Binding of gastrin to gastric transferrin

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Binding of ^{125}I-gastrin$_{2,17}$ to porcine gastric transferrin has been demonstrated by covalent cross-linking with disuccinimidyl suberate. The concentration of gastrin$_{17}$ required to reduce cross-linking by 50% was approx. 100 μM. The occurrence of both gastrin and gastric transferrin in porcine gastric mucosa and lumen suggests a novel synergistic role for the observed interaction in the uptake of dietary iron.

Gastrin *Iron uptake* *Transferrin*

1. INTRODUCTION

The hormone gastrin is synthesized in the antral mucosa of the stomach [1]. On release it stimulates the secretion of acid by the parietal cells of the gastric corpus [2]. The major circulating form of the hormone is a heptadecapeptide, containing an unusual sequence of five consecutive glutamic acids [3]. This negatively charged region is presumably responsible for the binding of divalent [4,5] and trivalent [5] metal ions by gastrin.

During a study of the gastrin binding proteins of porcine gastric mucosa we observed a cytosolic protein with many similar properties to serum transferrin [6]. The two proteins shared the same molecular mass (80 kDa), the same N-terminal sequence, and the ability to bind 2 molecules of Fe^{3+}. However the carbohydrate content of the gastric transferrin was 40% higher than porcine serum transferrin, and this difference, together with the abundance of the gastric protein, suggested that it was synthesized in the gastric mucosa. In this paper we describe the binding of gastrin to the gastric transferrin and postulate a role for this interaction in the uptake of dietary iron.

2. MATERIALS AND METHODS

$Na^{125}I$ (spec. act. 15.9 mCi/μg) was obtained from the Radiochemical Centre, Amersham, England. Disuccinimidyl suberate and trifluoroacetic acid were from Pierce, Rockford, IL.

Human [Met^{15}]gastrin$_{2,17}$ and human [Met^{15}]-gastrin$_{17}$ were from Research Plus, Bayonne, NJ. Human [Met^{15}]gastrin$_{2,17}$ was iodinated as described by Fraker and Speck [7] and the monoiodinated component (spec. act. 950 μCi/μg) purified by reverse-phase high-performance liquid chromatography as described by Baldwin and coworkers [8] except that the 0.1 M Na^+ phosphate buffer was replaced by 0.1% trifluoroacetic acid.

Porcine gastric transferrin was purified from the cytosolic fraction of the corpus of gastric mucosa by chromatography on lentil lectin-Sepharose and metal chelate-Sepharose as described [6]. Porcine serum transferrin was prepared from porcine plasma by the same method. Human serum transferrin was from Sigma, St. Louis, MO.

2.1. *Cross-linking of ^{125}I-gastrin$_{2,17}$ to gastric transferrin*

Mono human ^{125}I-gastrin$_{2,17}$ was reacted with 0.6 mM disuccinimidyl suberate in 50 mM Na^+ Hepes, pH 7.6 for 15 min at 0°C. 25 μl aliquots

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were added to 25 μl aliquots of samples containing gastric transferrin in the same buffer. After 20 min at 0°C reaction was stopped by addition of 50 μl of loading buffer [9]. The samples were heated at 95°C for 5 min and electrophoresed on SDS-10% polyacrylamide gels [9]. After staining with Coomassie blue, destaining and drying the gels were autoradiographed at −70°C with Dupont lighting plus intensifying screens and Kodak X-AR5 X-ray film.

3. RESULTS AND DISCUSSION

During a study of the gastrin-binding proteins of porcine gastric mucosa we purified a gastric variant of porcine serum transferrin by chromatography on lentil lectin-Sepharose and DEAE-Sepharose [6]. Binding of gastrin at various stages of the purification was assessed by covalent cross-linking of ^{125}I-gastrin$_{2,17}$ with disuccinimidyl suberate. Gel electrophoresis and autoradiography revealed a radioactive band in close association with the transferrin band under both reducing and non-reducing conditions (fig.1). (Presumably only a small proportion of the gastric transferrin was cross-linked to gastrin$_{2,17}$, so that the difference in molecular mass of approx. 2 kDa between the radioactive and Coomassie blue bands may be ascribed to the contribution of iodinated gastrin$_{2,17}$, which has a molecular mass of 2111 Da. A derivative (gastrin$_{2,17}$) lacking the N-terminal pyroglutamate residue of gastrin was used in this study because the intact hormone contains no free amino groups.)

The dissociation constant of the gastrin-gastric transferrin complex was estimated by inclusion of various concentrations of unlabelled gastrin$_{17}$ in the reaction. 50% inhibition of cross-linking of ^{125}I-gastrin$_{2,17}$ was observed at approx. 100 μM gastrin$_{17}$ (figs 2,3). Similar values were obtained for the binding of gastrin to porcine and human serum transferrins (fig.3). Preliminary ultracentrifugation experiments suggest that the true dissociation constant of the gastrin-gastric transferrin complex is at least an order of magnitude lower than this value (Baldwin, G.S., Howlett, G.J. and Knesel, J.A., unpublished). The difference between the two methods is presumably due to the irreversible nature of the cross-linking reaction.

