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Molecular Characteristics of the Transferrin-Receptor Complex of the Rabbit Reticulocyte

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A macromolecular complex of transferrin and a membrane component was isolated by gel filtration chromatography from Triton X-100-solubilized ghosts of reticulocytes previously incubated with ^{125}I-labeled transferrin. This complex is believed to be transferrin specifically associated with its primary receptor. Following the procedures of Clark [14], the complex in Triton X-100 was found to behave as an asymmetric molecule with a molecular weight of approximately 250,000 and an axial ratio of 9:1. On SDS-polyacrylamide gel electrophoresis the complex displays, in addition to transferrin, components of molecular weights 176,000 and 95,000, respectively. The larger component may be a dimer of the smaller. Each appears to cross-link, with dimethyl suberimidate, to transferrin. These results are compatible with the hypothesis that the transferrin receptor itself has a molecular weight near 175,000 and may be a dimer of two smaller components each of molecular weight near 95,000.

Key words: transferrin, receptor, reticulocytes, cross-link, membrane, detergent

Probably the most important, and certainly the best known, function of transferrin is to provide iron for the biosynthesis of hemoglobin. Indeed, transferrin is the only known effective source of iron for hemoglobin synthesis by reticulocytes [1], and presumably for other immature erythroid cells as well. The initial event in the interaction of transferrin with reticulocytes is the binding of the protein to specific receptors on the cell surface. These transferrin-binding receptors are lost, along with the capacity to synthesize hemoglobin, as the reticulocyte matures into a circulating erythrocyte [2]. They are vulnerable to proteolytic attack, so that cells treated with trypsin or Pronase lose the ability to bind transferrin and remove its iron [3, 4]. Until recently, little more was known about the chemistry of transferrin receptors and the nature of their interaction with the iron-binding protein.

The development of detergent-based methods for solubilizing membrane proteins with preservation of function has led to a resurgence of interest in the transferrin receptors of erythroid cells. A number of groups have reported studies of transferrin-binding

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components of red cell membranes [5–13]. As yet, however, an agreed-upon model of the transferrin receptor, and the chemical nature of the transferrin-receptor interaction, has not emerged. Our own work has been prompted by the recent studies of Clarke in which methods are developed for characterizing the molecular properties of impure membrane proteins in detergent solution [14]. In presenting our data, we will also try to point out explicitly areas of agreement and discord with the work of others.

EXPERIMENTAL PROCEDURES

Materials

Deuterium oxide (99.8%) was obtained from Stohler Isotope Chemicals. Triton X-100 was purchased from New England Nuclear, while dimethyl suberimidate and sodium dodecyl sulfate (Sequanal grade) were products of the Pierce Chemical Co. Carrier-free ^{125}I was supplied by Amersham/Searle. Ultrapure sucrose was from Schwarz/Mann, and Ultrogel AcA22 from LKB Instruments. Pig heart fumarase, porcine thyroglobulin, yeast alcohol dehydrogenase, bovine liver catalase, lactoperoxidase, o-nitrophenyl-β-D-galactopyranoside, L-malic acid, and β-nicotinamide adenine dinucleotide were supplied by the Sigma Chemical Co. Rabbit muscle phosphorylase a and E coli β-galactosidase were from the Worthington Biochemical Corp. Other chemicals were analytical grade, or the highest-quality commercially obtainable. Doubly distilled water was used for all experiments and preparations.

Preparation of ^{125}I-labeled Transferrin and the Putative Transferrin-Receptor Complex

