GUINEA PIG INTESTINAL IRON BINDING PROTEIN

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SUMMARY

An iron binding protein, isolated from guinea pig intestinal mucosa, was compared to guinea pig transferrin. Both had a molecular weight of approximately 80,000. The intestinal iron-binding protein consisted of 2 subunits of equal molecular weight; transferrin had no subunits. Transferrin showed an absorbance peak at 470 nm; the intestinal iron-binding protein had no visible absorbance but did have a peak at 336 nm. Electron spin resonance spectra of the two proteins differed. Significant differences on amino acid analysis were also identified.

INTRODUCTION

Iron absorption by the intestine is closely regulated. The means by which this control is effected has been the subject of extensive study. In earlier work we identified a guinea pig intestinal mucosal fraction which bound ^{59}Fe, given as an intraluminal pulse, and rapidly transferred the initially bound iron to the animal's carcass. We suggested that the fraction might, therefore, contain a carrier (1,2).

An apparently new iron-binding protein, GIBP*, was subsequently purified from this fraction (3). GIBP bound approximately 2 gram atoms of iron per mole with an apparent binding constant of $10^{19} M^{-1}$ at pH 7. It had a molecular weight of 78,000 and consisted of two subunits of equal molecular weight. It was distinguished from transferrin and lactoferrin by its differential elution from DEAE-Sephadex and its failure to cross react with antisera to these other proteins.

The possibility that GIBP was a proteolytically altered transferrin was considered because of the unavoidable contamination of the initial intestinal mucosal scrape with blood. Mixtures of ^{59}Fe labelled guinea pig

*GIBP: abbreviation for Gut Iron Binding Protein

serum and mucosal homogenate failed to generate a species resembling GIBP (3). The critical question of a proteolytically altered transferrin remained, however, since the in vitro control might have failed to precisely parallel the events in the actual preparation of GIBP and since a proteolytic modification might change antigenic properties and charge.

In the present study we describe a more efficient method for preparing GIBP and compare several of its properties to guinea pig transferrin. Differences on visible and UV spectroscopy and electron spin resonance spectroscopy of GIBP and transferrin are noted and amino acid analyses are presented. The amino acid analyses show significantly different contents of tyrosine, isoleucine and serine, and provide unequivocal proof that GIBP and transferrin are separate species.

METHODS

Preparation of GIBP

The method of purification used in our earlier work involved preparation of a 140,000 xg supernatant from a scrape of guinea pig intestinal mucosa followed by filtration through G100 Sephadex and then elution from DEAE-Sephadex. (3). We found that the G100 Sephadex columns gave poor separations after several runs and needed to be replaced. We found also that attempts to increase the concentration of protein in the mucosal supernatant, by reducing the amount of buffer in proportion to mucosa, gave poor separations even with fresh Sephadex, apparently because of formation of excessively viscous solutions during passage through the column.

The original sequence of preparation was revised to avoid these difficulties. The procedure involved the following sequence: preparation of a 140,000 xg supernatant from a scrape of guinea pig intestinal mucosa and partial purification by $(NH_4)_2SO_4$ precipitation; labelling of the $(NH_4)_2SO_4$ purified fraction with ^{59}Fe and elution of the labelled fraction from DEAE-Sephadex; isoelectric focussing in a preparative column and G100 Sephadex filtration of the count peak obtained from the isoelectric focussing experiment.

Preparation of Transferrin

Transferrin in guinea pig serum, labelled and saturated with nitrilotriacetate-chelated $^{59}Fe + FeCl_3$, was purified by $(NH_4)_2SO_4$ precipitation, Sephadex filtration, DEAE-Sephadex chromatography using a phosphate buffer at pH 7.0 - pH 7.5, and a second DEAE-Sephadex chromatography using HEPES* buffer at pH 8. After these steps the transferrin was pure as judged by electrophoresis on 4%, 5% and 6% polyacrylamide gels at pH 8.5.

*HEPES: abbreviation for N^2-hydroxyethylpiperazine $N^1$2 ethanesulfonic acid.

