USE OF SOLUBILIZED RADIOIODINATED THYROID PLASMA MEMBRANES FOR PURIFICATION OF TSH-RECEPTOR BY AFFINITY CHROMATOGRAPHY

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1. Introduction

The purification of the thyroid TSH receptor has been described using bovine thyroid plasma membranes and bovine TSH [1]. Studies on its characterization required a large amount of thyroid tissue because of the low yield of purified material in terms of protein concentration. The use of radiolabelled material for purification may be advantageous, since very small amounts of labelled protein can be easily detected. Radioiodination of cell surface components or plasma membrane purified materials by enzymic procedures has been widely used for the study of surface proteins of human erythrocytes [2] and human platelet membrane [3,4].

In the present report human thyroid plasma membranes were radioiodinated, solubilized and then used for the purification of the TSH receptor by affinity chromatography with TSH–Sepharose column. The radiolabelled plasma membrane material was also studied by conventional chromatography on agarose gel.

2. Materials and methods

2.1. *Preparation of thyroid plasma membranes*

Plasma membranes (PM) were derived from human thyroids obtained at surgery for Graves' disease or non-toxic goiter. The glands were immediately refrigerated to 0°C and processed within 1 h. PM was prepared as in [5]. Briefly, a membrane-rich pellet from the whole homogenate was obtained by centrifugation at 1250 × *g* for 30 min. The pellet was adjusted with sucrose to a final density of 1.22 and a discontinuous sucrose gradient (densities: 1.2; 1.18; 1.16) was layered above the mixture. Ultracentrifugation was carried out in a Spinco L2 centrifuge in the SW 27 rotor at 25 000 rev/min for 90 min. The membrane fraction was collected at the interface between 1.18 and 1.16 sucrose densities, washed and diluted in 10 mM Tris buffer, pH 7.5. The membrane preparation was stored at −70°C.

2.2. *Plasma membrane iodination*

Lactoperoxidase-catalyzed radioiodination was performed by a modification [2] adapted for isolated PM [3]. To a cellulose nitrate tube were added 0.9 ml 10 mM Tris buffer, pH 7.5, 100 μl PM suspensions (5 mg/ml), 1–4 mCi $Na^{125}I$ (Radiochemical Center, Amersham) at conc. 100 mCi/ml, 3 μl lactoperoxidase (Calbiochem; 1 mg/ml) and 10 μl 0.03% H_2O_2. The reaction proceeded for 4 min at room temperature. Subsequently this step was repeated twice with 3 μg lactoperoxidase and 10 μl H_2O_2 and then with 10 μl H_2O_2 alone. The reaction was terminated by dilution with 5 ml cold Tris buffer. The radioiodinated thyroid PM was centrifuged twice for 15 min at 98 000 × *g* and the supernatant was saved and counted. The pellet was used for subsequent solubilization.

2.3. *Plasma membrane solubilization*

Radiolabelled PM was solubilized as in [6] employing 0.1 M lithium diiodosalicylate (LIS). After centrifugation at 50 000 × *g* for 90 min the supernatant was dialyzed for 36 h against 20 mM $NaHCO_3$, pH 9.4, at 4°C. The solubilized material was stored at −70°C.

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2.4. *Preparation of TSH and HCG adsorbents*

Bovine TSH (NIH-TSH-B8) was coupled to CNBr activated Sepharose 4B as in [7]. HCG (APL Ayerst) was bound to activated Sepharose 4B with the same method.

2.5. *Affinity chromatography*

The coupled gels were used to form columns with 1.2 × 5 cm dimensions, saturated with 1 M glycine and extensively washed with 200 ml of each of the following solutions: 0.1 M Na acetate, pH 4, containing 1 M NaCl; 0.1 M Na borate, pH 8.5, containing 1 M NaCl (BBS). The eluates were evaluated for A_{280}. The radioiodinated solubilized thyroid PM preparation was passed through the column for at least 7 times. Non-specific adsorbed material was washed away with BBS until removed radioactivity was <0.01% of the total applied. Subsequent elution of the hormone-bound material was performed with 3 M KSCN.

