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Note

Isolation of the rat transferrin receptor by affinity chromatography

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The transferrin receptor (Tf-R), a glycoprotein, consists of two 90-kDa subunits, each of which is capable of binding one molecule of transferrin[1]. It has been found to be present on a variety of eukaryotic cells, and has now been isolated from several species, including chicken[2], man[3–10], mouse[11], rabbit[12–15], rat[16,17] and sheep[18,19]. The first step in Tf-R purification is membrane solubilization, done mostly by Triton X-100, but also by deoxycholate[7], Nonidet P-40[8], or Teric 12A9[13]. Subsequently, the receptor is separated from the lysate by one of the following techniques: (1) simple gel filtration in the presence of labelled transferrin[12,13]; (2) indirect immunoprecipitation by antibodies to transferrin[3,5,20,21] in the presence of a saturating amount of transferrin; (3) direct immunoprecipitation by antibodies to the Tf-R[8,21–24]; (4) immunoaffinochromatography using immobilized antibodies to the Tf-R[7,17] or to transferrin[4] and (5) affinity chromatography using immobilized diferric transferrin[2,14,16] or deoxycholate-coupled poly(L-lysyl)agarose[25].

The disadvantages of approaches 3 and 4 above are that dissociation of the receptor–antibody complex requires either harsh conditions with respect to pH[17,19] or the deployment of a mixture of detergents[7]. The other techniques accomplish dissociation of the transferrin–receptor complex by using excess transferrin[14] or 22% polyethylene glycol[10]; however, subsequent removal of these agents is cumbersome[26] and prolongs the procedure.

In the light of the above, the one-step procedure proposed by Van Driel *et al.*[11] (see Experimental) appeared a major simplification and advance because it takes into account the physiology of transferrin–Tf-R interactions as established by Morgan[27]: the high affinity of Tf-R for diferric transferrin at pH 7.4 and for apotransferrin at pH 5.0, and the diminished affinity of the receptor for apotransferrin at pH 7.4. However, on attempting to apply this procedure to the isolation of the Tf-R from rat reticulocytes, we encountered major problems. Considering the continuing interest in isolating Tf-Rs from new sources, we felt that the cause of our difficulties and their solution may be of wider interest. Therefore they are briefly described below.

EXPERIMENTAL

Washed reticulocytes were obtained from phenylhydrazine-treated rats (strain

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Sprague-Dawley or Wistar) according to a published procedure[28]. They were converted to ghosts by hypo-osmotic shock[29] before solubilization in phosphate-buffered saline (PBS), containing 1% Triton X-100 and aprotinin (Sigma; 15 μg/ml), at a final concentration of 1 mg cell protein/ml. After 1 h on ice under constant stirring, the insoluble material was removed by centrifugation (141 000 *g* for 1 h or 30 877 *g* for 2 h) at 2–4°C. The supernatant, diluted 1 in 5 with PBS, was used for affinity chromatography.

Affinity adsorbents were prepared by coupling[30] rat[31] or human[32] diferric transferrin to Sepharose 4B. Columns, containing *ca.* 10 mg of ligand per gram of wet gel, were operated at 5°C essentially as described by Van Driel *et al.*[11]. Briefly, the solubilized Tf-R was loaded and adsorbed at pH 7.4; then the column was rendered iron-free by washing with 500 ml of 0.1 *M* citrate buffer, pH 5.0, containing 0.2% Triton X-100 and 50 μg/ml of the specific iron chelator, deferoxamine mesylate (Ciba). Various alkaline buffers used to elute the adsorbed Tf-R are given in the Results and discussion. The efficacy of elution was monitored by measuring rat ^{125}I-labelled transferrin-binding activity in the fractions[4]. To do so, samples (0.2 ml) were incubated for 40 min at room temperature with the radioligand in the presence of 1% bovine serum albumin and then placed in an ice bath. Human immunoglobulin G (0.2 ml; 1.5 mg/ml of PBS) was added, followed by 0.2 ml of a saturated solution of ammonium sulphate, pH 7.4. Precipitates were separated 15 min later by filtration through Whatman GF/C microglass filters and two washings (4 ml each) with 40% ammonium sulphate, pH 7.4. A 20-fold excess of human diferric transferrin was used to measure non-specific binding. Filters were counted in a Packard Model 5986 multichannel analyzer.

Residual Tf-R that remained on the affinity columns after different elution schemes was assessed by polyacrylamide gel electrophoresis in sodium dodecyl sulphate as described by Janatova and Gobel[33].

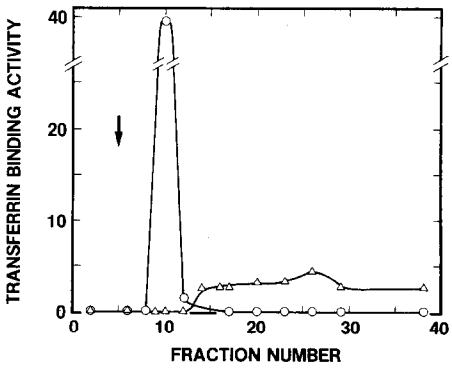

Fig. 1. Elution of the rat transferrin receptor from a column (11 cm × 1 cm I.D.) of rat transferrin-Sepharose by two different eluents. Before elution, the column was exhaustively washed at pH 5.0 to remove iron from transferrin as described in the Experimental section (not shown). Arrow denotes the beginning of specific elution either with 0.5 *M* ammonium carbonate, pH 7.8 (△), or 1 m*M* Tris–HCl, pH 8.0, containing 1 *M* sodium iodide (○). Both buffer systems included 0.2% Triton X-100 and 50 μg/ml of deferoxamine mesylate. Results are expressed as nanograms of ^{125}I-labelled transferrin bound specifically by 50-μl portions of the fractions tested.

