

Affinity labeling of the plasma membrane 3,3',5-triiodo-L-thyronine receptor in GH₃ cells

(thyroid hormone/*N*-bromoacetyl-3,3',5-triiodo-L-thyronine/membrane receptor/ligand–receptor covalent interaction)

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ABSTRACT The binding of 3,3',5-triiodo-L-thyronine (T3) to GH₃ rat pituitary tumor cells was studied at 15°C and was shown to be saturable, reversible, and stereospecific. Least-squares analysis of the binding data showed two classes of binding sites with dissociation constants of 1.8 ± 0.2 nM and 260 ± 30 nM and binding capacities of $(5.2 \pm 0.2) \times 10^4$ and $(1.6 \pm 0.2) \times 10^6$ sites per cell, respectively. Affinity labeling of intact cells was carried out by incubation of cells with 0.3 nM *N*-bromoacetyl-[¹²⁵I]T3 at 15°C for 1 hr. Analysis of the cellular extracts by sodium dodecyl sulfate gel electrophoresis showed three labeled protein bands with apparent molecular masses of 55, 47, and 33 kilodaltons (kDa) in a ratio of 86:7:7. The labeling of only the 55-kDa protein band was selectively reduced to 50% by 20 μM unlabeled T3. Highly purified plasma membranes of GH₃ cells were prepared and shown to be free of nuclei. Affinity labeling of the purified plasma membranes gave the same labeling pattern as with intact cells. Peptide mapping by *Staphylococcus aureus* V8 digestion of the 55-kDa protein from cells or plasma membranes gave the identical peptide fragments. Thus the 55-kDa protein labeled from intact cells is the same protein as that from purified plasma membranes. These results together with our earlier findings [Horiuchi, R., Cheng, S.-y., Willingham, M., & Pastan, I. (1982) *J. Biol. Chem.* 257, 3139–3144] suggest that the 55-kDa protein may be involved in mediating the uptake of T3 in GH₃ cells.

Thyroid hormones regulate differentiation and development and are important for the regulation of metabolic homeostasis (1). Over the past two decades it has been believed that the delivery of thyroid hormones into cells was by simple passive diffusion (2–4). However, recent evidence indicates that the uptake of thyroid hormones into cells occurs by a receptor-mediated process (5–12). We have shown that the uptake of 3,3',5-triiodo-L-thyronine (T3) into GH₃ rat pituitary tumor cells occurs by receptor-mediated endocytosis and that this mode of entry is physiologically significant (13).

To characterize further the plasma membrane T3 receptors of GH₃ cells, we employed radiolabeled T3 to measure the binding of T3 to the plasma membrane receptors. In addition the present work directly identifies the plasma membrane receptors by using affinity labeling methods to label them *in situ*. The reagent chosen to label the membrane receptors is *N*-bromoacetyl-[¹²⁵I]T3 (BrAc-[¹²⁵I]T3), which has been successfully used to label thyroid hormone rat liver nuclear receptors (14).

MATERIALS AND METHODS

Reagents. [¹²⁵I]T3 (3,380 μCi/μg; 1 Ci = 3.7×10^{10} becquerels) was purchased from New England Nuclear. T3, L-thyrox-

ine, D-T3, spermine, spermidine, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid, polyethylene glycol 6000, bovine gamma globulin (Cohn fraction II), and human transferrin were obtained from Sigma. Horse serum, fetal calf serum, and Ham's F-10 medium were purchased from GIBCO. Ham's F-12 medium was obtained from Microbiological Associates (Bethesda, MD). *Staphylococcus aureus* V8 protease was purchased from Miles. *N*-Bromoacetyl-3,3',5-tri[¹²⁵I]iodo-L-thyronine was synthesized as described (14) with modifications. Triethylamine was omitted and two equivalents of NaOH in 20 μl was used instead. Insulin was obtained from Eli Lilly. Thyrotropin-releasing hormone was obtained from Peninsula Laboratories (San Carlos, CA). Fibroblast growth factor was a gift from D. Gospodarowicz (University of California, San Francisco Medical Center). Bovine parathyroid hormone was a gift from A. Spiegel (National Institutes of Health).