Transferrin was isolated from rabbit serum by the method of Baker, Shaw, and Morgan [15] and radioiodinated to high specific activity using sepharose-bound lactoperoxidase as described by David and Reisfeld [16]. Reticulocytosis of 15–25% was induced in rabbits by repeated bleeding. Ghosts from reticulocytes previously incubated with ^{125}I-labeled transferrin were prepared by the method of Dodge, Mitchell, and Hanahan [17] and extracted with Triton X-100 according to the procedure of Leibman and Aisen [13]. A complex of transferrin and a membrane constituent, thought to be the primary transferrin receptor of the reticulocyte, was purified from such extracts by gel filtration chromatography on a 1.6 × 81 cm column of Ultrogel AcA22. The column was equilibrated with 0.2% Triton X-100 in pH 7.4, 5 imosm phosphate buffer [17] containing 0.02% sodium azide and eluted with the same buffer. Two peaks of radioactivity were observed in the chromatograms. The slower-moving peak eluted at the position of free transferrin, and it presumably represents uncomplexed protein released from its association with the cell membranes. The faster peak, accounting for about 60% of the radioactivity supplied to the column, moved with ferritin (MW ∿440,000; Stokes radius, 61 Å) and is considered to represent the complex of transferrin and its receptor. This peak was proportionately greater in columns run at 4°C than in columns at 18°, in keeping with the tendency of transferrin to remain associated with its receptor at low temperature [10]. For subsequent studies, fractions totaling about 6 ml in volume were taken from the crest of the faster-moving peak.

Sucrose Gradient Ultracentrifugation

The procedures of Clarke [14] were followed to obtain molecular parameters of the transferrin-receptor complex in detergent. Sucrose gradients 5–20% (w/v) in 5 imosm sodium phosphate and 0.1% Triton X-100, were prepared in H_2O and D_2O in cellulose nitrate tubes (0.5 X 2 inches, Beckman) and stored over night at 5°. Samples (100 μl) of the purified, concentrated transferrin-receptor were carefully layered on top of each gradient and centrifuged in the SW65 rotor of a Beckman L5-65 preparative ultracentrifuge at a speed of 34,000 rpm at 5°. A running time of 17 h was used for gradients in H_2O, and 26 h for gradients in D_2O. After the run, five-drop fractions were collected from the bottom of each tube for radioactivity counting, with aliquots taken for enzymatic assay of the marker proteins in 0.1 M sodium phosphate buffer, pH 7.6, at room temperature (following the Worthington Enzyme Manual) and for determination of density from refractive index measurements in a Zeiss Abbe refractometer.

Partial Specific Volume and Sedimentation Coefficient of the Transferrin-Receptor Complex

Again, the experimental and computational procedures developed by Clarke provided the model for our studies [14]. Assuming that the ^{125}I-transferrin-receptor complex binds the same amount of Triton X-100 in H_2O and in D_2O, its partial specific volume can be obtained from the measured sedimentation coefficients (s), densities (ρ), and viscosities (η) of the complex in the sucrose gradient at the position of half-distance of travel (r_{avg}), Equation 1:

$$\bar{v}_H = \frac{\dfrac{S_H \eta_H}{S_D \eta_D} - 1}{\rho_D \dfrac{S_H \eta_H}{S_D \eta_D} - \rho_H} \tag{1}$$

Here, subscripts H and D refer to quantities measured in H_2O and D_2O respectively. Sedimentation coefficients at the position r_{avg} were calculated from Equation 2:

$$S_{r_{avg}} = [(r - r_0)/t]/\omega^2 r_{avg} \tag{2}$$

where r_0 is the distance of the applied sample from the center of rotation, r is the distance of the peak of interest at time t, and ω is the angular velocity of the rotor. Since the density of the medium is a linear function of sucrose concentration, $\rho_{r_{avg}}$ may be determined from the measured refractive index of the fractions collected after ultracentrifugation and a reference plot of the densitites of standard solutions versus their refractive indices. The viscosity of the medium at the position r_{avg} of the transferrin-receptor complex was obtained from interpolation of the values for the marker protein calculated from Equation 3:

$$\frac{\eta_{r_{avg}}}{\eta_{20,w}} = \frac{s_{20,w}(1 - \bar{v}\rho_{r_{avg}})}{s_{r_{avg}}(1 - \bar{v}\rho_{20,w})} \tag{3}$$

Values of $s_{20,w}$ (Svedberg units) and $\bar{v}$ (cm^3/gm), respectively, for the marker proteins used in these calculations are: β galactosidase, 16.0 and 0.73 [18]; catalase, 11.4 and 0.73 [19]; fumarase, 9.09 and 0.738 [20]; alcohol dehydrogenase, 7.40 and 0.73 [21]; and transferrin, 4.97 and 0.725 [22, 23].