RESULTS AND DISCUSSION

The salient observation made in our study was that, contrary to any expectations, recovery of the rat Tf-R from homologous affinity columns was poor when chromatography was based on the unequal affinities of Tf-R for apo- and holotransferrin at pH 7.4. This is evident from the dragging appearance of small quantities of receptor activity on elution with ammonium carbonate as seen in Fig. 1. When human transferrin was used as the affinity adsorbent, yields were even less in that hardly any receptor appeared in the eluate after applying the same buffer (not shown). Examination of the contents of the affinity column by polyacrylamide gel electrophoresis[33] before and after elution showed a substantial quantity of Tf-R still adherent to the

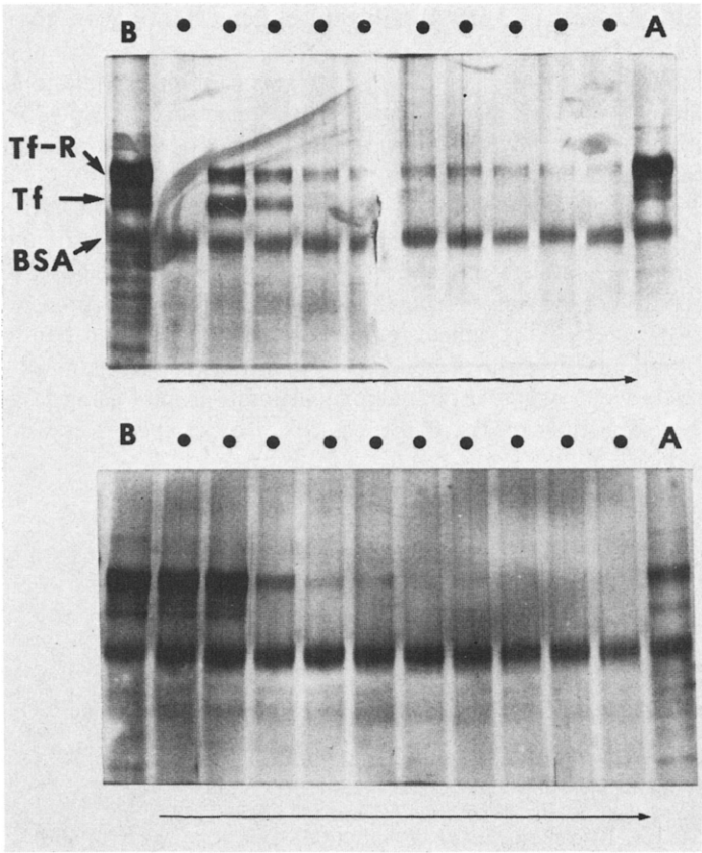

Fig. 2. Evaluation by polyacrylamide slab gel electrophoresis[33] of two methods of eluting the rat Tf-R from a column (11 cm × 1 cm I.D.) of rat transferrin-Sepharose. Elution in the top was by 0.5 *M* ammonium carbonate, pH 7.8, and in the bottom by 1 m*M* Tris–HCl, pH 8.0, containing 1 *M* sodium iodide. Migration from top to bottom. Silver staining[34]. Track B was loaded with a sample of the affinity gel before elution, and track A after elution. Tracks designated by dots in the upper gel were loaded with samples from fractions No. 12, 14, 16, 18, 20, 23, 26, 29, 31 and 33, and in the lower gel from fractions 9, 10, 12, 14, 16, 18, 20, 22, 24 and 26 of the chromatogram in the order as indicated by the arrows. Tf-R, transferrin receptor; Tf, transferrin; BSA, bovine serum albumin. Note the difference between both runs with respect to the Tf-R content of the fractions as well as of tracks A.

adsorbent after attempted elution (Fig. 2, top). On the other hand, elution of Tf-R from the apotransferrin column was greatly promoted by 1 *M* sodium iodide. As seen in Fig. 1, the receptor appeared in the chromatogram as a sharp peak. Complementary to this finding, little residual Tf-R remained electrophoretically detectable in the gel at the end of the run (Fig. 2, bottom).

The discrepancy between these results and those of Van Driel *et al.*[11] is likely explained by intrinsic differences in affinities among transferrins and Tf-Rs of various species. In contradistinction to our homologous system, the above authors worked with human transferrin and murine Tf-R. The realization that marked interspecies differences exist with respect to Tf-R–transferrin interactions, is very recent[31,35–37]. Not surprisingly therefore, the association in a heterologous affinity-chromatographic system may turn out to be either stronger[31] or weaker[35] than in a homologous setting. This may present an advantage, or a disadvantage, depending on the combination selected.

I^-, together with ClO_4^- and SCN^-, belongs to the group of chaotropic ions. These large negative anions, by virtue of their capability of electrostatic shielding and reducing hydrophobic bonding, have been successfully deployed previously for the unfolding of macromolecules and dissociating primary antigen-antibody complexes[38]. The present observation indicates that I^-, at the relatively low concentration of 1 *M*, is highly efficient in disrupting the bonding between rat transferrin and its receptor without causing irreversible denaturation. Potassium thiocyanate has also been used in a similar context[16]. However, the thiocyanate ion binds to proteins considerably more firmly, whereby its complete removal is tedious. This is a handicap, for proteins do resist iodination in the presence of even a trace of thiocyanate[39]. Further studies will have to decide whether chaotropic chromatography using I^- is similarly effective in the case of the analogous proteins from other species as well.

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