The Synthesis and Secretion of Rat Transferrin*

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Gerhard Schreiber,‡ Heide Dryburgh,‡ Anne Millership,‡ Yoshiko Matsuda,‡ Adam Inglis,§ John Phillips,‡ Kaylene Edwards,‡ and Judith Maggs‡

From the ¤Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, 3052, Victoria, Australia, and the §Division of Protein Chemistry, Commonwealth Scientific and Industrial Research Organization, Parkville, 3052, Victoria, Australia

Transferrin, isolated from plasma or serum, had a molecular weight of 76,500 and its NH₂-terminal amino acid sequence was Val-Pro-Asp-Lys-Thr-Val-Lys-Trp-(Cys)-Ala-Val-Ser-Glu-His-Glu-Asn-Thr-Lys-(Cys)-Ile-Ser-Arg-Asp-His-Glu-Asn-Thr-. About one-third of the total serum transferrin contained 3 mol of N-acetylenuraminic acid and about two-thirds of total contained 2 mol of N-acetylenuraminic acid/mol of transferrin. The former had an isoelectric point of 5.65, the latter one of 5.85. About 1% of total transferrin in serum had an isoelectric point of 5.35 and a trace amount had an isoelectric point of 6.1. The half-lives in serum were similar for transferrin containing 2 or 3 N-acetylenuraminic acid residues.

Characteristic differences in the labeling kinetics of transferrin, albumin, and α₁-acid glycoprotein with radioactive L-leucine or D-glucosamine suggested that these proteins were processed independently in the liver cell. The secretion of transferrin was inhibited by proteinase inhibitors but not by inhibitors of glycosylation.

A transferrin-like protein was isolated from liver. It possessed the same NH₂-terminal amino acid sequence as transferrin from serum but did not appear to contain N-acetylenuraminic acid. After injection of radioactive amino acids it was labeled earlier than the sialylated forms of transferrin.

A cell-free protein-synthesizing system from wheat germ, programmed with RNA isolated from liver, synthesized a protein precipitable with anti-transferrin antiserum. Compared to serum transferrin, it possessed an NH₂-terminal extension of 20 amino acids beginning with Met-Lys-. . . .

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

Isolation and Properties of Transferrin from Plasma—The purification of transferrin from plasma is summarized in Table I. The theoretical maximum of purification is 16.4-fold for a starting transferrin concentration of 1.4 mg/ml and a total protein concentration of 23 mg/ml in diluted plasma. This was achieved. The molecular weight of the isolated transferrin was determined by electrophoresis in 7.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. The molecular weight of transferrin was found to be 76,500. This is higher than the value of 68,000 reported previously for rat transferrin (2). However, our value agrees well with values of between 73,200 and 81,000 reported for human transferrin (3-6), 76,000 for ovotransferrin (7), 76,400 for porcine transferrin (8), and between 70,000 and 79,500 for transferrin in rabbit (4,7-9).

In recent years, major additions have been made to our knowledge about the mechanism of synthesis and secretion by the liver of albumin, the most abundant plasma protein. Albumin is initially synthesized as pre-proalbumin, which is converted into proalbumin and finally into albumin (for a recent review, see Ref. 1). The question arises as to whether similar mechanisms exist in the liver for the synthesis and secretion of other plasma proteins. The experiments reported in this paper describe aspects of the secretion of transferrin, another major, well-characterized serum protein which is made in the liver.

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† Experimental Procedures (including a figure) is presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document 9M-313, cite author(s), and include a check or money order for $1.50 per set of photocopies.

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Purification of transferrin from rat plasma

Plasma was obtained from 116 rats as described under “Methods,” in the miniprint. A total of 1075 ml of diluted plasma, in 0.76% trisodium citrate, was used for purification (for details see “Methods”).

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein</th>
<th>Transferrin</th>
<th>Transferrin/total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted plasma</td>
<td>24,700</td>
<td>1,510</td>
<td>6%</td>
</tr>
<tr>
<td>Fractionation with 45 to 58% saturated ammonium sulfate DEAE-cellulose chromatography, pH 7.7</td>
<td>6,130</td>
<td>1,100</td>
<td>18%</td>
</tr>
<tr>
<td>Sephadex G-100 chromatography, pH 7.7</td>
<td>921</td>
<td>823</td>
<td>89%</td>
</tr>
<tr>
<td>Polycrylamide gel electrophoresis, pH 8.9</td>
<td>637</td>
<td>600</td>
<td>94%</td>
</tr>
</tbody>
</table>

For alanine at position 16 and isoleucine for glutamate at position 20. The changes at positions 16 and 20 would involve a double base change in the codons for these residues. Thus, our sequence for rat transferrin would support the arrangement of residues obtained by Boutigue et al. (11) for human transferrin rather than the alternative proposed by Sutton and Brew (12). The sequence reported earlier for human transferrin (13) is completely different.

