Serum Transferrin Receptor Is a Truncated Form of Tissue Receptor*

Yuan J. Shih, Roy D. Baynes, Billy G. Hudson, Carol H. Flowers, Barry S. Skikne, and James D. Cook†

From the Departments of Medicine and Biochemistry, Kansas University Medical Center, Kansas City, Kansas 66100

Recent studies have provided immunological evidence for the existence of transferrin receptor in human serum and have revealed that its concentration is a sensitive measure of erythropoiesis and iron deficiency. The present study was undertaken to establish the molecular identity of this immunoreactive component. Purification from human serum was accomplished by immunoaffinity chromatography using, as the ligand, monoclonal antitransferrin receptor antibody. The receptor preparation contained two major components with $M_r$ of 75,000 and 85,000, which were identified as transferrin and transferrin receptor, respectively. The physicochemical and immunochemical properties of the 85,000 serum receptor were compared with those established for intact placental transferrin receptor. The serum receptor exhibited an apparent $M_r$ of 85,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under non-reducing conditions, as compared with 190,000 for placental transferrin receptor. Upon reduction, the $M_r$ of serum receptor was unaltered, whereas, the 190,000 placental receptor dimer decreased to the expected monomer value of 95,000. Amino-terminal amino acid sequence analysis revealed that residues 1–19 of serum receptor were identical to residues 101–119 of intact receptor. These findings provide physicochemical evidence for the existence of transferrin receptor in human serum, establish its molecular identity as a truncated form lacking the cytoplasmic and transmembrane domains (residues 1–100) of intact receptor, and demonstrate that it exists as a transferrin-receptor complex in serum.

The process by which cells acquire iron for growth and synthesis of iron-containing proteins has been defined extensively in recent years (1-3). Iron uptake is mediated by a specific receptor for transferrin, the transport protein for iron. Diferric transferrin binds to its receptor at the cell surface and is then rapidly internalized in nonlysosomal vesicles. The acidic environment in these compartments causes the iron to dissociate while transferrin remains bound to its receptor. Diferric transferrin binds to its receptor at the cell surface where the apotransferrin rapidly dissociates while transferrin remains bound to its receptor. Upon reduction, the receptor preparation contained two major components with $M_r$ of 75,000 and 85,000, which were identified as transferrin and transferrin receptor, respectively. The physicochemical and immunochemical properties of the 85,000 serum receptor were compared with those established for intact placental transferrin receptor. The serum receptor exhibited an apparent $M_r$ of 85,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under non-reducing conditions, as compared with 190,000 for placental transferrin receptor. Upon reduction, the $M_r$ of serum receptor was unaltered, whereas, the 190,000 placental receptor dimer decreased to the expected monomer value of 95,000. Amino-terminal amino acid sequence analysis revealed that residues 1–19 of serum receptor were identical to residues 101–119 of intact receptor. These findings provide physicochemical evidence for the existence of transferrin receptor in human serum, establish its molecular identity as a truncated form lacking the cytoplasmic and transmembrane domains (residues 1–100) of intact receptor, and demonstrate that it exists as a transferrin-receptor complex in serum.

The process by which cells acquire iron for growth and synthesis of iron-containing proteins has been defined extensively in recent years (1-3). Iron uptake is mediated by a specific receptor for transferrin, the transport protein for iron. Diferric transferrin binds to its receptor at the cell surface and is then rapidly internalized in nonlysosomal vesicles. The acidic environment in these compartments causes the iron to dissociate while transferrin remains bound to its receptor. The complex of transferrin and receptor is promptly returned to the cell surface where the apotransferrin rapidly dissociates at a neutral pH. Transferrin receptor has been purified, cloned, and its primary structure determined from cDNA analysis (4, 5). The receptor is a transmembrane glycoprotein composed of two disulfide-linked monomers. Each polypeptide subunit contains 760 amino acids which are divided into the following three major portions: a large C-terminal extracellular domain of 671 amino acids, a transmembrane domain of 28 amino acids, and an N-terminal cytoplasmic domain of 61 amino acids. Transferrin receptor is expressed on all dividing cells, and its rate of synthesis is closely linked to the requirements of the cell for iron.

Recently, evidence was obtained for the existence of transferrin receptor in human and rat sera (6-9). The evidence was based on the immunological reactivity of serum with monoclonal or polyclonal antibodies that were directed against intact transferrin receptor. The concentration of this serum receptor appears to be related to tissue receptor mass and thus provides a clinical measure of erythropoiesis or iron deficiency (7-11). Potentially, this serum receptor may provide important clues about the overall metabolism of transferrin receptor, and its concentration will assume importance in the detection and management of patients with hematological disease (12).