TABLE I

GUINEA PIG INTESTINAL IRON BINDING PROTEIN

	Protein (mgm)	n Mole Fe / mg protein
Mucosal scrape	29.1×10^3	
140,000 xg supernatant	9.2×10^3	
$(NH_4)_2SO_4$ precipitation	2.5×10^3	
DEAE-Sephadex elution	32.	1.4
Isoelectric Focussing electrophoresis and G100 Sephadex Filtration.....	0.238	21.10

Molecular weight determinations using gel filtration chromatography followed the method of Andrews (4). The G200 Sephadex column was 1 cm X 54 cm and was calibrated with aldolase, bovine serum albumin, ovalbumin, chymotrypsinogen and ribonuclease. Gel electrophoresis with SDS* followed the procedure of Fairbanks (5). Molecular weight estimation followed the procedure of Weber (6). The visible and ultraviolet absorbance of the protein was scanned in a Cary 14 Spectrophotometer using a 1 cm light path. Protein was quantified by the Lowry method following precipitation with CCl_3COOH, using crystalline bovine serum albumin as a standard (7). Electron spin resonance spectroscopy was performed with a Varian E-9 spectrometer. Isoelectric focussing was carried out in an LKB Column (8100-20) using 1% Ampholine in a gradient of pH 5 - pH 8.

Samples for amino acid analysis were hydrolyzed with 6 N HCl containing 0.9 mg % phenol in vacuum sealed ampoules kept at 110°C for 22 hours. Amino acid analysis was performed on a JEOL amino acid analyzer, Model JLC-6AH. The average of the analysis of duplicate samples is reported.

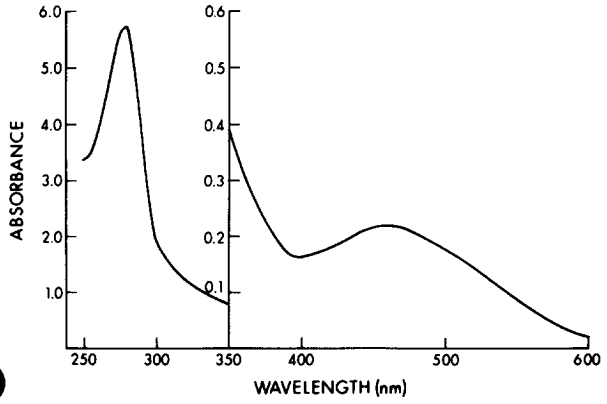
RESULTS AND DISCUSSION

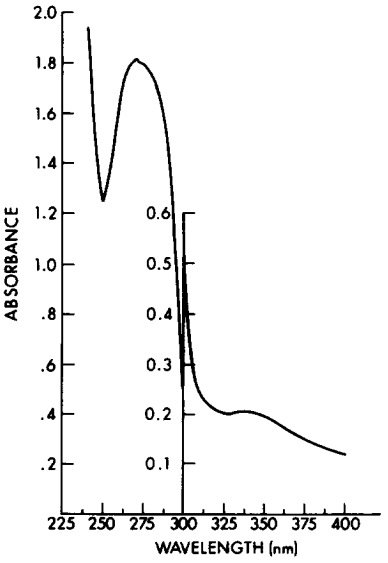
The protein content of fractions during the purification of GIBP is described in Table 1.

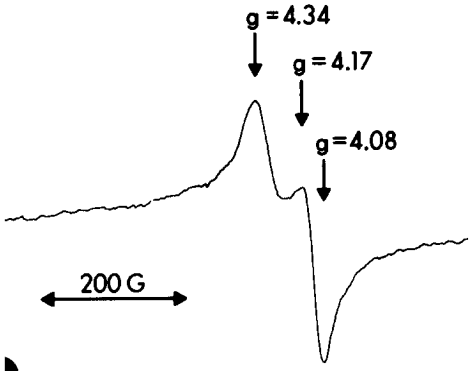
Guinea pig transferrin has a molecular weight of 80,000 as measured by gel filtration and no subunits on SDS gel electrophoresis. GIBP has a similar molecular weight but consists of two subunits of equal molecular weight(3).

Guinea pig transferrin has the 470 nm absorbance characteristic of transferrin from other species (Fig. 1). GIBP has no 470 nm absorbance but has a

* SDS: abbreviation for sodium dodecylsulfate







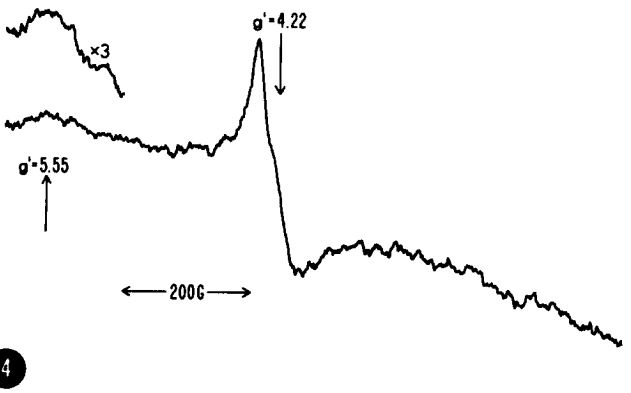


Figure 1: Spectrum of guinea pig transferrin in 0.1 M NaCl. Scanned in a Cary Spectrophotometer with a 1 cm light path. Iron bound to transferrin was 1.1 X 10^{-4} M.

