Transferrin Receptor in Human Placental Brush Border Membranes

STUDIES ON THE BINDING OF TRANSFERRIN TO PLACENTAL MEMBRANE VESICLES AND THE IDENTIFICATION OF A PLACENTAL BRUSH BORDER GLYCOPROTEIN WITH HIGH AFFINITY FOR TRANSFERRIN

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Evidence is presented demonstrating the existence of a receptor specific for transferrin on the brush border membrane of the human syncytiotrophoblast. Equilibrium-binding studies were conducted using a placental vesicle preparation to demonstrate the presence of a high affinity, saturable binding site for 125I-ferrotransferrin on placental membranes. The $K_a$ for this binding site was determined by Scatchard analysis to be $3.6 \pm 1.5$ S.D. × 10² M⁻¹. The specificity of the high affinity transferrin binding site was examined by competition studies showing that purified human IgG, human albumin, and ovalbumin did not compete for the binding sites. The protein nature of the binding site was suggested by its sensitivity to trypsinization of the membrane vesicles. Solubilization of the membranes with Triton X-100 did not impair the binding of 125I-ferrotransferrin or the $K_a$ of the soluble receptor. The transferrin binding of the complex was stable at pH 5.0 and reversible at pH 7.4. Using stabilization at pH 5.0, the solubilized membrane-transferrin complex was immunoprecipitated with anti-transferrin antibodies in the presence of saturating concentrations of transferrin using Staphylococcus aureus as an immunoadsorbant. Polyaacrylamide gel electrophoresis of this complex in the presence of sodium dodecyl sulfate demonstrated a single polypeptide of $M_r = 90,000 \pm 5,000$ and $pI = 6.6$ associated with transferrin in the immunoprecipitates of the membrane extracts. These values for the putative receptor were the same as for sialoglycoprotein 15b previously identified in a study characterizing the surface sialoglycoproteins of the term human placenta (Wada, H. G., Gornicki, S. Z., and Sussman, H. H. (1977) J. Supramol. Struct. 6, 473-484).

The demonstration of the transferrin receptor in the human placenta is of potential importance for studying the molecular mechanism involved in transplacental iron transport during fetal development.

The growing human fetus consumes as much as 300 mg of iron during the course of gestation (1). Ferrokinetic data from studies in guinea pigs, rabbits, rats, and humans indicate the transferrin-bound iron of the maternal plasma is the source of iron actively transported across the placenta. Once in the fetal circulation, the iron reassociates with fetal transferrin which carries it to the fetal tissues for incorporation primarily into hemoglobin and other iron-containing proteins and for storage in the fetal liver (2-4).

It has been postulated that the first step in placental transport of iron involves binding of maternal ferrotransferrin to placental membrane receptors. This would be similar to the transport of iron into reticulocytes where the initial event is transferrin binding to reticulocyte membrane receptors (5). Evidence for placental binding of transferrin was first obtained from animal studies which demonstrated the concentration and binding of radiolabeled transferrin in placental tissues following intravenous injection into the maternal circulation (5-8). Following incubation with placental tissue, transferrin elutes in a higher molecular weight complex by gel filtration, suggesting an interaction with a tissue receptor (9). Immunohistochemical techniques utilizing peroxidase-labeled antibodies have shown that the transferrin bound to human placental tissue is present on the surface of the syncytiotrophoblast (10). This cell surface is the placental brush border in contact with the maternal circulation and is the most probable site for the transferrin receptor.

The evidence for transferrin binding to placental tissue and brush border membranes strongly supports the contention that receptors for transferrin exist on these membranes; however, direct evidence for specific transferrin membrane receptors has not yet been provided. In the present investigation, a membrane vesicle preparation was used to study the binding of 125I-radiolabeled transferrin to placental membranes. A saturable, high affinity ($K_a = 3.6 \pm 1.5$ S.D. × 10² M⁻¹) binding site which appears to be specific for transferrin was demonstrated. Furthermore, by detergent solubilization of the transferrin-receptor complex from 125I-radiolabeled placental membrane vesicles and precipitation of the complex with anti-transferrin antibodies, a polypeptide with a molecular weight of 90,000 $M_r = 5,000$ and a $pI = 6.6$ was identified as the putative transferrin receptor. Based on the $M_r$, $pI$, and two-dimensional electrophoretogram pattern, the putative transferrin receptor is identical with a sialoglycoprotein designated 15b in a previous study which characterized the brush border membrane sialoglycoproteins of the term human placenta (11).