Cell Culture. GH₃ cells were cultured as described (13). Cultures used to isolate the plasma membrane were prepared by subculturing cells in 150-cm² flasks at a density of 3×10^6 cells per flask and harvesting them in the late logarithmic phase of growth.

Binding of [¹²⁵I]T3 to cells and computer analysis of the binding data. Binding of [¹²⁵I]T3 to cells at 15°C was carried out as described (13). The computer analysis of the experimental data was performed by a least-squares program (15). These least-squares analyses were performed by fitting the concentration of bound hormone as a function of the concentration of free hormone as described by

$$\text{bound} = \sum \frac{n[f]/K_d}{1 + [f]/K_d}, \quad [1]$$

in which $[f]$ is the free concentration, n is the concentration of binding sites of a given class, and K_d is the dissociation constant of the sites. The summation refers to being summed over multiple classes of sites.

Isolation of the Plasma Membrane from GH₃ Cells. Cells (30 flasks) were rinsed twice with Dulbecco's Mg²⁺- and Ca²⁺-free phosphate-buffered saline (P_i/NaCl) and detached from the flasks by incubation for 2–3 min with the same buffer at 37°C. All subsequent steps were carried out at 4°C. After centrifugation at $200 \times g$ for 3 min, the cell pellet was suspended in 30 ml of 10 mM Tris·HCl/2 mM MgCl₂/1 mM EDTA, pH 7.4, and allowed to stand for 10 min. The cells were homogenized with a type A Dounce homogenizer (seven strokes). Nuclei and unbroken cells were immediately removed by centrifugation at $200 \times g$ for 5 min. The supernatant was

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Abbreviations: T3, 3,3',5-triiodo-L-thyronine; BrAc, bromoacetyl; P_i/NaCl, Dulbecco's phosphate-buffered saline; STME buffer, sucrose/Tris/Mg/EDTA buffer; kDa, kilodalton(s).

centrifuged at $2,500 \times g$ for 20 min. The pellet was resuspended in 30 ml of 10 mM Tris-HCl/2 mM $MgCl_2$ /1 mM EDTA/0.3 M sucrose, pH 7.4 (STME buffer), and centrifuged at $3,000 \times g$ for 15 min. The pellet was resuspended in 30 ml of STME buffer and centrifuged at $2,200 \times g$ for 15 min. This step was repeated to remove mitochondria and other intracellular organelles.

The resulting pellet was suspended in 11 ml of STME buffer and layered on a step gradient consisting of 14 ml of 36% sucrose over 12 ml of 38.5% sucrose. After centrifugation in a Beckman SW 28 rotor for 90 min ($100,000 \times g$), the plasma membrane fraction that sedimented to the 36/38.5% sucrose interface was removed, diluted with 30 ml of STME buffer, and pelleted by centrifugation at $12,000 \times g$ for 15 min. The pellet was resuspended in 2.5 ml of STME buffer and 0.5- to 1-ml aliquots were stored in liquid nitrogen.

As measured by adenylate cyclase activity, the membrane preparation was enriched 11-fold over the total cell homogenate (16). The possible contamination of the purified membrane preparation by mitochondria was evaluated by assaying the mitochondrial marker enzyme succinate dehydrogenase, using the method of Veeger *et al.* (17); no succinate dehydrogenase activity was detectable. An examination of the purity of the membranes by electron microscopy showed that the preparation consisted of large ghost-like structures and small vesicles. Contamination by mitochondria was less than 1%. In the preparation 1.5% of total cellular protein and 16% of total adenylate cyclase activity were recovered.

Binding of [^{125}I]T3 to the Plasma Membrane. Membranes (15 μg of protein) were incubated with 0.5 nM [^{125}I]T3 in 0.5 ml of P_i /NaCl (pH 7.4) at 15°C for various lengths of time. The bound and free [^{125}I]T3 were separated according to Mashio *et al.* (18). At the end of incubation, 0.3 ml of gamma globulin (5 mg/ml) and 0.8 ml of polyethylene glycol (25%, wt/vol) in P_i /NaCl were added and the incubation mixture was mixed with a Vortex mixer. After centrifugation at $1,000 \times g$ for 7 min, the pellet was washed twice with 2 ml of 12.5% polyethylene glycol and the radioactivity associated with the pellet was determined with a Beckman Gamma 8000 spectrometer.