Once $\bar{v}$ is calculated from Eq (1), $s_{20,w}$ of the transferrin-receptor complex can be obtained from Equation 4:

$$s_{20,w} = s_H(\eta_H/\eta_{20,w})(1-\bar{v}_H\,\rho_{20,w})/(1-\bar{v}_H\,\rho_H) \qquad (4)$$

The peak of interest in these studies is taken to be the faster-moving, ^{125}I-bearing peak of Figure 1.

The Stokes Radius of the Transferrin-Receptor Complex

The Stokes radius of the transferrin-receptor complex in Triton X-100 was determined by gel filtration on a column of Ultragel AcA22 equilibrated with 5 imosm sodium phosphate buffer (pH 7.4) containing 0.1% of the detergent, 10% sucrose (the concentration at the position of the transferrin-receptor complex in the sucrose gradient centrifugation study), and 0.02% sodium azide. The column was run and calibrated with standard proteins at 18° (Fig 2). The buffer used for equilibration was also used for elution. For better accuracy, elution positions were measured by weight rather than volume [24]. Void weight was estimated with blue dextran, and included weight with DNP-alanine. Results were expressed as $erf^{-1}(1-K_d)$, of which the Stokes radius is generally a linear function:

$$R_s = A + B\,erf^{-1}(1-K_d) \qquad (5)$$

where A and B are empirical constants for a given chromatographic system [24].

Cross-Linking of the Transferrin-Receptor Complex

Reaction conditions were similar to those of Wang and Richards [25]. The purified complex of ^{125}I-labeled transferrin and its receptor was concentrated about five times by ultrafiltration with the Schleicher and Schuell ultrafiltration apparatus, and made 25 mM in sodium phosphate buffer (pH 8.0). Dimethyl suberimidate was added to a concentration of 3 mg/ml. The reaction mixture was kept at 0° for 20 min, and at room temperature for 30 min. The cross-linking reaction was then stopped by addition of ammonium acetate to a final concentration of 0.2 M, with stirring for 10 min. After removing Triton X-100 with Bio-Beads SM-2 [13], the preparation was taken for electrophoresis in SDS polyacrylamide gel.

SDS Polyacrylamide Gel Electrophoresis

The methods of Fairbanks, Steck, and Wallach [26] were followed, with minor modifications. Gels measured 0.5 × 11 cm, and contained 4% polyacrylamide. Samples were in electrophoresis sample buffer containing 2% SDS, with or without 3% β-mercaptoethanol, and were incubated at 37°C for 2 h before electrophoresis. Gels were calibrated for estimations of molecular weight with thyroglobulin (subunit MW 335,000),

β-galactosidase (130,000), phosphorylase a (92,000), transferrin (77,000), catalase (57,000), and ovalbumin (43,000). Gels containing ^{125}I-labeled transferrin, or its complex with the transferrin receptor, were cut into 3.0-mm slices and counted for ^{125}I activity in a Searle model 1195 automatic gamma counter.

RESULTS

Molecular Size and Shape of the Transferrin-Receptor Complex

Results of sucrose gradient ultracentrifugation studies are shown in Figure 1 and Table I. Water-soluble proteins, which in general do not bind Triton X-100 [14], were used as markers to calibrate the gradients. The peaks of greater density are taken to represent the transferrin-receptor complex, while the peaks of lower density correspond to free transferrin, presumably dissociated from the receptor during the course of experimental manipulation.