EXPERIMENTAL PROCEDURES

Placental Membrane Vesicle Preparation—The membrane vesicles used in the binding studies were prepared from freshly delivered human placentas by the method of Smith (12). This method isolates the fast sedimenting microsomes obtained by isotonic saline (0.9% NaCl solution) extraction of trophoblastic tissue washed free of ma-
ternal blood with 100 mM CaCl₂. These membrane vesicles are produced when brush border microvilli vesiculate and separate from the basol plasma membrane of the trophoblast forming large, oblong vesicles.

**Assay for Specific Transferrin Binding to Membrane Vesicles—**

Human transferrin (Sigma) was chemically iodinated with [125I] for use in the binding assay. The transferrin was saturated with iron by adding 2 mol of FeCl₃/mole of transferrin in 5 mM NaHCO₃, pH 8.1, buffered and dialyzing against 0.15 M NaCl, 10 mM NaHCO₃, pH 8.1, overnight. The ferrotransferrin, 20 μg, was then iodinated by the chloramine-T method of Mehdi and Nussey (13) using 1 mCi of sodium [125I]iodide (New England Nuclear). The iodinated transferrin was separated from free [125I] and other minor protein contaminants by gel filtration on Sephadex G-100 equilibrated with 0.15 M NaCl, 10 mM NaPO₄, pH 7.4. The peak fractions of transferrin eluted from the column were collected in tubes containing 250 μg of bovine serum albumin (Sigma, radioimmunossay grade) for use in the binding assay.

The standard binding assay contained 200 to 400 μg of membrane protein in a total volume of 0.5 ml of 0.15 M NaCl, 10 mM NaPO₄, pH 7.4, containing 2 mg/ml of ovalbumin (Sigma), 10,000 to 20,000 cpm of labeled transferrin with unlabeled transferrin of the assay concentration. The incubation was conducted at 37°C for 20 min and stopped by chilling on ice. The preparation was washed once with 1.0 ml of 18% (w/v) polyethylene glycol-6000 (Baker, analyzed reagent grade) in 0.15 M NaCl, 0.5% Triton X-100, pH 5.0, prior to elution of the bound immune complexes with sodium dodecyl sulfate polyacrylamide gel electrophoresis sample buffer containing 2% SDS for 10 to 20 min at room temperature. Polyclonal antiserum to Staphylococcus aureus was added to the sample to saturate membrane binding sites, the suspension was chilled on ice, and Triton X-100, pH 5.0, was added to 0.1% SDS (w/v). This mixture was allowed to stand at 4°C overnight. The ferrotransferrin, 20 pg, was then iodinated by the method of Davis (16) with the inclusion of 0.1% SDS (w/v) in the top electrode reservoir, the stacking gel, and 8% acrylamide running gel. Glycitol (10% v/v) was included in the running gel and improved the sharpness of the protein bands. The samples eluted from the S. aureus immunoprecipitate were analyzed in 8% acrylamide gel electrophoresis according to the method described by Kessler (15), utilizing formaldehyde-fixed Staphylococcus aureus to absorb the IgG immune complexes selectively. The reacted S. aureus was washed three times with 50 mM NaPO₄, 0.15 M NaCl, 0.5% Triton X-100, pH 5.0, prior to elution of the bound immune complexes with sodium dodecyl sulfate polyacrylamide gel electrophoresis sample buffer containing 2% SDS for 10 to 20 min at room temperature.

The binding between transferrin and placental membrane vesicles was determined by chromatofocusing with polyethylene glycol-6000. The reaction was conducted at 10°C for 20 min in the presence of 10% β-mercaptoethanol prior to electrophoretic analysis. The gels were fixed and stained with Coomassie blue, destained, and dried down for autoradiography with Kodak RP X-Omat medical X-ray film using intensifying screens at −76°C. Subunit molecular weights were estimated by the method of Weber and Osborn (17) using protein standards: β-galactosidase, 130,000; phosphorylase a, 90,000; human transferrin, 77,000; and ovalbumin, 43,500.

Two dimensional electrophoresis of the Triton-solubilized membranes was conducted according to Wada et al. (11). The first dimension was isoelectric focusing in 4% polyacrylamide gel rods containing 8 M urea, 0.5% Triton X-100, 1% ampholyte, pH 3.5 to 10 (LKB). The second dimension was SDS-polyacrylamide gel electrophoresis in 8% acrylamide gel slabs as described above.