Affinity Labeling of Plasma Membrane T3 Receptors in Intact Cells or Purified Plasma Membrane Preparation. Cells in suspension ($2-4 \times 10^6$ cells) or purified plasma membranes (50–100 μg of protein) were incubated with 0.3 nM BrAc-[^{125}I]T3 in 0.5 ml of P_i /NaCl containing glucose at 1 mg/ml, pH 7.4 at 15°C, for various lengths of time depending upon the purpose of experiments. Nonspecific labeling was determined under identical conditions except with 20 μM unlabeled T3. At the end of the incubation, cells were pelleted at $200 \times g$ for 3 min and membranes were centrifuged at $12,000 \times g$ for 12 min. After resuspension of cells or membranes in 0.1 ml of P_i /NaCl, an equal volume of buffer containing 22.4% (vol/vol) glycerol, 10% (vol/vol) 2-mercaptoethanol, 6% NaDodSO₄, 0.14 M Tris-HCl, and 0.02% bromophenol blue, pH 6.8, was added. After the mixture had been heated at 100°C for 5 min, the cellular extracts were analyzed by NaDodSO₄/10% polyacrylamide slab gel electrophoresis according to Laemmli (19). The labeled proteins were identified by autoradiography. For quantification, the labeled bands were cut from the dry gel and the radioactivity was determined with a Beckman Gamma 8000 spectrometer.

Affinity labeling experiments were also carried out with cells that were grown in serum-free hormone-supplemented media. GH₃ cells were inoculated in 150-cm² flasks at a density of 3×10^6 cells per flask. After culturing in serum-containing medium (13) for 6 days, the medium was removed and cells were rinsed three times each with 50 ml of P_i /NaCl. Cells were cul-

tured in Ham's F-12 medium supplemented with 30 pM T3, 1 nM thyrotropin-releasing hormone, bovine parathyroid hormone at 0.5 ng/ml, human transferrin at 5 μg /ml, insulin at 5 μg /ml, and fibroblast growth factor at 1 ng/ml (20). In one experiment, cells were cultured in the same serum-free medium except without T3. The medium was changed after culturing for 24 hr. After 48 hr the cells were washed and affinity labeling experiments were carried out as described above.

Peptides Mapping of Plasma Membrane T3 Receptors. The 55-kilodalton (kDal) protein labeled either from intact cells or purified plasma membranes was partially digested with *S. aureus* V8 protease. The peptide mapping of the proteolytic digests was carried out similarly, according to Cleveland *et al.* (21) with minor modification as follows.

After one-dimensional gel electrophoresis as described above, the gel slab was dried. The 55-kDal band was cut from the dry gel, the backing paper was removed, and the dry gel slice was loaded onto the top of the stacking gel. After the gel slice had been soaked in a buffer of 0.125 M Tris-HCl/0.1% NaDodSO₄, pH 7.0 (soaking buffer), for 1 hr, the soaking buffer was removed. The swollen gel slices were covered with heat-solubilized 1% low-temperature agarose in the soaking buffer and 0.5 ml of *S. aureus* protease V8 (0.03 mg/ml) in 0.1% NaDodSO₄/0.125 M Tris-HCl/10% glycerol/0.01% bromophenol blue, pH 7.0, was added. Electrophoresis was carried out for 18 hr with a current of 3 mA and thereafter the current was increased to 35 mA until the electrophoresis was completed. Autoradiography was used to locate the labeled peptide fragments.

Binding of [^{125}I]T3 to GH₃ Cells at 15°C. Fig. 1 shows the time-dependent binding of [^{125}I]T3 to cells. The binding was rapid and reached equilibrium after 50 min. The kinetics of binding was similar to that at 0°C (13). It has previously been shown that the amount of [^{125}I]T3 bound by cells at 15°C is twice that at 0°C (13). Therefore the binding studies were carried out at 15°C to facilitate accurate measurements.