The total isolated serum transferrin could be resolved into two fractions by chromatography on Concanavalin A-Sepharose (Fig. 1, in miniprint). The first peak, amounting to about 70% of total serum transferrin, was characterized by a single band in the isoelectric focusing gel (Fig. 2). The second peak, which was about 30% of total serum transferrin, represented material with 2.8 mol of N-acetylneuraminic acid/mole of transferrin and was designated Tfa.

The isoelectric points of the four serum transferrin species were altered in a stepwise fashion after neuraminidase treatment. The results obtained are depicted in Figs. 2 and 3. The minimum secretion time after injection of L-leucine was about 21 min for transferrin and 15 min for albumin. The ratio of the specific radioactivity of leucine-labeled albumin over the specific radioactivity of leucine-labeled transferrin decreased steadily from about 11.6 after 21

Table I

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>No. residues per molecule of transferrin (nearest integer)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This work</td>
</tr>
<tr>
<td>Lysine</td>
<td>635.5</td>
</tr>
<tr>
<td>Histidine</td>
<td>208.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>284.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>93.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>829.9</td>
</tr>
<tr>
<td>Threonine</td>
<td>422.8</td>
</tr>
<tr>
<td>Serine</td>
<td>499.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>739.1</td>
</tr>
<tr>
<td>Proline</td>
<td>516.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>662.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>650.9</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>390.2</td>
</tr>
<tr>
<td>Valine</td>
<td>527.6</td>
</tr>
<tr>
<td>Methionine</td>
<td>55.4</td>
</tr>
<tr>
<td>Isoeucine (alloiso-eucine)</td>
<td>205.5</td>
</tr>
<tr>
<td>Leucine</td>
<td>672.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>247.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>363.6</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>22</td>
</tr>
</tbody>
</table>

for alanine at position 16 and isoleucine for glutamate at position 20. The changes at positions 16 and 20 would involve a double base change in the codons for these residues. Thus, our sequence for rat transferrin would support the arrangement of residues obtained by Boutigue et al. (11) for human transferrin rather than the alternative proposed by Sutton and Brew (12). The sequence reported earlier for human transferrin (13) is completely different.

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Fig. 2. Specific radioactivities of transferrin and albumin in plasma after intraportal injection of L-[1-14C]leucine. Eight male Buffalo rats were injected with 10 μCi/100 g body weight of L-[1-14C]leucine. Blood was withdrawn from the caval vein of each rat at the times shown and the specific radioactivities of albumin and transferrin were determined in the samples as described under “Methods.” Measurement at each time point was carried out on combined plasma from the eight rats.

Fig. 3. Specific radioactivities of transferrin, albumin, and α1-acid glycoprotein in plasma after intraportal injection of D-[1-14C]glucosamine. Five male Buffalo rats were each injected with 50 μCi of D-[1-14C]glucosamine and blood was withdrawn from the caval vein at the times shown. The specific radioactivities of transferrin, albumin, and α1-acid glycoprotein were determined in the plasma samples as described under “Methods.” Measurement at each time point was carried out on combined plasma from the five rats.

min to about 2.9 after 63 min (Fig. 2), indicating that the kinetics of processing in liver cells is quite different for albumin and transferrin. After injection of D-[1-14C]glucosamine, radioactivity began to appear in α1-acid glycoprotein after about 15 min and in transferrin after about 25 min. The ratio of the specific radioactivity of α1-acid glycoprotein to the specific radioactivity of transferrin increased steadily from 18 min to about 40 min after injection, indicating that the kinetics of carbohydrate attachment differed markedly for the two glycoproteins (Fig. 3). Albumin was not labeled with D-[1-14C]glucosamine. These results suggest that for these serum proteins, the processing of polypeptide chains and carbohydrate moieties occurs independently for the individual proteins, or perhaps different classes of proteins. This is in contrast to the situation for pancreatic proteins where a constant ratio of various excretory proteins has been observed and “co-transport” of excretory proteins has been proposed (14).