**RESULTS AND DISCUSSION**

**Characterization of Transferrin Binding to Placental Membranes—** The initial binding studies utilizing placental membrane vesicles washed in 10 mM NaPO₄, 0.15 M NaCl, pH 7.4, as described by Smith et al. (12), gave variable results and low levels of specific transferrin binding. Prewashing of the membrane vesicles with 5 mM NaHCO₃, pH 7.4, and resuspension in 10 mM NaPO₄, 0.15 M NaCl, pH 7.4, prior to use in the binding assay was found to greatly enhance the specificity of transferrin binding, increasing it from 2 to 4% to 15 to 25% for 200 μg of membrane protein. The vesicles were also changed in their surface properties by the bicarbonate wash, which rendered them more easily resuspended and less aggregated. This effect of low ionic strength extraction may be due to the removal of material adsorbed to the vesicles. The level of transferrin binding was also found to be sensitive to the temperature at which the membranes were pelleted from the diluted assay medium. Lower temperatures, 0–4°C, gave the higher specific binding values.

A time course study of transferrin binding to membrane vesicles at 37°C indicated binding was initially high but decreased to a relatively constant amount within 10 min of incubation (Fig. 1A). A slow progressive decrease in binding was observed after 1 h at 37°C. These observations were reproducible and subsequently all measurements were made at 20-min incubation time. The greater binding in the initial
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Fig. 1. Studies of the binding of 125I-transferrin to placental membrane vesicles. A, time course of transferrin binding to placental membrane vesicles. Membrane vesicles were added to the assay medium containing transferrin at 10^{-10} M at time zero and incubated at 37°C. Aliquots were withdrawn at time intervals for assay of percentage of transferrin specifically bound as described under "Experimental Procedures." The initial sample, at zero time, was taken as the reference value and subsequent values were expressed as percentage of 125I-transferrin binding remaining. Equilibrium binding was attained within 20 min at 37°C. B, dependence of specific transferrin binding on the amount of placental membranes. The amount of specific transferrin binding at 10^{-10} M transferrin under equilibrium conditions, incubation at 37°C for 20 min, was measured with increasing amounts of placental membrane protein. C, competition by human transferrin, albumin, and γ-globulin for 125I-transferrin binding to membrane vesicles. The effect of various concentrations of ferrotransferrin (O---O), albumin (■■■■), and γ-globulin (Δ---Δ) were tested for their effect on 125I-transferrin binding to placental membrane vesicles under equilibrium conditions described under "Experimental Methods." The binding values were normalized by calculating per cent of 125I-transferrin binding remaining using binding with no additions of competing protein as reference value. D, equilibrium binding curves for transferrin interaction with placental membrane vesicles. The amount of 125I-transferrin and membrane vesicles was held constant and the concentration of unlabeled ferrotransferrin was varied in assays for transferrin binding under equilibrium conditions, 20 min at 37°C. The picomoles of transferrin bound was calculated from the per cent bound by correction for isotope dilution of unlabeled transferrin added, which gave total transferrin bound (■). Nonspecific binding (---) was calculated by taking the slope of the total binding curve between 10^{-7} and 10^{-5} M transferrin concentration and extrapolating through the origin. Specific binding (O---O) was calculated by taking the difference between the total and nonspecific binding curves.

The amount of transferrin binding under equilibrium conditions was found to be dependent on the amount of membrane protein (Fig. 1B), which indicated the transferrin binding site to be membrane-associated.

The competition of unlabeled transferrin with labeled transferrin for binding to placental membrane vesicles is illustrated in Fig. 1C. Competition experiments using apotransferrin gave results very similar to those with ferrotransferrin. Human γ-globulin did not compete for transferrin binding, even at the highest concentration of 10^{-5} M; nor did ovalbumin, which was present in the assay medium at a concentration of 5 × 10^{-5} M.

The human albumin preparation showed competition at 5 × 10^{-5} M; however, radioimmunoassay for transferrin in this preparation revealed transferrin contamination which could account for the competition observed at this concentration. These results indicated that binding of transferrin to placental membranes is specific.