The molecular identity of human serum transferrin receptor is unknown. On the basis of SDS-PAGE analyses, Kohgo et al. (6) concluded that the serum receptor (non-reduced) is a polypeptide of $M_r = 80,000-100,000$, a value which is compatible with a monomeric form of the membrane-bound transferrin receptor. Based on a subsequent study of the electrophoretic behavior of the reduced receptor, these workers suggested that it represents nicked dimers of intact receptor (7). Huebers et al. (9) concluded that the serum receptor is an intact transferrin receptor based on the behavior of the receptor-transferrin complex on gel filtration chromatography. At present, no definitive information is available regarding the chemical nature of this serum transferrin receptor or its structural relationship with the membrane-bound transferrin receptor.

The present study was undertaken to establish the molecular identity of this putative transferrin receptor in serum. Our findings provide physicochemical evidence for the existence of transferrin receptor in human serum, establish its molecular identity as a truncated form lacking the cytoplasmic and transmembrane domains (residues 1–100) of intact receptor, and demonstrate that it exists as a transferrin-receptor complex in serum.

EXPERIMENTAL PROCEDURES

Materials—Serum and plasma were obtained from normal subjects and patients with iron deficiency or sickle cell anemia. Plasma samples were prepared from blood collected into tubes containing heparin. Diethanoldamine, Tween 20, Tween 80, Tris, diisopropyl fluorophosphate, phenylmethylsulfonyl fluoride, sodium azide, 4-

* This work was supported by National Institutes of Health Grant DK38246 and AID Cooperative Agreement DAN-5115-A-00-7908-00. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom reprint requests should be addressed: Division of Hematology, Kansas University Medical Center, 39th & Rainbow, Kansas City, KS 66103.

The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunoassay.
chloro-l-naphthol, concanavalin A, peroxidase, cyanogen bromide-activated Sepharose 4B, and transferrin were obtained from Sigma.

Monoclonal antibodies against transferrin receptor were developed against soluble transferrin-saturated receptor purified from human placenta as described previously (11). The following two monoclonal antibodies were employed: A4A6 that is used in the enzyme-linked immunosorbent assay (ELISA) and D3A12 that is equally immunoreactive in this assay. The antibodies were conjugated to horseradish peroxidase as described previously (13). Monoclonal antibodies against human transferrin were prepared as previously described (14).

Preparation of Human Transferrin Receptor-Human transferrin receptor was isolated from sera by affinity chromatography using, as the ligand, monoclonal antibodies against transferrin receptor. Human serum was prepared for chromatography by diluting it with an equal volume of equilibrium buffer containing phosphate-buffered saline, pH 7.2, 0.5% Tween 80, 0.5 mM diisopropyl fluorophosphate or 0.5 mM phenylmethylsulfonyl fluoride and 0.01% sodium azide. The diluted serum was centrifuged at 10,000 X g for 20 min at 4 °C and the pellet discarded.

Monoclonal antibody to transferrin receptor was coupled to cyanogen bromide-activated Sepharose 4B according to the manufacturer's instructions. Serum was mixed with the derivitized Sepharose 4B resin at an approximate ratio of 1.5 parts antibody to 1 part resin. The mixture was placed on an end-over-end rotor overnight at 4 °C, poured onto a column, and washed with the same buffer until the A280 approached zero.

Quantitative recovery of the receptor from the affinity column was monitored with an antibody (A4A6) that is specific to the transferrin receptor. Recovery varied when eluted from the affinity column using the elution buffer. Preliminary studies were performed to determine the stability of serum receptor in different buffer systems. Human serum was diluted 1:5 with phosphate-buffered saline, pH 7.2, 0.5% Tween 20 and mixed in a ratio of 1:3 with the test buffer. Three acetic buffers (0.1 M glycine, pH 2.5; 0.1 M acetic acid, pH 2.9; 0.1 M sodium acetate, pH 4.0) gave recoveries of less than 3%. A polarity reducing agent, 10% dioxane, gave a somewhat higher recovery of 7.7%. Recoveries with high salt concentration buffers varied widely from 0% with 5 M KI and 27% with 3 M NaCl, to 61.9% with 2.8 M MgCl2. Recoveries with basic buffers were consistently higher. With 1 M NH4OH, recovery was 69.9% whereas 0.05 M diethanolamine, 0.5% Tween 80, pH 11, resulted in no loss of receptor as measured by the ELISA. Consequently, elution of the receptor from the affinity column was accomplished by incubating the affinity resin in this buffer system for 10 min and then collecting the eluate in a test tube containing 0.5 M Tris, pH 7.5.