(i) **Saturability and specificity.** Fig. 2 shows that binding of [^{125}I]T3 to cells was competitively inhibited by increasing concentrations of unlabeled T3. Least-squares analysis of this data indicated a K_d of $0.26 \pm 0.03 \mu M$ and a binding capacity of $(1.6 \pm 0.3) \times 10^6$ sites per cell. A similar experiment designed to measure high-affinity sites is shown in Fig. 3. The K_d of this

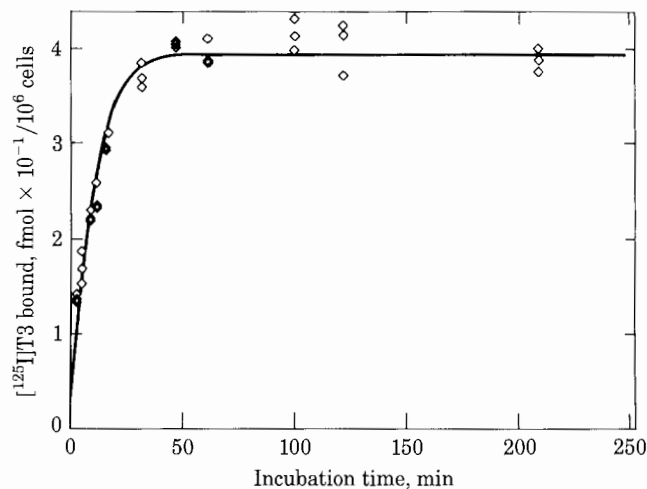


FIG. 1. Time-dependent binding of [^{125}I]T3 to GH₃ cells. Cells (3.4×10^6) were incubated with 0.5 nM [^{125}I]T3 (1 ml) at 15°C. At the time indicated free and cell-bound [^{125}I]T3 were separated by the oil-centrifugation method (13). The line is a theoretical curve corresponding to a first-order rate constant of $0.12 \pm 0.02 \text{ min}^{-1}$.

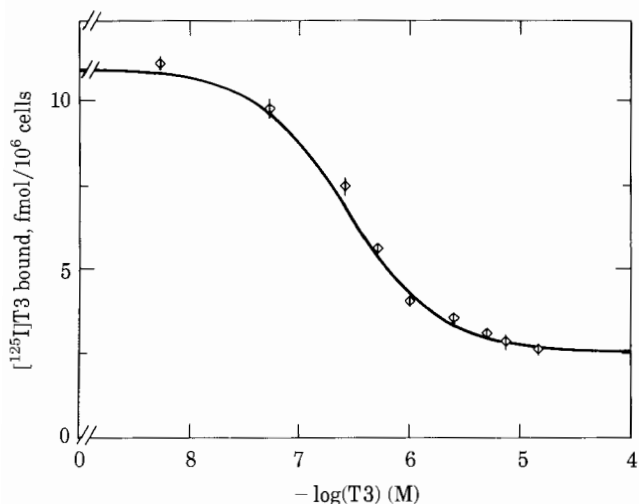


FIG. 2. Competition of [¹²⁵I]T3 and unlabeled T3 for binding to cells. Cells (1.6×10^6) were incubated with 1 ml of 0.1 nM [¹²⁵I]T3 at 15°C for 2 hr in various concentrations of unlabeled T3. Free and cell-bound [¹²⁵I]T3 were separated by the oil-centrifugation method. Each point was a mean of four determinations. The vertical lines designate the standard deviations. Nonspecific binding was not subtracted. The line is a theoretical curve calculated by using Eq. 1.

high-affinity site is 1.8 ± 0.2 nM with a binding capacity of $(5.2 \pm 0.2) \times 10^4$ sites per cell.

L-Thyroxine and D-T3 had a lower affinity for the low-affinity site than did T3 (Fig. 4). The K_d s were calculated as 3.5 ± 1.6 μ M and 1.78 ± 0.5 μ M for D-T3 and L-thyroxine, respectively. L-Thyroxine, D-T3, and 3,3',5-triiodo-L-thyropionic acid (data not shown) are all less effective than T3 in competing with [¹²⁵I]T3 for the binding sites. One important finding is that D-T3 has only approximately 1/10th of the affinity of T3. These results indicate that the binding of T3 to plasma membrane receptors is stereospecific.