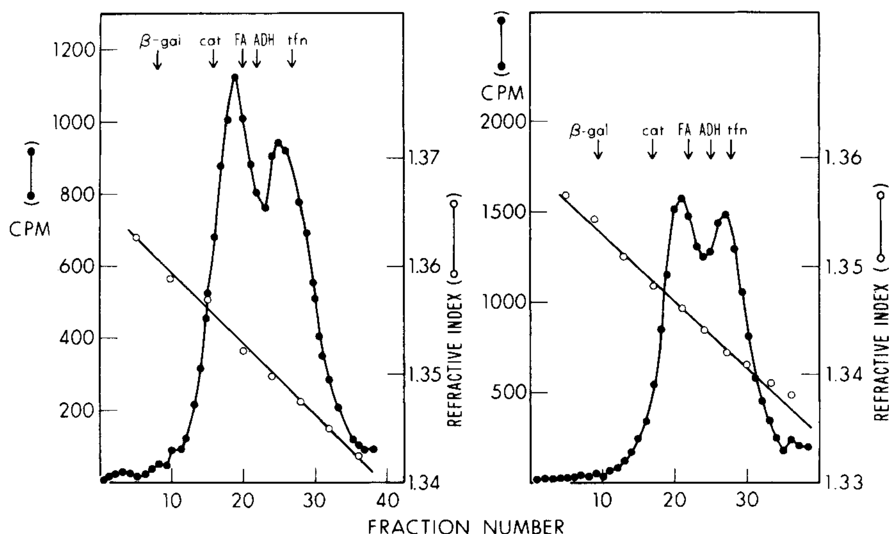

Fig 1. Ultracentrifugation profile of transferrin-receptor complex of rabbit reticulocytes in 5–20% (w/v) sucrose gradient containing 5 imosm sodium phosphate and 0.1% Triton X-100 (pH 7.4) in H_2O (left) and D_2O (right) at 5°. β-gal) β-galactosidase, cat) catalase, FA) fumarase, ADH) alcohol dehydrogenase, tfn) transferrin.

TABLE I. Parameters Obtained for Calculation of the Partial Specific Volume of the Transferrin-Receptor Complex by Sucrose Gradient Ultracentrifugation in 0.1% Triton X-100

Parameter	H_2O	D_2O
Sedimentation coefficient, s (S)	5.062	3.037
Density, ρ (g/cm^3)	1.035	1.135
Viscosity, η (cP)	1.692	1.953

The transferrin-receptor complex migrated to a position between catalase and fumarase, the relative position of the complex with respect to these markers being virtually the same in H_2O as in D_2O. Thus, the partial specific volume of the complex must be similar to those of the marker enzymes. The sedimentation coefficients, densities, and viscosities for the transferrin-receptor complex at the half-distance of travel in H_2O and D_2O, calculated from the ultracentrifugation data as indicated under Methods, are shown in Table I. The partial specific volume, $\bar{v}$, was then obtained from these calculated values according to Equation 1, assuming that the complex binds Triton X-100 equally in H_2O and D_2O. With $\bar{v}$ known, the sedimentation coefficient $s_{20,w}$, shown in Table II, is obtained.

In order to estimate the molecular weight of the transferrin-receptor complex from the Svedberg equation

$$M = \frac{6\pi\eta_{20,w} R_s N S_{20,w}}{1-\bar{v}\,\rho_{20,w}} \tag{6}$$

the Stokes radius of the complex must be known. This was obtained (Table II) by gel filtration using an Ultrogel AcA22 column calibrated with soluble protein standards. Since high concentrations of sucrose may change the amount of Triton X-100 bound to proteins or their state of aggregation [27], these gel filtration studies were carried out in the same buffer used for ultracentrifugation, made 10% (w/v) in sucrose, the approximate concentration at the position of the transferrin-receptor peak after ultracentrifugation. The elution profile of the column is shown in Figure 2, and its calibration plot in Figure 3. The elution weight of the transferrin receptor complex remains very close to that of apoferritin, the same result obtained in earlier studies when the column buffer contained no sucrose [13]. It seems likely, therefore, that the perturbing effects of sucrose on the complex, if any, are minimal, and its Stokes radius is therefore taken as that of apoferritin – 61 Å as calculated from the hydrodynamic data in Crichton [28]. The molecular weight of the complex, as given in Table II, may then be calculated from Equation 2. Two other physical parameters of interest, the frictional ratio f/f_0 and the diffusion coefficient $D_{20,w}$, are also shown in Table II. These were obtained from knowledge of its Strokes radius, partial specific volume and molecular weight by the following relationships:

$$D_{20,w} = \frac{kT}{6\pi\eta_{20,w} R_s} \tag{7}$$

$$f/f_0 = \frac{R_s}{(3\bar{v}M/4\pi N)^{1/3}} \tag{8}$$

where k is the Boltzmann constant and T the absolute temperature. The high frictional ratio of the transferrin-receptor complex, 1.46, suggests that it is an asymmetric molecule