The results from the competition experiments using unlabeled transferrin plotted as picomoles bound versus concentration of transferrin demonstrated a saturable, specific binding site and unsaturable, nonspecific binding sites (Fig. 1D). The nonspecific binding curve was calculated from the slope of the total binding curve at the concentrations of transferrin above specific receptor saturation (10^{-7} to 10^{-5} M). The specific binding curve was obtained by subtraction of the nonspecific binding curve from the total curve. Analysis of these data by the methods of Scatchard (20) gave a plot of bound/free transferrin versus concentration of bound transferrin which was linear in the lower range of transferrin concentration, indicating a single type of high affinity binding site (Fig. 2). The equilibrium constant for association (K_a) was calculated from the slope of the regression line of the Scatchard plots (r^2 = 0.98) derived from three sets of data giving a K_a of 3.6 ± 1.5 (S.D.) 10^{11} M^{-1}.

The protein nature of the transferrin binding site was sug-
gested by the effect of protease treatment on the binding of transferrin to placental membrane vesicles. Incubation of placental membranes with trypsin, 200 μg/ml for 30 min at 37°C destroyed 64% of the specific transferrin-binding activity relative to control membranes incubated without trypsin. This inhibitory effect was not due to residual proteolytic activity associated with the washed, trypsin-treated membranes since 1 mm TosLysCH₂Cl, a trypsin inhibitor, was included in the buffer used for the transferrin-binding assay.

Stabilization of the Solubilized Transferrin-Rceptor Complex—Preliminary experiments indicated that placental transferrin receptor retains its affinity for transferrin after solubilization from the membrane with Triton X-100. Evidence for this was obtained using polyethylene glycol-6000 (PEG) to precipitate the solubilized transferrin-receptor complex. PEG had been used previously to precipitate the human chorionic gonadotropin and insulin hormone receptor complexes (21, 22). Fig. 3A illustrates the precipitation of labeled transferrin-receptor complexes at increasing concentrations of PEG. Labeled transferrin alone did not precipitate with these levels of PEG and nonspecific, co-precipitation of transferrin with membranes in the PEG assay system was determined by the addition of saturating levels of unlabeled transferrin to the assay system (10⁻⁶ M). The specific precipitation of transferrin-receptor complex was the difference between total precipitation and nonspecific precipitation.

In order to isolate the transferrin receptor, an attempt was made to stabilize the transferrin-receptor complex. Ecarot-Charrier has reported that lowering the pH of detergent-solubilized rabbit reticulocyte membranes stabilized the transferrin-reticulocyte receptor complex (23).

The effect of pH on the stability of the placental transferrin-receptor complex was evaluated by studying the exchange between unlabeled transferrin and labeled transferrin bound to receptor complexes solubilized in Triton X-100 at pH 7.4 and at pH 5.0. As shown in Figure 3B, at pH 7.4, there is a rapid decrease in polyethylene glycol-precipitable radioactivity when unlabeled transferrin is added to the Triton X-100 extract of membranes previously incubated with ¹²⁵I-labeled transferrin. The decrease in PEG precipitable counts demonstrated a loss of label by the complex, indicating that the added unlabeled transferrin exchanged with the ¹²⁵I-transferrin in the initial complex.

At pH 5.0, the PEG-precipitable counts were not decreased by addition of unlabeled transferrin with incubation up to 1 h at room temperature. These results indicate that lowering of the pH does appear to stabilize the placental receptor-transferrin complex.

Immunoprecipitation of the Transferrin-Rceptor Complex—In order to identify the transferrin receptor of the human placental brush border membrane, the detergent-solubilized transferrin-receptor complex was immunoprecipitated using rabbit anti-human transferrin serum. This approach had been previously used by Sullivan et al. (24) and Ecarot-Charrier et al. (23) in attempts to identify the transferrin receptor of reticulocytes.