3. Results and discussion

3.1. *Radioiodination of thyroid plasma membranes*

Iodination conditions established for platelet membrane [3,4] were initially employed. Incorporation of ^{125}I into thyroid PM, was <10% when lactoperoxidase and H_2O_2 were added only once. Repeated additions of lactoperoxidase and H_2O_2 increased the incorporation to 60%. These findings suggest that H_2O_2 was consumed during the incubation and that self iodination of lactoperoxidase may inhibit the catalyzation of the reaction. Repeated addition of the enzyme and H_2O_2 may circumvent these problems and reinitiate the reaction.

3.2. *Solubilization of radioiodinated thyroid plasma membranes*

Triton X-100 was preliminary used and gave good results in term of solubilization of radioiodinated PM, but removal of this detergent by dialysis resulted in considerable aggregation of the solubilized material. Furthermore Triton X-100 interfered with the binding of solubilized PM to TSH–Sepharose. The use of 0.1 M LIS proved to be advantageous. Solubilization of radioiodinated PM was in several experiments approx. 80%. A loss of ^{125}I, probably a consequence of LIS treatment, was found during dialysis of solubilized PM: virtually all (98%) the radioactivity retained by the dialyzed material was trichloroacetic acid (TCA) precipitable, but accounted for only 20% of initial radioactivity.

3.3. *Affinity chromatography*

The elution pattern of the radioiodinated solubilized thyroid PM after adsorption, washes and elution on TSH–Sepharose is illustrated in fig.1. The total adsorption of the labelled material was 88%; the washes with BBS detached 61% of the radioactivity. A further detachment of 32.9% was obtained with 3 M KSCN. Both the BBS and the KSCN eluted radioactivities were > 95% TCA precipitable. These treatments were shown in previous experiments to produce no detachment of radioiodinated TSH covalently bound to Sepharose. These results are in agreement with the findings [8] of a purification of solubilized TSH receptor by affinity chromatography, using the same conditions. When the ^{125}I-solubilized thyroid PM was passed through the HCG–Sepharose column, an adsorption of 85% was found (fig.1). The washes with BBS removed 68% applied material and only

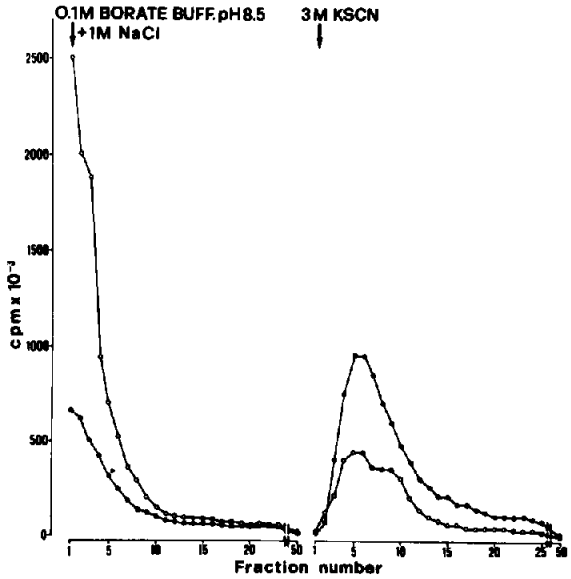

Fig.1. Affinity chromatography of solubilized [^{125}I]thyroid plasma membranes on TSH (●—●) or HCG (○—○) coupled to Sepharose. The arrows indicate the addition of eluting agents. Fraction vol. 0.5 ml.

16.4% was eluted by 3 M KSCN. Thus, in spite of similar initial adsorption to TSH and HCG adsorbents, specific binding to TSH was twice higher. The observation that, although less active than TSH, HCG also bound [^{125}I]thyroid PM is consistent with recent data indicating that the thyroid stimulator of molar pregnancy is in fact HCG [9]. Furthermore HCG inhibited [10] the binding of [^{125}I]TSH to bovine PM.