TABLE II. Molecular Characteristics of Putative Transferrin-Receptor Complex of Rabbit Reticulocyte in Triton X-100

Physical parameter	Transferrin-receptor complex	Receptor
Partial specific volume, $\bar{v}$ (cm^3/g)	0.735	0.739
Sedimentation coefficient, $s_{20,w}$ (S)	9.49	
Stokes radius, R_S (Å)	61	
Molecular weight, M	250,000	175,000
Frictional ratio, f/f_0	1.46	
Diffusion coefficient, $D_{20,w}$ (cm^2/s)	3.50×10^{-7}	

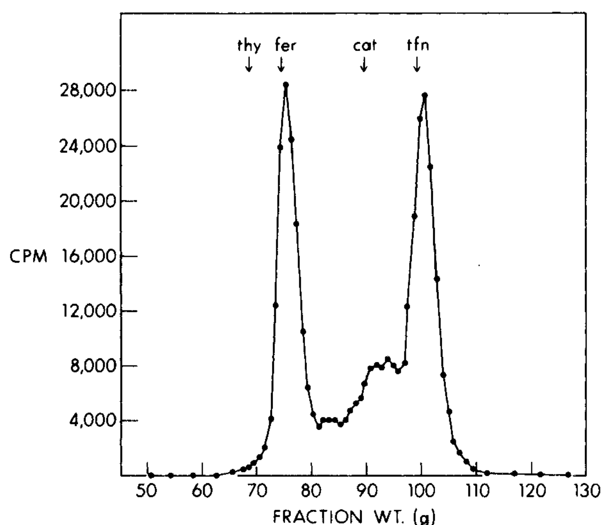

Fig 2. Gel filtration chromatogram of Triton X-100-solubilized rabbit reticulocyte membranes prepared from cells incubated with ^{125}I-labeled rabbit transferrin on Ultrogel AcA22 (1.6 × 81 cm) column. The column was equilibrated and eluted with 5 imosm sodium phosphate buffer (pH 7.4) containing 0.1% (v/v) Triton X-100 and 10% (w/v) sucrose at 18°; thy) thyroglobin, fer) apoferritin, cat) catalase, tfn) transferrin.

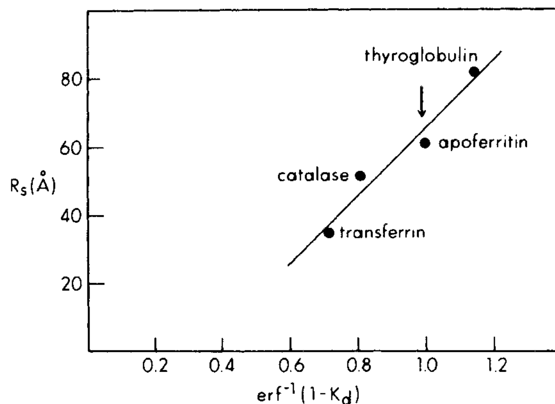

Fig 3. Calibration plot for determination of the Stokes radius of the transferrin receptor complex by gel filtration on Ultrogel AcA22. The arrow indicates the position of the transferrin-receptor complex.

deviating substantially from globular shape. Assuming no solvation by H_2O, the axial ratio of an ellipsoid of revolution approximating the shape of the complex is about 9, as obtained from Perrin plots [29]. If one molecule of receptor is taken to bind one molecule of transferrin, the partial specific volume and molecular weight of the receptor itself in Triton X-100 can be deduced, and these too are shown in Table II.