(ii) *Reversibility.* The data in Table 1 show that the binding of [¹²⁵I]T3 to cells is reversible at 15°C. GH₃ cells were incubated with 0.1 nM [¹²⁵I]T3 at 15°C for 2 hr. After the removal of the excess ligand, cells to which [¹²⁵I]T3 was bound were in-

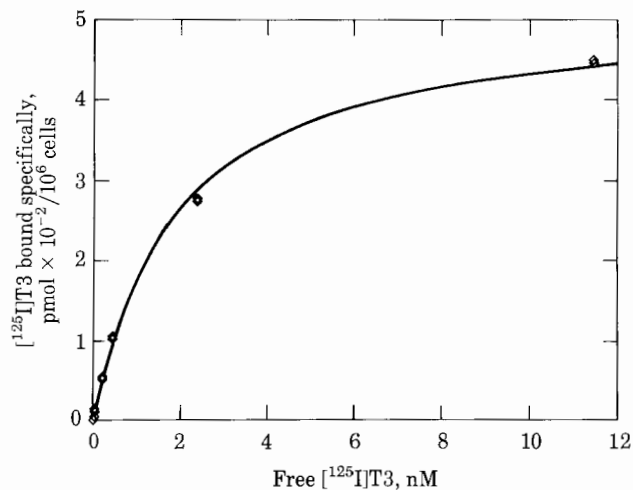


FIG. 3. Binding of [¹²⁵I]T3 to the high-affinity sites of plasma membrane T3 receptors in intact cells. Cells (1×10^6) were incubated with 1 ml of various concentrations of [¹²⁵I]T3 (total concentration: 2.5 pM to 25 nM) at 15°C for 2 hr. Free and cell-bound [¹²⁵I]T3 were separated by the oil-centrifugation method. Each point is a mean of triplicate determination. The line is a theoretical curve calculated from Eq. 1. Nonspecific binding was 20%.

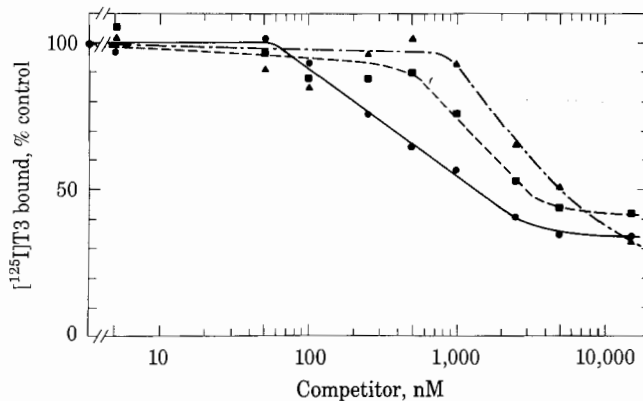


FIG. 4. Competition of [¹²⁵I]T3 and T3 analogs for binding to cells. Cells (1.2×10^6) were incubated with 0.1 nM [¹²⁵I]T3 in the presence of various concentrations of T3 (●), D-T3 (▲), and L-thyroxine (■) at 15°C for 2 hr. Free and cell-bound [¹²⁵I]T3 were separated by the oil-centrifugation method. The line is the theoretical curve calculated by using Eq. 1. Nonspecific binding was not subtracted.

incubated with 15 μ M unlabeled T3. After three changes of incubation medium, 85% of cell-associated [¹²⁵I]T3 was released. Thus, nearly all of the cell-associated [¹²⁵I]T3 remains on the cell surface at 15°C and can be displaced by unlabeled T3.

Competitive Inhibition of the Binding of [¹²⁵I]T3 to the Plasma Membranes at 15°C by Unlabeled T3. Fig. 5 shows the time-dependent binding of 0.5 nM [¹²⁵I]T3 to purified plasma membranes with or without 16 μ M unlabeled T3. With 0.5 nM [¹²⁵I]T3 binding reached steady state after 120 min. With 16 μ M unlabeled T3, \approx 50% of the binding was blocked. The nonspecific binding was more than that of intact cells. These results demonstrate the presence of T3 receptors in a highly purified preparation of plasma membranes.