Existence and Properties of a Transferrin-like Protein in the Liver which is a Precursor for Transferrin in the Bloodstream—In contrast to the single peak obtained for transferrin isolated from serum, two distinct peaks of anti-transferrin-precipitable protein were obtained when a buffer extract of an acetone-dried powder from whole rat liver was analyzed by ion exchange chromatography on DEAE-cellulose (Fig. 4). The first peak, which was eluted at about 55 mM NaCl at pH 7.7, was unique to liver, while the second peak, eluted at about 70 to 75 mM NaCl, corresponded on isoelectric focusing to the bands found in serum.

At 25 min after intraportal injection of L-[1-14C]leucine, most of the anti-transferrin-precipitable radioactivity was
found in the liver-specific transferrin-like protein following analysis by DEAE-cellulose chromatography of an acetone-dried powder extract from total liver homogenate (Fig. 4A). Five hours after injection, anti-transferrin-precipitable radioactivity was observed in both the liver-specific transferrin-like protein and in the protein corresponding to serum transferrin (Fig. 4B). The isoelectric point of transferrin-like protein was 6.3 when analyzed by isoelectric focusing. The first three amino acids obtained by Edman degradation at the NH$_2$ terminus of the transferrin-like protein were Val-Pro-Asp. . . . Its molecular weight was 76,500, which did not differ from that of serum transferrin. Both transferrin and transferrin-like protein gave positive staining with periodic acid Schiff's reagent. It seems that the transferrin-like protein is an asialotransferrin with some carbohydrate attached. In the following, transferrin-like protein will be abbreviated as Tfo just as the transferrin with some carbohydrate attached. In the following, two major serum forms of transferrin containing either 2 or 3 N-acetylneuraminic acid residues have been abbreviated as Tfs and Tfs, respectively, the subscript referring to the number of residues of N-acetylneuraminic acid per molecule of transferrin.

Structure of the NH$_2$ Terminus of the Earliest Detectable Precursor of Transferrin in Liver—The subcellular fraction in which the earliest precursor forms of transferrin are most likely to appear is the microsomal fraction. Therefore, microsomes were prepared from the livers of rats killed at various times from 2% to 300 min after intraportal injection of L-[1-14C]leucine. An acetone-dried powder was prepared from the microsomes and exhaustively extracted with Tris-HCl buffer. Proteins were transferrin-precipitable protein peaks were identified as Tfo and in the (TfZ + Tfs) peak is plotted against time after injection in Fig. 5. Maximum labeling occurred in microsomal Tfo 15 min after injection and in (TfZ + Tfs) 20 min after injection. The values found for the specific radioactivity of (TfZ + Tfs) were probably too high to account for the overlap by the Tfo peak. Fig. 6 shows the elution pattern of anti-transferrin-precipitable protein in the DEAE-cellulose chromatography for the 10-min and the 25-min time points. It is clear that after 10 min only minimal labeling, if any, occurred in the second peak (TfZ + Tfs). Therefore, a 10-min labeling period was chosen to prepare radioactive Tfo for investigation of the NH$_2$ terminus of the molecule by radioactive sequencing. From the data on the amino acid sequence of transferrin in the serum (described above) we expected that valine would be most appropriate for the identification of transferrin since it occurs in three positions near the NH$_2$ terminus (positions 1, 6, and 11). Phenylalanine, which does not occur until position 22 of the serum transferrin, was chosen as a second amino acid for the determination of the NH$_2$ terminus of early labeled Tfs. Tfo was isolated by ion exchange chromatography of the acetone-dried powder extracts of microsomes prepared from the livers of rats removed 10 min after injection of either L-[3,4(4u)-3H]valine or L-[2,6-3H]phenylalanine. The Tfo obtained was subjected to sequential degradation from the NH$_2$ terminus in an Edman-Begg-type sequenator and the radioactivity released after each step was determined. The results are shown in Fig. 7. Valine was found in positions 1, 6, and 11, phenylalanine in position 22. Incomplete cleavage of the peptide bond after proline (position 2 of serum transferrin) led to the appearance of radioactivity in positions following the valine peaks and following phenylalanine in position 22. Our results suggested that within 10 min of synthesis, microsomal Tfo already possesses the NH$_2$-terminal amino acid sequence found in transferrin in the serum. No “pro”-segment, comparable to that described for albumin (for summary see Ref. 1), seems to exist in transferrin-like protein.