Cross-Linking of the ^{125}I-Transferrin-Receptor Complex With Dimethyl Suberimidate

In previous work, three prominent components were identified by SDS-gel electrophoresis in dithiothreitol of the transferrin-receptor complex: a band of molecular weight 77,000, corresponding to free transferrin; another at molecular weight 95,000; and a third at molecular weight 176,000 [13]. To determine which, if any, of these are directly complexed to transferrin, ^{125}I-transferrin-receptor complexes, purified by gel filtration, were treated with the cross-linking reagent dimethyl suberimidate and then subjected to SDS-gel electrophoresis. Three radioactive components were found (Fig 4). In the absence of the disulfide-disrupting reagent β-mercaptoethanol, the apparent molecular weight of these were 60,000, 128,000, and 260,000. Incubation with β-mercaptoethanol before electrophoresis shifted the apparent molecular weight of these components to 76,000, 180,000, and 280,000 respectively. In a control study, free transferrin at concentrations of 0.2–0.5 mg/ml (much greater than that in transferrin-receptor complex) was treated with dimethyl suberimidate and in each case gave only one radioactive band on SDS gel. This had an apparent molecular weight of 60,000 in the absence of β-mercaptoethanol, and 77,000 when preincubated with the sulfhydryl reagent. We believe, therefore, that the three radioactive bands observed in dimethyl suberimidate-treated transferrin-receptor complexes correspond to free transferrin, transferrin linked to the 95,000 mol wt component, and possibly transferrin linked to the 176,000 mol wt component. In one experiment, the relative amount of the 176,000 mol wt component in the

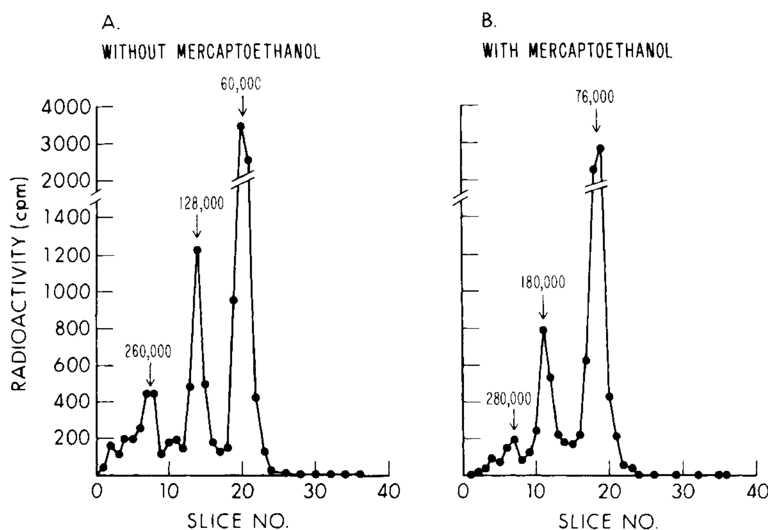

Fig 4. Radioactivity patterns of sodium dodecyl sulfate gel electrophoresis of transferrin-receptor complex purified by gel filtration and treated with dimethyl suberimidate. A) Without β-mercaptoethanol; B) with β-mercaptoethanol in sample buffer.

transferrin-receptor complex increased on prolonged preincubation without β-mercaptoethanol, while the 95,000 mol wt component became hardly detectable, perhaps suggesting that the smaller component may dimerize to form the larger.

DISCUSSION

The Existence of Surface Receptors for Transferrin on Erythroid Cells

By now, the existence of specific transferrin-binding receptors on the membranes of developing, hemoglobin-synthesizing erythroid cells is beyond question. These receptors are specific for transferrin [10]; they are vulnerable to attack with proteases [3], and hence a) they are located at the external surface of the cell membrane and b) they have protein moieties as essential components, and they have been identified on circulating reticulocytes, on erythroid cells of bone marrow, and on Friend erythroleukemic cells [2, 30, 31]. Evidence has also been obtained for similar transferrin-receptors on the surface of placental cells [32]. Since the first stage in the interaction of transferrin with erythroid cells to provide iron for the biosynthesis of hemoglobin entails specific binding of the protein to its receptor, the fundamental importance of the transferrin receptor in the physiology of iron metabolism is clear.