Figure 2: Spectrum of GIBP in 0.1 M NaCl. Scanned in a Cary Spectrophotometer with 1 cm light path. Iron bound to GIBP was 0.2 X 10^{-4} M.

Figure 3: EPR spectrum obtained at 77°K of guinea pig transferrin in 0.1 M NaCl. Microwave power 20 mW. Modulation amplitude 10 Gauss. Microwave frequency 9.118 GHZ. Spectrometer gain 2.5 X 10^{3}. Iron bound to transferrin was 1.1 X 10^{-4} M.

Figure 4: EPR spectrum obtained at 77°K of GIBP in 0.1 M NaCl. Microwave power - 30 mW. Modulation amplitude 10 Gauss. Microwave frequency 9.118 GHZ. Spectrometer gain 1.25 X 10^{4}. Iron bound to GIBP was 0.2 X 10^{-4} M.

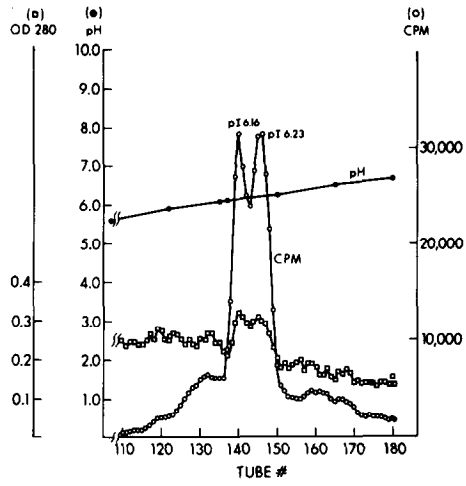


Figure 5: Partially purified GIBP was subjected to isoelectric focussing in a gradient of pH 5 - pH 8. The major count peaks (shown in the figure) contained 33% of the counts put on the column; 19% of the counts emerged as a peak with alkaline (cathodal) electrode solution. The remainder of the counts were spread diffusely through the column.

distinctive absorbance at 336 nm (Fig. 2). EPR spectroscopy of guinea pig transferrin shows a signal near g' = 4.17, characteristic of Fe (III) with structure showing deviation from complete rhombic symmetry (Fig. 3). GIBP has its major EPR peak near g' = 4.22 and a minor peak at g' = 5.55 (Fig. 4). The former, nearly isotropic line, is indicative of Fe (III) with almost complete rhombic symmetry. The origin of the g' = 5.55 signal is obscure.

On isoelectric focussing of partially purified GIBP a bifid count peak, coincident with a bifid 280 nm peak, is demonstrated. The first peak is at pH 6.16; the second peak is at pH 6.23 (Fig. 5).

Amino acid analysis of transferrin and GIBP reveals significantly different contents of tyrosine, isoleucine and serine (Table II). These differences indicate that the new iron-binding protein, GIBP, is not a modified transferrin. The similarity in overall amino acid composition between GIBP and transferrin is perhaps as striking as the differences and suggests that the two proteins, though different, may be closely related. Transferrin appears

TABLE II

Amino Acid Analysis of Guinea Pig Intestinal Iron Binding Protein and Guinea Pig Transferrin. (Grams of Amino Acid residue per 100 grams protein).

	Guinea Pig Transferrin	Guinea Pig Intestinal Iron-Binding Protein
Lysine	10.8	11.5
Histidine	4.5	3.6
Arginine	5.9	5.3
Aspartic Acid	10.9	11.3
Threonine	4.5	5.0
Serine	6.7	4.1
Glutamic Acid	16.2	15.6
Proline	4.4	3.4
Glycine	4.6	4.9
Alanine	6.2	5.2
Valine	5.0	5.0
Isoleucine	3.4	6.3
Leucine	9.0	7.8
Tyrosine	2.9	5.5
Phenylalanine	5.1	5.5

to have two similar (though not identical) iron binding sites (8,9) and appears, by tryptic digestion, to have extensive internal homology (10) though the homology is far from complete (II). It is tempting to speculate that GIBP, which consists of two subunits, each approximately half the molecular weight of transferrin, may be a primitive precursor of transferrin.

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