Translocation of mRNA from Rat Liver in Cell-free Protein Synthesizing Systems from Wheat Germ—Many studies of the early processing of secretory proteins have successfully utilized cell-free protein-synthesizing systems, which are low in protease activity, for the process of mRNA coding for secretory proteins. Such a cell-free protein-synthesizing system was prepared from wheat germ, programmed with RNA isolated from rat liver as described under “Methods,” in the miniprint, and the incorporation of radioactive methionine, valine, and lysine into anti-transferrin-precipitable protein was studied. In an Edman degradation of anti-transferrin-precipitable protein synthesized in the wheat germ system, radioactive methionine was released in Cycle 1 and lysine was released in Cycle 2. The first valine was observed in Cycle 21. These data are consistent with the structure Met-Lys-Val . . . at the NH$_2$ terminus of the nascent transferrin molecule. A “pre”-sequence of 19 amino acids with Met-Lys-Val . . . at the NH$_2$ terminus of the nascent transferrin molecule has recently been reported for “pre”-transferrin in chicken (15).

Effect of Proteinase Inhibitors and Inhibitors of Glycosylation on the Synthesis of Transferrin in the Liver—The subcellular fraction in which the earliest precursor forms of transferrin are most likely to appear is the microsomal fraction. Therefore, microsomes were prepared from the livers of rats killed at various times from 2% to 300 min after intraportal injection of L-[1-14C]leucine. Male Buffalo rats were injected with 5 μCi/100 g body weight of L-[1-14C]leucine and livers were removed at the following times, the figure in parentheses referring to the number of rats used per time point: 2% (11), 5 (11), 10 (15), 15 (15), 20 (15), 25 (15), 50 (9), 120 (11), and 300 (10) min after injection. A buffer extract of an acetone-dried powder of liver microsomes was chromatographed on DEAE-cellulose and the specific radioactivities of the peaks of Tfo and of (TfZ + Tfs) were determined as described under “Methods.” For explanation of the terms Tfo, Tfs, and Tfs, refer to “Existence and Properties of a Transferin-Like Protein in the Liver which is a Precursor for Transferrin in the Bloodstream.”
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The Influence of N-Acetylenuraminic Acid Content on the Turnover of Transferrin in the Bloodstream—It has been suggested that N-acetylenuraminic acid at the periphery of the carbohydrate moiety of glycoproteins influences the rate of elimination of glycoproteins from the bloodstream (17). We therefore injected L-[1-14C]leucine into rats and isolated Tfi and Tfs from plasma obtained 120 and 300 min after injection of the radioactive amino acid. For both times the specific radioactivity of Tfs equaled that of Tfi. The half-life of L-[1-14C]leucine-labeled Tfs when injected into rats was found to be about 40 h. A similar value was obtained in an analogous experiment with Tfi. The injected radioactive Tfs could be isolated as such from the serum. No significant amount of radioactivity had been transferred to Tfi. Similar results were obtained upon injection and isolation of radioactive Tfs. Thus, Tfi and Tfs did not differ in their rate of elimination from the bloodstream.

Acknowledgments—We are very grateful to Dr. H. Morgan, University of Western Australia, Perth, Australia, for providing us with some anti-transferrin antisera in the very early stages of our work and to Dr. R.L. Hamill, Eli Lilly and Co., Indianapolis, for a gift of tunicamycin. We would like to thank Dr. C. Ruxburgh for performing the amino acid analysis and Mr. M.R. Rubira for his technical assistance.

REFERENCES

Additional references appear on p. 12019.
The Synthesis and Secretion of Rat Transferrin

Gerhard Schreiber, Heide Dreyburgh, Anne Miller, Peter Drueckhammer and Judith Meyer

SYNOPSIS AND SECRESSION OF RAT TRANSFERRIN

Buffalo rats from an inbred colony kept in our laboratory were fed on a 209 protein diet with free access to water and food.

Materials

BUFFALO RATS

Sera were removed from the caudal vein of male buffalo rats which had been starved overnight and were then mixed with trisodium citrate (final concentration 0.7%). Plasma obtained by centrifugation at 25,000 g for 15 min. was extracted with 100 mM Tris-Cl, pH 7.7, dialyzed against 10 mM Tris-Cl, pH 7.7, and applied to a 75 x 4 cm DEAE-cellulose column which had been eluted with 10 mM Tris-Cl, pH 7.7.

