 8 x 10^{-10} M, a one-half maximal effect was seen at 5 x 10^{-8} M, and maximal effects at 10^{-5} M (Fig. 3a). Scatchard plots of the data were compatible with two orders of binding sites (Fig. 3b,

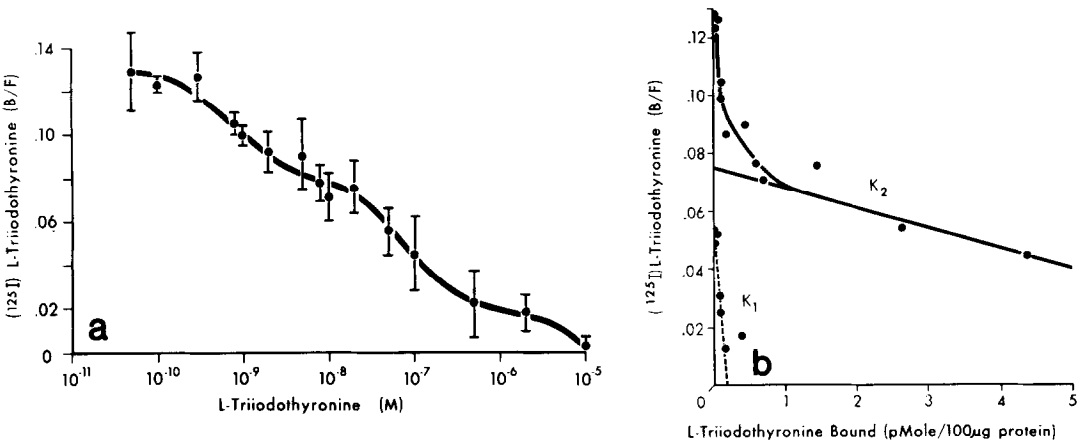


Figure 3a. The effect of increasing T_3 concentrations on the specific binding of [^{125}I]L-triiodothyronine. The duration of incubation was 30 min. Each value represents the mean ± standard deviation for triplicate determinations.

Figure 3b. Scatchard plot of data shown in Fig. 3a. The high affinity site, K_1, was obtained by subtracting the contribution of the low affinity site, K_2, from the curve.

Table I). A high affinity, low capacity site was calculated having a Kd of 3.2 ± 0.5 nM with a binding capacity of 0.250 ± 0.05 pMoles/100 µg protein, and a lower affinity, higher capacity site was calculated having a Kd of 220 ± 50 nM with a binding capacity of 11.0 ± 0.5 pMoles/100 µg protein (Table I).

Both D-T_3 and L-T_4, two iodothyronines with reduced biological activities (10), were less potent than L-T_3 in inhibiting the specific binding of [^{125}I] L-triiodothyronine to plasma membranes (Fig. 4). Based on their ability to one-half maximally inhibit the specific binding of [^{125}I]L-triiodothyronine, unlabeled L-T_4 was approximately 25% as potent, and unlabeled D-T_3 was 5% as potent as unlabeled L-T_3. The relative binding potencies of both L-T_4 and D-T_3 were greater at lower hormone concentrations (Fig. 4). This finding suggests that the higher affinity binding site was less able to discriminate amongst the thyroid hormone analogues than was the lower affinity site.

TABLE I

Affinities and Binding Capacities of T_3 Binding to Rat Liver Plasma Membranes

	Site I	Site II
Affinity Constant (nM)	3.20 ± 0.5	220 ± 50
Binding Capacity (pMoles/100 µg protein)	0.25 ± 0.05	11 ± 0.5

In the above studies, four separate experiments were performed; in each individual experiment, triplicate determinations were made. The values represent the mean value ± standard error of the mean.

DISCUSSION

The present studies demonstrate that high affinity, saturable binding sites for L-T_3 are present on plasma membranes prepared from rat liver. The Kd of the higher affinity, L-T_3 binding site seen on rat liver plasma membranes, 3.2×10^{-9} M, is similar to the Kd of the L-T_3 binding site on rat liver nuclei, as reported by Tata (15) and Surks et al. (3), but is tenfold lower than the Kd reported by Spindler et al. (6), DeGroot and Torresani (5), and Samuels and Tsai (4). The L-T_3 binding sites detected on plasma membranes differ in several respects from those found on nuclei. First with plasma membranes, multiple orders of binding sites are seen; in contrast with nuclei, only one order of binding sites is discerned (3-6). Second, with plasma membranes, the L-isomer of T_3 is more potent than the D-isomer, whereas in nuclei the two isomers are almost equally potent (3,5). Finally, the total number of binding sites per milligram protein is far greater on plasma membranes than on nuclei.

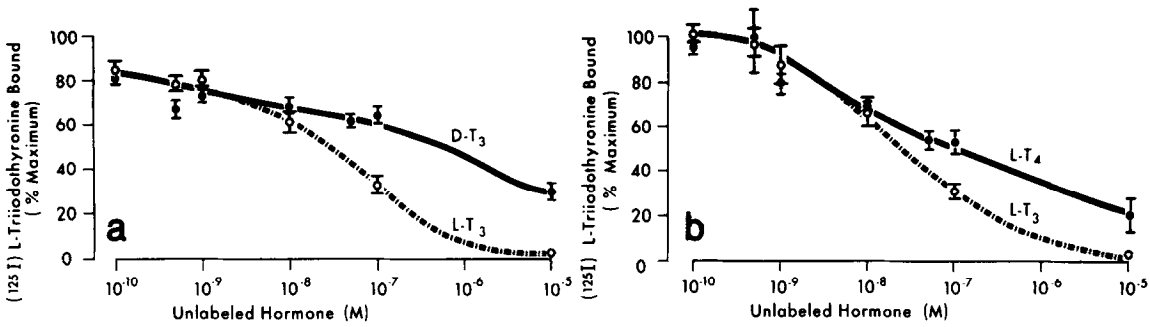


Figure 4a. Effect of unlabeled L-T_3 and D-T_3 on the specific binding of [^{125}I] L-triiodothyronine. The duration of incubation was 30 min. Four separate experiments were performed; in each individual experiment, triplicate determinations were made. The values represent the mean value ± standard error of the mean.

Figure 4b. Effect of unlabeled L-T_3 and L-T_4 on the specific binding of [^{125}I] L-triiodothyronine. The duration of incubation was 30 min. Three separate experiments were performed; in each individual experiment, triplicate determinations were made. The values represent the mean value ± standard error of the mean.

The biological role of L-T_3 binding sites on plasma membranes is unknown. One possibility is that these binding sites are involved in thyroid hormone action at the cell surface. Studies with rat and chicken cartilage and rat thymocytes indicate that thyroid hormones stimulate the transport of several neutral amino acids (8-11). In addition, studies with cultured fetal chicken heart cells indicate that thyroid hormones can directly stimulate the uptake of glucose (12,13). These studies suggest, therefore, that thyroid hormones may have direct effects on membrane functions and that the binding sites described herein may be involved in this process.

It is now evident that thyroid hormones themselves may be transported into the interior of target cells via specific components of the plasma membrane. Recent studies from this laboratory indicate that membrane transport in both cultured human lymphocytes and rat hepatocytes regulates the cellular uptake of thyroid hormones (17). Also, Rao and co-workers, employing isolated rat hepatocytes, have published data indicating that thyroid hormones are taken

up via a specific plasma membrane transport system (18). It is possible, therefore, that these binding sites seen on plasma membranes may also be involved in the transport of thyroid hormones into the interior of target cells. Further studies, however, will be necessary to establish the exact role of these binding sites for L-T_3 detected on purified rat liver plasma membranes.

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