For control purposes, intact transferrin receptor was prepared from human placenta using the same affinity purification method. The placental tissue was homogenized and solubilized with 1% non-fat dry milk in phosphate-buffered saline. The latter was then incubated with concanavalin A at a concentration of 10 μg/ml in phosphate-buffered saline containing 10 μM Ca2+, Mg2+, Mn2+, and 0.5% Triton X-100 for 1 h followed by extensive washing in the above buffer. After incubating the membranes with horseradish peroxidase (50 μg/ml), the same buffer, the glycoprotein was detected by adding 4-chloro-1-naphthol as the substrate for the peroxidase.

Detection of Glycoprotein—Glycoprotein was detected using the method of Clegg (17) after transblotting to nitrocellulose membranes as described above. The membrane was first blocked by incubating with 2.5% bovine serum albumin in phosphate-buffered saline, pH 7.2, for 1 h at room temperature. The membrane was then incubated with monoclonal antibody. The latter was conjugated to horseradish peroxidase as described previously. The antibodies were conjugated to horseradish peroxidase as described previously (13). Monoclonal antibodies against human transferrin were prepared as previously described (14).

In the purification of serum transferrin receptor, we observed that repeated elution of the receptor from the affinity column progressively decreased the amount of contaminating transferrin in the eluant. By performing repeated elutions with the same elution buffer, receptor was obtained which was essentially free of transferrin. The receptor was then electrophoresed on a 7.5–20% gradient of polyacrylamide and transblotted onto polyvinylidene difluoride membrane for sequencing according to the procedure of Matsudaira (18). The sequencing was performed at University of California, Los Angeles, Department of Biological Chemistry, UCLA School of Medicine.

RESULTS

Purification of Serum Transferrin Receptor—In preliminary studies, we attempted to purify serum receptor with techniques that are commonly used to isolate intact receptor but these methods were unsuitable. Affinity chromatography using, as the ligand, human transferrin (19) yielded a recovery of only 6.7% and less than 5% of the purified protein was immunoreactive as measured by ELISA. We also tried the combined use of DEAE-Sephadex anion-exchange and gel filtration chromatography (20) which yielded a somewhat higher recovery of 36%. However, less than 1% of the isolated protein was immunoreactive by ELISA. Affinity chromatography using, as the ligand, monoclonal antibody (A4A6 or D3A12) directed against solubilized intact placental receptor was highly satisfactory, yielding recoveries of over 80%. The high recovery indicated that the monoclonal anti-transferrin receptor antibodies extracted virtually all of the immunoreactive transferrin receptor present in the serum. This immunoreactive receptor preparation was used in subsequent studies to determine its molecular identity.

SDS-PAGE Analysis of Serum Transferrin Receptor Preparation—This receptor preparation was analyzed using SDS-PAGE to determine the number, identity, and Mr of protein components (Fig. 1). Under non-reducing conditions, the preparation was found to be composed of two major components with apparent Mr of 75,000 and 85,000, along with a faint diffuse component of Mr = 180,000. The 75,000 component was identified as transferrin, the 85,000 component as serum transferrin receptor and the 180,000 component as contaminating monoclonal antibody, as described below. Under reducing conditions, the Mr of the 85,000 transferrin receptor was unaltered. In contrast, the electrophoretic profile of intact transferrin receptor preparation from placenta, under non-reducing conditions, revealed a 75,000 transferrin component, the expected 190,000 transferrin receptor dimer and a minor 85,000 component. Upon reduction, the 190,000 dimer yielded a monomer component of Mr = 95,000. These
results showed that the $M_r$ of the serum receptor is about 10,000 less than that of the monomeric form of the intact receptor. Moreover, the results suggested that the serum receptor 1) is a single polypeptide chain, because its size is not influenced by reduction of disulfide bonds as is the intact placental transferrin receptor, and 2) exists in serum as a complex with transferrin because transferrin copurifies with it on the immunoaffinity column.