Protein which had been equilibrated with 16 mM ammonium acetate buffer was applied to the column and was eluted with 10 mM Tris-Cl, pH 7.7, containing 150 mM NaCl in 20 mM Tris-Cl, pH 7.7. All fractions from the DEAE-cellulose chromatography which contained anti-transferrin precipitable protein were combined, ultrafiltrated into 150 mM NaCl, 0.1 M NaCl and chromatographed on Concanavalin A-Sepharose, as described in Methods. A total of 124 mg protein in 5.6 ml was applied to the column. The protein content of anti-transferrin precipitable protein and the absorbance at 280 nm were measured in each of the fractions. The total isolated serum transferrin could be resolved into two fractions. The first peak, non-bound protein, contained 2.8 mol of N-acetylneuraminic acid per mol transferrin and was designated Tf3. The second peak, bound protein, contained 1.9 mol of N-acetylneuraminic acid per mol transferrin and was designated Tf2.

Determination of Molecular Weight

Molecular weights of proteins were determined by chromatography on sodium dodecyl sulfate (SDS) polyacrylamide gels. Gels, 8.0% (v/v) cast in a Tris-glycine (pH 6.8) buffer system containing 0.1% SDS in 100 mM sodium phosphate buffer, pH 7.2, and with polyacrylamide gels containing 0.01% methylene blue dye (TEMED) and an excess of peroxidase. Samples, 10 µl, were added to a cuvette containing 0.1 M sodium phosphate buffer, pH 7.2, 2.5 µl of mercaptoethanol and 0.1% SDS were homogenized with a 20-gauge needle. Dodecyl sulfate 0.1% SDS and mercaptoethanol were added to a cuvette containing 10 µl of a solution of dodecyl sulfate. The remaining proteins were precipitated by adding an equal volume of 10% trichloroacetic acid. The pellets were washed with 10% trichloroacetic acid and dissolved in a solution containing 10% acetic acid and 20% methanol. Standard proteins were used to calibrate the gels: phosphorylase b (molecular weight 97,000), bovine serum albumin (molecular weight 67,000), ovalbumin (molecular weight 44,000) and glyceraldehyde-3-phosphate dehydrogenase (molecular weight 35,000).

Determination of Protein

Protein was determined using either the method of Lowry (2) or a modification of the method of Beisenherz (36, 3).

Amino Acid Analysis

Amino acid analysis of plasma transferrin was carried out by a Beckman 1209 amino acid analyzer on protein hydrolyzed in 6 N HCl for 5 hr. (308 mg transferrin) 48 h 1.4 mg transferrin and 72 h (256 mg transferrin).

Determination of Partial Amino Acid Sequence

The N-terminal amino acid sequences were determined automatically by Edman degradation (9) using the modification of Ingalls and Barber (10). The initial experiments with transferrin indicated that sequence yields fell to zero after a few cycles; presumably, because the formation of carboxylic acid p-nitroanilides was not extended beyond the side of the cup upon addition of formic acid (99.0% conversion was obtained by addition of formic acid) (99.0% conversion was obtained by addition of formic acid). This was overcome by addition of phosphoric acid (12). Samples containing sodium phosphate buffer, 72 h (256 mg transferrin).

The trichloroacetic acid-fixed gels were washed thoroughly, then stained in a 1% aqueous solution of acetic acid/methanol, 15:1 (v/v). Staining was performed by washing the gel in the same solution without methanol.

Gels to be fixed with antiserum were covered with the antiserum until immunoprecipitates appeared. They were then thoroughly washed with 0.1 N NaCl.

Storage of Carbohydrate

Proteins which had been electrophoresed as described above were tested for the presence of carbohydrate by washing the gels in a solution containing 0.05% trichloroacetic acid/0.001 M NaCl/94.9 g/l NaCl and 0.4 g/l of NaCl. The protein precipitate was collected by centrifugation and washed with 0.05% trichloroacetic acid/0.001 M NaCl/94.9 g/l NaCl and 0.4 g/l of NaCl. The protein was then resuspended in 0.05% trichloroacetic acid/0.001 M NaCl/94.9 g/l NaCl and 0.4 g/l of NaCl. The protein was then resuspended in 0.4 N NaOH and 1.0% sodium dodecyl sulfate. The samples were then applied to a Bio-Gel P-30 column, which had been equilibrated with 0.1 N NaOH and 1.0% sodium dodecyl sulfate. The samples were then applied to a Bio-Gel P-30 column, which had been equilibrated with 0.1 N NaOH and 1.0% sodium dodecyl sulfate. The samples were then applied to a Bio-Gel P-30 column, which had been equilibrated with 0.1 N NaOH and 1.0% sodium dodecyl sulfate.
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Determination of Secretion Kinetics of Plasma Proteins

(a) Using L-[1-14C]leucine: Eight non-starved male Buffalo rats were injected with 5 ml of 5 Ci/100 g body weight, into the portal vein. At intervals of 7 min from the time of injection, 5 ml of blood was withdrawn from the caval vein into a syringe containing 0.1 ml of 2.5% trichloroacetic acid. After 0.5 ml of 0.9% NaCl was injected into the caval vein. For a given time point, samples from the eight rats were combined and plasma was obtained by centrifugation at 2000 x g for 15 min.