Because of its accessibility in the peripheral circulation the reticulocyte, induced by bleeding or by injection of acetyl phenylhydrazine, is often taken as a model of the hemoglobin-synthesizing, iron-requiring cell. Transferrin-specific receptors of the reticulocyte are saturable so that their behavior can be described by the Michaelis-Menten formalism commonly applied to enzymic reactions. By such analyses, the number of binding sites for homologous transferrin on the reticulocyte membrane has been variously estimated as 50,000 for the human cell, and from 26,000 to 560,000 for the rabbit cell [2, 33, 34]. The reasons for this variability are not clear, although recently it has been suggested that artifactual binding of transferrin to plastic ware may be at fault [35]. The avidity of red cell precursors for transferrin also varies with their stage of maturation, earlier cells binding more protein [36]. The means by which reticulocytosis is brought about is another factor affecting transferrin binding [37]. In our laboratory we generally find that reticulocytes induced by bleeding bind, at saturation, about 125,000 transferrin molecules per cell, and we take this to be the number of available receptor sites.

As the reticulocyte develops into a mature erythrocyte, functioning surface receptors for transferrin disappear [2] and transferrin-binding activity is no longer demonstrable in detergent-solubilized membranes [13]. The fate of the receptor during maturation is unknown, although it has been suggested that an alteration in its carbohydrate content may be associated with its loss of function [13].

Transferrin-Binding Components of Erythroid Cell Membranes

Probably the first to utilize detergents to solubilize membranes of reticulocytes incubated with labeled transferrin in a search for the transferrin receptor were Garrett, Garrett, and Archdeacon [5]. Using Triton X-100, deoxycholate, and SDS, these investigators found by gel filtratron chromatography a macromolecular complex of transferrin and a membrane component(s) which may have included the primary transferrin receptor. Pursuing this approach with Triton X-100, the gentlest and most effective detergent used by Garrett and associates for solubilizing transferrin-bearing com-

plexes, Speyer and Fielding identified a transferrin-bearing component from membranes of human reticulocyte incubated with labeled transferrin [6]. This fraction had an apparent molecular sizeof 230,000 by gel chromatography and was thought to represent the transferrin-receptor complex. An obligatory step in the Speyer-Fielding isolation entailed passage of the detergent-solubilized reticulocyte membranes through a column of Sepharose 2B, a procedure not found necessary by most other investigators [10]. However, the findings of Speyer and Fielding were substantially confirmed by van der Heul, Kroos, and van Eijk [38], using rat reticulocytes.

In general, remarkably little agreement has prevailed among the several groups attempting to characterize the transferrin receptor of erythroid cells. Sly, Grohlich, and Bezkorovainy found a 200,000 mol wt complex of transferrin and a membrane component from rabbit reticulocytes induced by injection of acetyl phenylhydrazine [8]. On SDS-urea gel electrophoresis this complex yielded bands of molecular weight 120,000, 77,000 (free transferrin), and 60,000, and showed the presence of carbohydrate not accounted for by the transferrin. Witt and Woodworth [7], in contrast, found a 35,000 mol wt protein in chick embryo red cells, capable of binding to photoaffinity-labeled ovotransferrin. Using affinity chromatography, Light [12] identified, in Triton X-100 solubilized rabbit reticulocyte ghosts, a 35,000 mol wt protein which formed a dimer capable of associating with transferrin, and argued that this represented the basic unit of the transferrin receptor [12]. Ecarot-Charrier et al, taking a different tack, used antibodies to rabbit transferrin to precepitate the apparent transferrin-receptor complex from solubilized rabbit reticulocyte membranes [39]. The nontransferrin component of this complex migrated electrophoretically as a single peak, but no estimate of its molecular weight was published. Finally, Morgan's group identified a macromolecular complex of transferrin and a component of the rabbit reticulocyte membrane by gel chromatography using the detergent Teric 12A9 (polyethoxylated lauryl alcohol) [10]. This component was shown to be present in reticulocytes and not in mature erythrocytes, and it bound transferrin in reversible and saturable manner. The complex migrated on column chromatography with an apparent molecular weight of 350,000, so that the receptor itself appeared to be a protein of molecular weight near 275,000.

Some Molecular Characteristics of the Putative Transferrin-Receptor Complex

Because of these inconsistencies in the reported properties of these transferrin-associating membrane components we have undertaken to characterize the transferrin-receptor complex by the more rigorous approaches of Clarke [14]. Our starting material was a complex of transferrin and a component of the rabbit reticulocyte membrane, isolated by Ultrogel AcA22 chromatography in Triton X-100 [13]. Over 60% of the radioactivity of ^{125}I-labeled transferrin bound to reticulocyte ghosts is recoverable in this complex, in which transferrin is associated in a reversible and saturable manner. We believe it likely, therefore, that we are dealing with the specific complex of transferrin and its membrane receptor.