The electrophoretic patterns (non-reduced and reduced) of the serum receptor were unaltered by a variety of conditions. These included 1) the use of different monoclonal antibodies, directed against transferrin receptor, as the ligand for the affinity purification of the serum receptor, 2) the addition of proteolytic inhibitors to serum or whole blood prior to harvesting serum or plasma, or 3) the use of plasma rather than serum. Moreover, ELISA measurements using monoclonal antibody revealed that the recovery of receptor from serum by the immunoaffinity chromatography procedure was consistently over 80%. Thus, the major component of serum that is detected by monoclonal antibodies directed against intact transferrin receptor is the 85,000 component.

![FIG. 4. Glycoprotein detection using concanavalin A and horseradish peroxidase. Serum transferrin receptor was transblotted onto a nitrocellulose membrane after SDS-PAGE. The glycoproteins were detected by sequential incubation with concanavalin A, peroxidase, and 4-chloro-1-naphthol. Lanes 2 and 4 were non-reduced and reduced samples, respectively. Lanes 1 and 3 were molecular weight standards.](image1)

Identification of the Components of the Serum Receptor Preparation—The identity of the two major (75,000 and 85,000) components and the minor (180,000) component of the immunoaffinity purified preparation of serum transferrin receptor was established by Western blotting analysis using antibodies directed against transferrin receptor, transferrin, and rabbit anti-mouse IgG. The 75,000 component comigrated with transferrin (Fig. 2, lanes 3 and 7), purified transferrin, and lanes 4 and 8, monoclonal anti-transferrin receptor antibody. Lanes 1 and 5 contained molecular weight standards. Lanes 2-4 were electrophoresed under non-reducing conditions, and lanes 6-8 under reducing conditions.

![FIG. 5. Resolution of the 85,000 serum receptor from transferrin. The 85,000 receptor was dissociated from the immunoaffinity column with stepwise elution. Elution fraction numbers 2-9 were analyzed by SDS-PAGE under non-reducing conditions. Fraction numbers 7-9 contained receptor which was essentially transferrin-free. Lane 1 was the molecular weight standard.](image2)

Hence, these results established the identity of the 85,000 component as the serum transferrin receptor. The minor components of the preparation, $M_r$ 180,000 (non-reduced) and 52,000 and 25,000 (reduced) corresponded to impurities derived from the monoclonal antitransferrin receptor antibody that was used as the ligand for affinity chromatography as shown in Fig. 2 (compare lane 2 with lane 4 and lane 6 with lane 8). Overall, these results revealed that 1) the component of serum which reacts with monoclonal antitransferrin receptor antibody is an 85,000 protein and 2) that the affinity purified preparation of serum receptor contains about equal amounts of transferrin and transferrin receptor. In addition,
the 85,000 component was shown to be a glycoprotein (Fig. 4).

Sequence Analysis of the 85,000 Serum Transferrin Receptor—The amino-terminal amino acid sequence of the 85,000 serum transferrin receptor was determined to chemically identify the receptor and to investigate its primary structure in relation to the intact transferrin receptor. Serum receptor was resolved from the transferrin component of the receptor preparation by successive elutions of the affinity column (Fig. 5). After seven successive elutions, the 85,000 receptor was essentially transferrin-free. The N-terminal sequence of the serum receptor was compared with that deduced from the cDNA data of the intact transferrin receptor (5). Sequence identity was observed between residues 1–19 of the 85,000 serum transferrin receptor and residues 101–119 of the intact receptor. Xs designate the non-identified amino acid residues.

**DISCUSSION**

The present study provides physicochemical evidence for the existence of transferrin receptor in human plasma and serum and establishes its molecular identity. The size and behavior of the serum receptor on SDS-PAGE, under reducing and non-reducing conditions, contrast sharply with that previously established for intact placental transferrin receptor. The serum receptor exhibits a $M_r$ of 85,000 under non-reducing or reducing conditions. In contrast, placental transferrin receptor exhibits a $M_r$ of 190,000 for the dimer under non-reducing conditions and 95,000 for the monomer upon reduction (4, 21). These results indicate that the serum receptor is a single polypeptide chain and that its $M_r$ is about 10,000 less than that of the monomeric form of intact placental receptor.