Affinity Labeling of the Plasma Membrane T3 Receptors in Intact Cells and Plasma Membranes. Intact cells or purified plasma membranes were incubated with 0.3 nM BrAc-[¹²⁵I]T3 with or without 20 μ M T3 at 15°C. The solubilized cell extracts were analyzed by NaDodSO₄ gel electrophoresis (Fig. 6A). Three radioactive protein bands with apparent molecular masses of 55, 47, and 33 kDal in a ratio of 86:7:7 were detected, although the exposure used for this figure reveals only one band (lane 1). Only the labeling of 55-kDal protein band was selectively reduced by 20 μ M unlabeled T3 (lane 2). Fig. 7 shows that the labeling of the 55-kDal band occurred rapidly during the first hour and thereafter increased very slowly up to 5 hr. Fig. 6A further shows that affinity labeling of the purified plasma membranes under the same conditions as with intact cells gave identical labeling patterns (lanes 7 and 8). Under the present experimental conditions, 5.8 fmol of 55-kDal protein

Table 1. Reversibility of cell binding of [¹²⁵I]T3 at 15°C

| Dissociation by 15 μ M T3 | Cell-bound [¹²⁵ I]T3 | |
|-------------------------------|----------------------------------|------------|
| | fmol/10 ⁶ cells | % of total |
| None | 25.7 | 100 |
| First incubation (60 min) | 18.0 | 70.6 |
| Second incubation (50 min) | 10.9 | 42.3 |
| Third incubation (30 min) | 3.7 | 14.4 |

Cells (2×10^6) were incubated with 0.1 nM [¹²⁵I]T3 at 15°C for 2 hr. After free [¹²⁵I]T3 was removed by centrifugation, cells with bound [¹²⁵I]T3 were incubated with 15 μ M unlabeled T3 (1 ml) in Ham's F-10 medium at 15°C. After 1 hr, the same process was repeated once for 50 min. The third dissociation was carried out by incubation of cells with 15 μ M unlabeled T3 (1 ml) in 0.8% NaCl/10 mM Tris, pH 10.0, at 0°C for 30 min. Cell-bound [¹²⁵I]T3 was determined by the oil-centrifugation method.

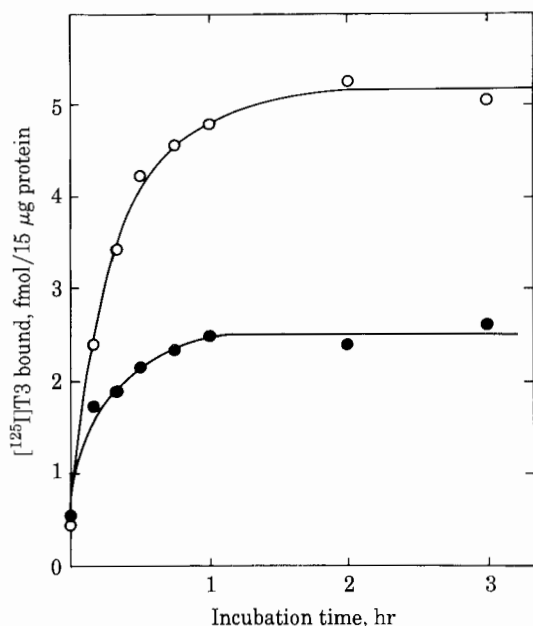


FIG. 5. Time-dependent binding of [125 I]T3 to purified plasma membranes. Fifteen micrograms of purified plasma membranes was incubated with 0.5 ml of 0.5 nM [125 I]T3 in P_i /NaCl, pH 7.3, at 15°C in the absence (○) or presence (●) of 16 μ M unlabeled T3. At the times indicated, membrane-bound [125 I]T3 was separated from free [125 I]T3 and the radioactivities were determined. Each point is a mean of duplicate determinations with variations less than 5%.

was labeled per 10^6 cells. These results provide direct evidence for the presence of T3 receptors in the plasma membrane of intact cells and in purified plasma membranes.