3.4. *Gel chromatography of [^{125}I] thyroid plasma membranes*

The elution pattern on Sepharose 4B chromatography column of [^{125}I]thyroid PM is illustrated in fig.2A. When the KSCN eluted peak from TSH–Sepharose was passed through the same column, the radioactivity distributed itself into 2 major peaks (fig.2B). The first was in the void volume, probably representing aggregated material, and was present in both cases. The second peak accounted for a much larger proportion of the retarded material than in the unpurified PM preparation. These results indicate that the purification step on TSH–Sepharose eliminated a large amount of thyroid PM components unrelated to TSH binding activity. The use of radioiodinated thyroid PM appears to be useful for the purification and characterization of the TSH receptor.

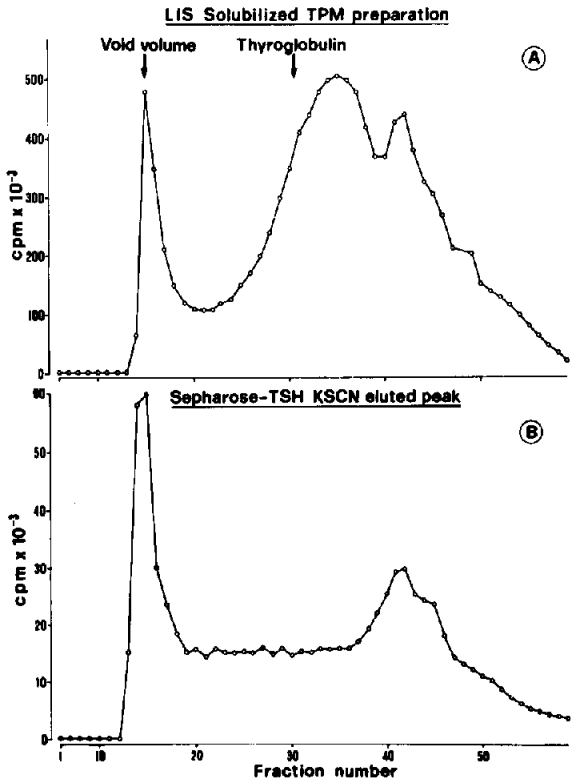

Fig.2. Chromatography on Sepharose 4B: (A) lithium diiodosalicylate (LIS) solubilized [^{125}I]thyroid plasma membrane (TPM); (B) LIS solubilized [^{125}I]TPM adsorbed on TSH–Sepharose and eluted by 3 M KSCN. The arrows indicate the Blue Dextran 2000 and the thyroglobulin peaks. Bed dimensions: 1.8 × 65 cm. Eluant: 20 mM $NaHCO_3$ buffer, pH 9.4. Flow rate: 3 ml/cm^{2}h.

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References

[1] Tate, R. L., Schwartz, H. I., Holmes, J. M., Kohn, L. D. and Winand, R. J. (1975) J. Biol. Chem. 250, 6509.
[2] Phillips, D. R. and Morrison, M. (1971) Biochemistry 10, 1766.
[3] Nachman, R. L., Hubbard, A. and Ferris, B. (1973) J. Biol. Chem. 248, 2928.
[4] Phillips, D. R. (1972) Biochemistry 11, 4582.
[5] Fenzi, G. F., Macchia, E., Bartalena, L., Mazzanti, F., Baschieri, L. and DeGroot, L. J. (1978) J. Endocrinol. Invest. 1, 17.
[6] Tate, R. L., Holmes, J. M., Kohn, L. D. and Winand, R. J. (1975) J. Biol. Chem. 250, 6527.
[7] Tate, R. L., Winand, R. J. and Kohn, L. D. (1974) 34, 692.
[8] Tate, R. L., Winand, R. J. and Kohn, L. D. (1976) in: Thyroid Research: Proc. 7th Int. Thyroid Conf.,Boston, MA, June 9–11, 1975 (Robbins, J. and Braverman, L. eds) pp. 57–60, Excerpta Medica, Amsterdam.
[9] Cave, W. T. and Dunn, J. T. (1976) Ann. Intern. Med. 85, 60.
[10] Amir, S. M., Uchimura, H. and Ingbar, S. H. (1977) J. Clin. Endocrinol. Metab. 45, 280.