The structural relationship between the serum and tissue forms of the receptor was established by amino-terminal amino acid sequence analysis and molecular weight values. Residues 1–19 of the N-terminal sequence of the 85,000 serum receptor were found to be identical to residues 101–119 of intact placental receptor, indicating that residues 1–100 are absent from the serum receptor. The $M_r$ of the serum receptor of 85,000 agrees well with a value of 83,900 calculated from the $M_r$ of the intact receptor monomer minus the first 100 amino acid residues. Glycoprotein analysis indicated that the $M_r$ of the serum receptor included its carbohydrate residues. The lack of an effect of reduction on apparent $M_r$ is also consistent with the absence of cysteine residues at positions 89 and 98 which are involved in intermolecular disulfide bonds (22) in the intact receptor. Thus, these findings establish that serum transferrin receptor lacks the first 100 amino acid residues of intact receptor. The truncation occurs in the extracellular domain between arginine 100 and leucine 101 (Fig. 7). A related fragment has been generated by tryptic digestion in vitro in which the main cleavage site is at residue 121 (19, 23).

The identification of transferrin receptor in human serum by Kohgo and co-workers (6) was prompted by an earlier observation that in vitro culture of sheep erythrocytes is accomplished by the release of vesicles termed exosomes containing transferrin receptor (24). These vesicles have been shown recently to contain a number of plasma membrane activities including acetylcholine esterase, cytochalasin B binding, nucleoside binding, and Na$^+$-independent amino acid transport as well as transferrin receptor (25). These findings differ from ours because the exosomal receptor had the same electrophoretic mobility on SDS-PAGE as intact receptor isolated from reticulocyte membranes.
of 186,000 and 93,000). While the soluble form of the receptor
identified in the current study is clearly distinct from the
intact membrane-bound form in the exosome, the latter may
possibly contribute to the production of the soluble receptor.

A number of other cell surface receptors have been shown
to exist in soluble or truncated form. These include the insulin
receptor (26), HLA antigens (27), the epidermal growth factor
receptor (28), the interleukin-2 receptor (29), and the IgM
immunoglobulin molecule (30). The most extensively studied
of these is the interleukin-2 receptor with findings very similar
to ours with the serum transferrin receptor. The truncation
of the interleukin-2 receptor occurs just distal to the trans-
membrane region of the molecule and involves the Cys-Leu
residues at positions 192 and 193 (31). Several mechanisms
have been proposed to account for these soluble forms. Some
workers have suggested that they are truly secretory forms of
the protein, lacking transmembrane regions and produced by
either multiple genes (27) or alternate mRNA splicing path-
ways (30). It has also been suggested that they arise from
proteolytic cleavage of the intact peptide (31, 32). This latter
mechanism may be operative in the case of transferrin recep-
tor because significant proteolytic activity exists in the low
pH environment or the prelysosomal endocytotic vesicle (33,
34). However, the mechanism of production of soluble trans-
ferrin receptor in human serum remains an important unan-
swered question.

In serum, the truncated receptor exists as a transferrin-
receptor complex. This conclusion is based on the findings
that transferrin co-purified with serum receptor in roughly
equal amounts on the monoclonal antibody affinity column.
The affinity of the truncated form of the receptor for trans-
ferrin has not been studied to date, but it is of interest that
<1% of circulating transferrin is complexed with soluble
receptor. Although transferrin complexed with its receptor
form is likely to impair delivery of iron to cells, the propor-
tion of transferrin complexed in this manner is too small to
influence iron transport significantly. Additional studies on
the kinetics of serum receptor and its binding characteristics
with transferrin are needed.

Acknowledgments—We would like to acknowledge the technical
dvice of Dr. Sripad Gunwar and the assistance of Leslie Kuharich
in the preparation of this manuscript.

REFERENCES
562
12888-12892
267-274
6. Kohgo, Y., Nishitani, N., Kondo, H., Bechtolt, N., Niitsu, Y.,
9. Huebers, H. A., Beguin, Y., Poupart, P., Einspahr, D., and
(1986) J. Biol. Chem. 211, 3368-3372
19. Purkis, A. P., Amatoura, J. E., Borebani, D., Harrison, S. C.,
23. Schneider, C., Sutherland, K., Newman, R., and Greaves, M.
25. Johnstone, R. M., Adam, M., Hammond, J. R., Orr, L., and
189-196
135, 3172-3177
30. Early, P., Rogers, J., Davis, M., Calame, K., Bone, M., Waller, R.,
82, 6172-6175
15317
Serum transferrin receptor is a truncated form of tissue receptor.
Y J Shih, R D Baynes, B G Hudson, C H Flowers, B S Skikne and J D Cook


Access the most updated version of this article at http://www.jbc.org/content/265/31/19077

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/265/31/19077.full.html#ref-list-1