To evaluate the possibility that the 55-kDal protein labeled with BrAc-[125 I]T3 consists of contaminating serum thyroxine-binding proteins tightly adhering to the cells, the cells were grown in serum-free medium supplemented with various hormones at 37°C for 2 days to allow the release of possible serum contaminants. The labeling patterns (lanes 3 and 4) were virtually identical to those of cells grown in serum-containing medium. In another experiment, the identical labeling patterns were also obtained with cells that were grown in serum-free medium supplemented with various hormones at 37°C for 30 days and had undergone three passages. These results show that the 55-kDal protein probably is not a contaminating serum thyroxine-transport protein. Lanes 5 and 6 show the labeling patterns of cells grown for 2 days in a defined medium without T3. The labeling pattern is unchanged, suggesting that T3 plasma membrane receptors are not affected by the absence of T3 for 2 days.

Peptide Mapping of the Labeled 55-kDal Protein. To assess whether the specifically labeled 55-kDal protein from intact cells is the protein that is labeled in purified plasma membranes, peptide mapping of the 55-kDal labeled protein with *S. aureus* V8 was carried out. Fig. 6B shows that four major labeled fragments with molecular masses of 31, 21, and 11 kDal were obtained from intact cells or purified plasma membranes. Three minor peptides with molecular masses of 28, 27, and 16 kDal were also obtained from both preparations, although they are not visible in the figure. The identical pattern indicates that the 55-kDal protein labeled in purified plasma membranes is the same protein labeled in intact cells.

DISCUSSION

Recent studies have indicated that the uptake of T3 by GH₃ cells is receptor mediated (13). In this present study, the in-

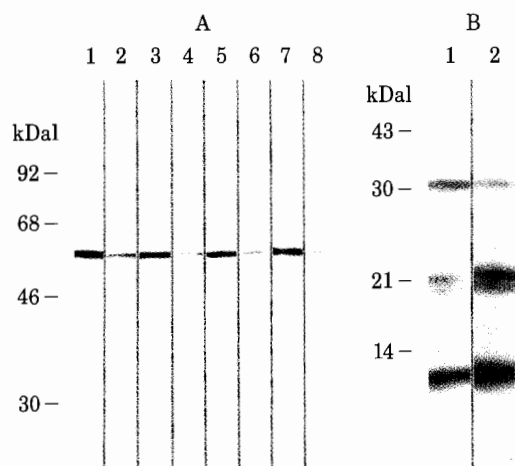


FIG. 6. Autoradiogram of affinity-labeled plasma membrane T3 receptors in intact cells and purified plasma membranes (A) and peptide maps of 55-kDal protein (B). (A) Cells (2×10^6) or purified plasma membranes (50 μ g of protein) were incubated with 0.3 nM BrAc-[125 I]T3 at 15°C for 2 hr. After labeling, cellular extracts or membranes were analyzed by NaDodSO₄/10% polyacrylamide gel electrophoresis. The positions of marker proteins are shown on the left. Lanes 1 and 2 were proteins from intact cells labeled in the absence and presence, respectively, of 20 μ M unlabeled T3. Lanes 3 and 4 were proteins from cells grown in serum-free hormone-supplemented media and labeled in the absence or presence, respectively, of 20 μ M T3. Lanes 5 and 6 are the same as lanes 3 and 4 except that T3 was not present in the growth media. Lanes 7 and 8 were proteins from purified plasma membranes labeled in the absence or presence of 20 μ M unlabeled T3. With this kind of exposure, only the major band, with the apparent molecular mass of 55 kDal, is visible. (B) The 55-kDal protein bands were excised and peptide maps were prepared. Lanes 1 and 2 are peptide maps of labeled protein from intact cells and purified membranes, respectively.

teraction of T3 with its plasma membrane receptors was further characterized by directly measuring the binding of [125 I]T3 under equilibrium conditions at 15°C. The binding of T3 to GH₃ cells was saturable, reversible, and stereospecific. Binding of T3 to cultured mouse fibroblasts has similar properties (unpub-