(b) Using D-[1-14C]glucosamine: Five male Buffalo rats which had been starved overnight, were injected intraperitoneally with 500 Ci of D-[1-14C]glucosamine hydrochloride in 0.9% NaCl. At time 0 min, 0.4 ml of blood was removed from the caval vein into a syringe containing 0.1 ml of 3.9% trichloroacetic acid. After each blood sample was removed, remaining blood was withdrawn from the caval vein into a syringe containing 0.1 ml of 3.9% trichloroacetic acid. For a given time point, samples from the five rats were combined and plasma was obtained by centrifugation at 2000 x g for 15 min.

The concentrations of albumin, transferrin and α1-acid glycoprotein in the samples were determined by single radial immunodiffusion in agar gels (9). Radioactivity in albumin, transferrin or α1-acid glycoprotein was determined using immunoprecipitation with specific antisera raised in rabbits to each plasma protein. The immunoprecipitates were washed 7 times with tri-HCl, pH 7.7, solubilized using 1 M p-diisobutylcresoxyethoxyethyl dimethylbenzyl ammonium hydroxide in methanol and counted in a scintillation cocktail which has been described previously (10).

The Kinetics In Vivo of Transferrin Synthesis

Male Buffalo rats which had been starved overnight were injected intraperitoneally with either 60 Ci/100 g body weight of L-[1-14C]leucine. Livers were removed 2.5, 5, 10, 15, 20, 25, 50, 120 and 300 min after injection. For each time point, buffer extract of an acetone-kried powder was prepared from liver specimens, and chromatographed on (2.5 x 90 cm DEAE-cellulose columns. The columns were eluted with 2500 ml of a linear gradient from 0-160 mM NaCl in 20 mM tri-HCl, pH 7.7, at a flow rate of 120 ml/h and fractions of 1 ml were collected. The concentration of anti-transferrin precipitable protein and radioactivity in anti-transferrin precipitable protein were determined as described above under "Secretion Kinetics of Plasma Proteins".

Preparation of [3H]Methionylalanine and [3H]Valine Labeled Transferrin from Liver

Liver DNA was isolated according to Keller and Taylor (11), except that heparin was omitted from the homogenization buffer and the RNA precipitates were washed four times with 70% ethanol containing 0.2 M NaCl.

Isolation of RNA from Liver

Liver RNA was isolated according to Heider, R.J., and Novelli, G.D. (1961) Arch. Biochem. Biophys. 100, 2330-2334.

REFERENCES


Studies of Transferrin Turnover in the Blood

For the preparation of radioactively labeled serum transferrin, rats were injected with L-[1-14C]leucine, 5 Ci/100 g body weight, and blood was taken from the caval vein 200 min later. Transferrin was purified from the serum by DEAE-cellulose, Sepharose-6B and concanavalin A-Sepharose chromatography, as described above. In the concanavalin A-Sepharose chromatography, transferrin was separated into two peaks. The first peak not bound by concanavalin A was designated "Tf1. Tf1 was released from the lectin by washing the column with 10 ml 1 M methyl-α-D-glucopyranoside. This transferrin was defined as Tf1. Two fractions could be distinguished in isoelectric focusing gels, by their different transferrin points.

In a similar experiment using the radioactively labeled Tf2, which had a specific radioactivity of 2800 dpm/mg, three male rats were each injected with 5.2 µg or 72 µg per 100 g body weight. In this case, the serum obtained 96 h after injection was used to determine the specific radioactivities of Tf1 and Tf2, which were 19 dpm/mg and 2.2 dpm/mg, respectively. Less than 4% of the radioactivity present in total serum transferrin was found in Tf1, and this could probably be accounted for by contamination of the Tf2 with Tf1.

For the estimation of the half-lives of injected transferrin in the blood, the radioactivity of total transferrin was determined using the method of Mandl and Novelli (14), either in the liquid phase or on filter paper discs.

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