The molecular parameters of this complex, in Triton X-100, are presented in Table II. Perhaps of greatest interest, its molecular weight is 250,000, or, subtracting the contribution of transferrin and assuming a 1:1 stoichiometry of receptor binding to transferrin, 175,000 for the receptor itself. The hydrodynamic or Stokes radius of the complex, has a value measured by gel filtration chromatography (Fig 2) close to that of ferritin, a hollow spherical molecule 61 Å in radius and of about 440,000 mol wt. Since this is probably a

lower limit of the true Stokes radius [40], it is evident that the transferrin-receptor complex departs greatly from spherical symmetry. Assuming that water of hydration makes a negligible contribution to hydrodynamic behavior, which seems probable for a complex largely composed of a membrane protein, the axial ratio for a hydrodynamically equivalent prolate ellipsoid of revolution is about 9:1 [29]. Such an ellipsoid would be 40 Å × 360 Å, so that it could readily span the cell membrane. The asymmetric nature of the transferrin-receptor complex may also help account for the apparent discrepancies in its molecular size reported by various groups, since small variations in methods used for solubilization might induce appreciable changes in hydrodynamic properties.

As much as 6% of the total membrane protein of a reticulocyte bearing 125,000 transferrin receptors of 175,000 mol wt could, from the estimate of total membrane protein in the erythrocyte ghost given by Fairbanks, Steck, and Wallach [26], consist of the specific transferrin receptor.

The partial specific volume of the transferrin-receptor complex, 0.735 cm^3/g, falls in the range of most proteins. Since the partial specific volume of Triton X-100 is 0.91 cm^3/g, it appears that, once solubilized, the complex binds very little detergent. Attempts to verify this experimentally were unsuccessful, as it was not possible to attain concentrations of the complex sufficiently high to measure reliably its ability to bind detergent. It seems likely, however, that the transferrin receptor falls into the class of peripheral or extrinsic membrane proteins [41] which bind only small amounts of detergent, and in monomeric rather than micellar form [42].

Subunit Structure of the Transferrin Receptor

In earlier SDS gel electrophoresis studies of the transferrin-receptor complex, three major protein components were identified: a band of 176,000 mol wt, another of 95,000 mol wt, and a band corresponding to free transferrin. The experiments with dimethyl suberimidate reported in this paper provide evidence that the 95,000 mol wt component is in sufficiently close proximity to transferrin to be cross-linked to it. Our results are not sufficiently compelling, however, to indicate whether the same is true of the 176,000 mol wt component. Since the molecular weight of the transferrin receptor itself is about 175,000, it is unlikely that the receptor is composed of two different subunits of 95,000 and 176,000 mol wt respectively. A more attractive possibility is that the heavier component is a dimer of the smaller, incompletely dissociated by the procedure for SDS-gel electrophoresis, and that the functional receptor consists of this dimer. It should again be noted that both the 95,000 and 176,000 mol wt components can be identified in membrane fractions of mature erythrocytes which no longer bind transferrin, and no longer cross-link to it [11]. We have previously suggested that the loss of transferrin-binding activity may be due to alteration in the carbohydrate content of these components [13], but definitive evidence for this is still not available.

Our results are not in complete accord with those reported by Nunez et al in studies of transferrin cross-linked to intact reticulocytes [11]. These investigators found a 125,000 mol wt complex of transferrin and a membrane component in the absence of mercaptoethanol, migrating with an apparent molecular weight of 140,000 in its presence. In our studies, in which the apparent molecular weight of a cross-linked product increased from 128,000 to 180,000 after reduction, the treatment with sulfhydryl reagent was carried out for 2 h rather than 20 min, perhaps accounting for some of the discrepancy. We have also chosen to work with purified transferrin-receptor complexes rather than whole cells in

the belief that nonspecific cross-linking would be minimized. It is possible, however, that a cross-linking component is present in intact membranes which we fail to detect in purified complexes.

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