The proteins of the placental membrane vesicles were labeled with ¹¹¹In by lactoperoxidase-catalyzed iodination and then incubated with unlabeled transferrin to saturate the membrane binding sites and then solubilized with Triton X-100. The isolation of the transferrin receptor by immunoprecipitation was facilitated by stabilizing the solubilized receptor-transferrin complex at pH 5.0 with citrate buffer. SDS-polyacrylamide gel electrophoresis of immune complexes extracted from S. aureus demonstrated the presence of two major ¹²⁵I-labeled polypeptide bands (Fig. 4A). One band with a Mₗ of was determined by calculation of the difference between total and nonspecific precipitation. B, time course for exchange of unlabeled transferrin into solubilized ¹²⁵I-transferrin-receptor complexes. Unlabeled ferrotransferrin was added to 10⁻⁶ M at time zero to Triton X-100-solubilized ¹²⁵I-transferrin-receptor complexes at pH 7.4 (ΔΔΔΔ ΔΔΔΔΔΔ) and to an aliquot of the solubilized receptor complex which was titrated to pH 5.0 with 1 M sodium citrate, pH 5.0 (O---O). The time course for the exchange of unlabeled and labeled transferrin on the receptor complex was monitored by precipitation of the complex with 12% PEG as described under "Experimental Procedures."
The co-migration of glycoprotein 15b and the putative transferrin receptor complex was observed in the electrophoreogram of the whole membrane extract (Fig. 4B). When immunoprecipitation of transferrin was conducted at pH 7.4 the relative amount of putative receptor obtained was decreased 75% relative to precipitated transferrin (Fig. 4A). This was determined by scanning the autoradiogram of an SDS-polyacrylamide gel electrophoretogram with a scanning densitometer and integrating the peaks of transferrin and receptor. The relative loss of putative transferrin receptor at pH 7.4 was consistent with the PEG assay results, which indicated the receptor-transferrin complex was exchangeable at pH 7.4 and stabilized at pH 5.0. Immunoprecipitation with nonimmune serum and 5, unprecipitated Triton X-100 solubilized membranes. The gels were dried down and autoradiographed for 1 to 4 days to give optimum exposures.

The estimated molecular weight of the putative placental transferrin receptor, 90,000, closely resembles the molecular weight reported by Sullivan et al. for human reticulocyte transferrin receptor isolated by affinity chromatography, 95,000 (24), and by Hu and Aisen for rabbit reticulocyte receptors cross-linked to transferrin, 95,000 (25). These workers found a second polypeptide band at 145,000 and 176,000, respectively, associated with transferrin-binding activity. We have also noted a larger molecular weight component with affinity for transferrin; however, this component may represent a dimer of the 90,000 polypeptide, since it was approximately 200,000 in M, and only appeared in SDS-polyacrylamide gels of samples which were inadequately reduced and denatured prior to electrophoresis. Hu and Aisen share this interpretation in that they conclude that the larger subunit is a dimer of the smaller subunit. Their conclusion was based on the molecular weight of the solubilized transferrin-receptor complex, 250,000, which is compatible with a receptor molecular weight of 176,000. Since the 95,000 subunit was in sufficient proximity to the transferrin to be cross-linked using dimethylsuberimidate and the cross-linking to the 176,000 subunit was questionable, they concluded that the receptor was a dimer of the smaller subunit which is resistant to dissociation by SDS.

The question of whether the human reticulocyte and placental transferrin receptors represent the same gene product has not been investigated by the criteria of subunit molecular weight, it is possible.

The two components present in the electrophoreogram of the immunoprecipitate qualitatively showed the high degree of purification that was obtained in comparison with the whole membrane extract (Fig. 4B). When immunoprecipitation of transferrin was conducted at pH 7.4 the relative amount of putative receptor obtained was decreased 75% relative to precipitated transferrin (Fig. 4A). This was determined by scanning the autoradiogram of an SDS-polyacrylamide gel electrophoretogram with a scanning densitometer and integrating the peaks of transferrin and receptor. The relative loss of putative transferrin receptor at pH 7.4 was consistent with the PEG assay results, which indicated the receptor-transferrin complex was exchangeable at pH 7.4 and stabilized at pH 5.0. Immunoprecipitation with nonimmune serum did not precipitate any bands, and adsorption of the anti-transferrin antiserum with purified transferrin destroyed its ability to precipitate both 125I-labeled transferrin and putative transferrin receptor (Fig. 4B). These two controls indicated that the precipitation of putative receptor was due to its association with the transferrin-antibody complex rather than with the IgG itself. The substitution of human-IgG and anti-human IgG (light and heavy chain) for transferrin and anti-transferrin also did not result in the immunoprecipitation of the putative transferrin receptor (Fig. 4B).