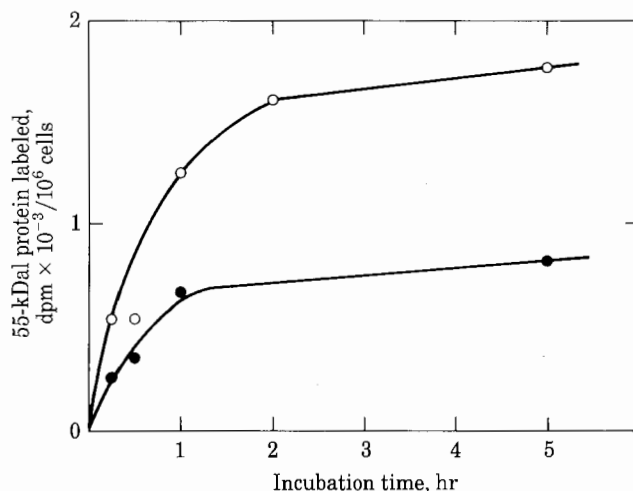


FIG. 7. Time-dependent labeling of plasma membrane T3 receptors in intact cells. Cells (2×10^6) were incubated at 15°C with 1 ml of 0.3 nM BrAc-[125 I]T3 in P_i /NaCl, pH 7.4, containing glucose at 1 mg/ml. Incubation was in the absence (○) or presence (●) of 20 μ M unlabeled T3. At the time indicated, labeled cells were washed with 2 ml of P_i /NaCl and cellular extracts were analyzed by NaDodSO₄ gel electrophoresis. The 55-kDal bands were excised and the radioactivity was determined.

lished data). Least-squares analysis of the binding data showed two classes of binding sites. The high-affinity sites have an apparent K_d of 1.8 ± 0.2 nM and a binding capacity of $(5.2 \pm 0.2) \times 10^4$ sites per cell. The low-affinity binding sites have an apparent K_d of 260 ± 30 nM and a binding capacity of $(1.6 \pm 0.3) \times 10^6$ sites per cell. The apparent K_d s for the binding of T3 to intact GH₃ cells are similar to those reported for purified rat liver plasma membranes by Pliam and Goldfine (22). In their studies, the K_d s are 3.2 ± 0.5 nM and 220 ± 50 nM for high- and low-affinity binding sites, respectively.

The purity of the isolated membranes was examined by electron microscopy to ensure no contamination by intracellular organelles, including nuclei. Because T3 binding sites were reported to be present on mitochondria (23, 24), the purity of the membrane preparation was further assessed for the possible presence of a mitochondrial marker enzyme, succinate dehydrogenase. No such enzymatic activity was detectable. Binding of [¹²⁵I]T3 to the purified membranes was subject to competition by unlabeled T3. The nonspecific binding was higher than that of intact cells (an increase from 30% to 50%; see Figs. 4 and 5); this is apparently due to binding to components on the inner surface of the membrane. Earlier, Pliam and Goldfine reported that the nonspecific binding of T3 to isolated rat liver plasma membranes was as high as 50% (22).

Affinity labeling of the purified plasma membranes identified one major protein with an apparent molecular mass of 55 kDal as determined by NaDodSO₄ gel electrophoresis. Although the molecular mass of the native protein is not yet available, it is of great interest that the apparent molecular mass of the denatured plasma membrane T3 receptor is similar to the masses reported for human thyroxine-binding prealbumin (25), human thyroxine-binding globulin (26), and rat liver T3 nuclear receptors (14, 27). It is highly unlikely that the membrane protein identified by affinity labeling is a contaminant of a serum thyroid hormone transport protein because affinity labeling of cells grown in medium with or without serum gave virtually identical patterns. It is also highly unlikely that the protein identified by affinity labeling is the T3 nuclear receptor because electron microscopic examination revealed an absence of nuclei in the plasma membrane preparation. Moreover, affinity labeling of intact cells also identified a major protein with the same molecular mass as that of the plasma membrane preparation. By peptide mapping these two proteins appear identical. Because the affinity labeling of intact cells was carried out under conditions in which most of T3 remained on the plasma membrane and was not internalized, we conclude that the 55-kDal protein is a membrane protein.

In the present study we have demonstrated the presence of plasma membrane T3 receptors by equilibrium binding analysis and have labeled a receptor protein molecule with an apparent molecular mass of 55 kDal. In earlier studies (13), it was shown that the uptake of T3 into GH₃ cells occurs by a receptor-mediated process. Because only one major protein was labeled, it

is probable that the 55-kDal protein is the receptor molecule that mediates the uptake of T3. However, further studies are needed to verify this hypothesis.

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