This finding demonstrates that the affinity of this membrane component is specific for the transferrin immune complex. The possibility that the putative receptor is cross-reacting with specific anti-transferrin antibodies is unlikely and inconsistent with the finding that less receptor protein is present in the precipitate when the immune complex is precipitated by anti-transferrin at pH 7.4 than at pH 5.0. This is unlike antibody-antigen interactions which are extremely high affinity interactions and which are stable at neutral pH. The most satisfying conclusion is that the putative receptor reversibly binds to transferrin itself, and that binding is considerably diminished at pH 7.4 as compared to pH 5.0.
Sulfate. The dried gels were autoradiographed for 24 h. B, the transphoresis in 8% acrylamide gel slabs containing 0.1% sodium dodecyl
4% polyacrylamide gel containing 8 M urea, 1% ampholytes, and 0.5%
The first dimension was isoelectric focusing in a 3.5 to 10 pH gradient,
the second dimension was polyacrylamide gel electrophoresis in 8% acrylamide gel slabs containing 0.1% sodium dodecyl
sulfate. The dried gels were autoradiographed for 24 h. B, the trans-
ferrin-receptor complex was precipitated at pH 5.0 from a Triton X-
100 extract of 125I-labeled placental membranes with anti-transferrin
in serum as described under “Experimental Procedures,” and eluted from
immune precipitate using 2% SDS-sample buffer. After reduction
with 10% β-mercaptoethanol at 100°C for 2 min, the extract was
diluted 1:5 with 9 M urea, 5 mM NaPO4, pH 8.0, 1% Triton X-100, 5% β-
mercaptoethanol, and concentrated to 100 μl for analysis by two-
dimensional electrophoresis. The gel was stained with Coomassie
blue, destained, and dried down for 3 days of autoradiography. C, the
Coomassie blue-stained gel described in B shows the immunoprecip-
itated unlabeled transferrin which was added to the Triton extracts
of labeled placental membranes prior to precipitation of the transferrin-
receptor complexes. The stained transferrin spots co-migrate with
Spot 22 on the autoradiogram of the same gel (see B).

Fig. 5. Two-dimensional electrophoresis of the immunopre-
cipitated transferrin-receptor complex. A, unprecipitated Triton
X-100 extract of 125I-labeled placental membranes was made 9 M in
urea by adding solid urea and 0.1% in SDS by adding 10% SDS. The
solution was concentrated to 100 μl, reduced with 5% P-mercapto-
etanol at room temperature for 10 min, and analyzed by two-dimen-
sional electrophoresis according to the method of Wada et al. (11).

A recent study comparing the antigenic glycoprotein com-
ponents of human placental, liver, and kidney plasma mem-
branes indicated that glycoprotein 15b was only found in the
placental membranes (19). In our studies of the sialoglycopro-
teins of the placental brush border membranes, glycoprotein
15b has been reproducibly demonstrated in its characteristic
position in two-dimensional polyacrylamide gel electrophoret-
ogram using either sialic acid labeling (11, 19) or by iodination
(19). Use of both labeling methods has been useful in estab-
lishing that it is a glycoprotein. The demonstration that
glycoprotein 15b is a specific transferrin receptor has identified
the specialized function of this membrane component. This
finding is of potential importance for investigating the molec-
ular mechanisms of transplacental transport of iron. The
placental transferrin receptor may represent one of a series of
cell surface, placental glycoproteins involved in the transport
of the many solutes and ions required for fetal growth and
development.

Note Added in Proof—Further work in this laboratory has identi-
fied a transferrin receptor on the membrane surface of cultured
human cells which has properties identical to the receptor described
in this paper (Hamilton, T. A., Wada, H. G. and Susman, H. H.,
receptor interaction (3.6 ± 1.5 (S.D.) × 10⁹ M⁻¹) in the present study
was derived from experiments using membrane vesicles which con-
tained endogenous bound transferrin. Subsequent studies in which
the endogenous transferrin has been removed have given a Kd value of
1.5 ± 0.4 (S.D.) × 10⁹ M⁻¹, which is in accordance with the value of
4.2 ± 2.1 (S.D.) × 10⁹ M⁻¹ for cultured cells grown in the absence of
human transferrin.

REFERENCES
58-64, University of California Press, Berkeley, CA
(Jacobs, A., and Worwood, M., eds) pp. 39-71, Academic Press,
N.Y.
62, 271-279
186
47-58
in Proteins of Iron Storage and Transport in Biochemistry and
Medicine (Crichton, R. R., ed) pp. 111-119, North Holland,
Amsterdam
Supramol. Struct. 6, 473-484
190-196
Commun. 40, 284-289
Struct. 10(3), 287-305
Chem. 248, 6973-6982
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