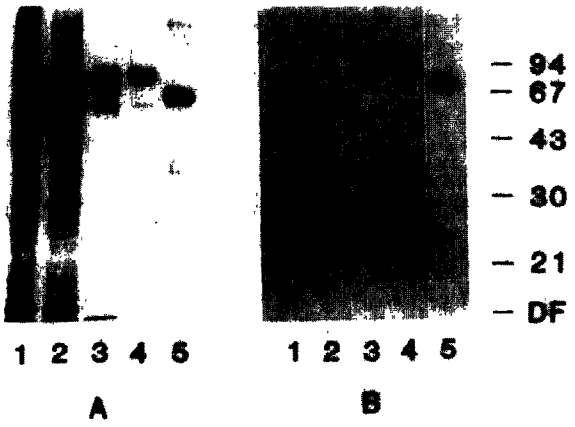

Fig.1. Binding of gastrin to gastric transferrin. Gastric transferrin was purified from the corporeal mucosa of porcine stomach as described [6]. Samples were removed at each stage of the preparation, cross-linked to ^{125}I-gastrin$_{2,17}$, reduced and electrophoresed on SDS-10% polyacrylamide gels, and autoradiographed as described in section 2. (A) Coomassie blue stain; (B) autoradiograph. Lanes: 1, cytosol; 2, redissolved 55-95% $(NH_4)_2SO_4$ pellet; 3, lentil lectin-Sepharose eluate; 4, metal chelate-Sepharose eluate; 5, as 4, but without reduction.

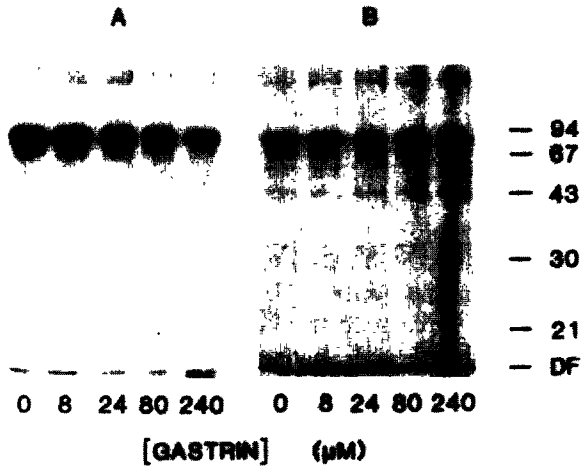

Fig.2. Inhibition of cross-linking by unlabelled gastrin$_{17}$. Aliquots of gastric transferrin were preincubated with the indicated concentrations of gastrin$_{17}$ for 15 min at 0°C, cross-linked to ^{125}I-gastrin$_{2,17}$, electrophoresed on SDS-10% polyacrylamide gels and autoradiographed as described in section 2. A, Autoradiograph; B, Coomassie blue stain.

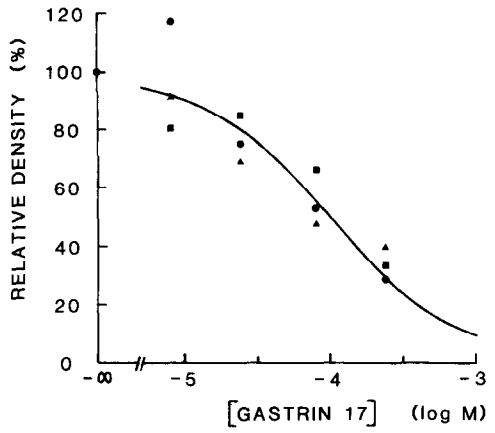

Fig.3. Inhibition of cross-linking by unlabelled gastrin$_{17}$. Aliquots of porcine gastric (▲), porcine serum (■), and human serum (●) transferrin were cross-linked to ^{125}I-gastrin$_{2,17}$ in the presence of various concentrations of gastrin$_{17}$ as described in the legend to fig.2. The samples were electrophoresed on SDS-10% polyacrylamide gels and autoradiographed as described in section 2. The amount of radioactivity associated with the transferrin band was determined by densitometer scanning of the autoradiograph, and was expressed as a percentage relative to the sample with no unlabelled gastrin$_{17}$. The theoretical curve for an IC_{50} value of 100 μM is shown.

We have previously proposed a model for the involvement of gastric transferrin in the uptake of dietary iron [6]. In the model gastric transferrin is released into the stomach lumen in response to ingestion of food, binds dietary iron as the pH of the luminal contents rises during the entry of food into the small intestine and is finally absorbed together with its bound iron by the intestinal mucosa. The role of gastric transferrin in iron uptake is thus postulated to be analogous to the role of intrinsic factor in the uptake of vitamin B_{12} [10]. This mechanism provides an explanation for the iron deficiency frequently observed in patients with atrophic gastritis or following partial gastrectomy [11–13]. In either case the reduced volume of the gastric mucosa would lead to a reduction in gastric transferrin output with a consequent reduction in iron uptake and hence ultimately to iron deficiency.

The interaction between gastric transferrin and gastrin revealed by covalent cross-linking (fig.1) suggests that the hormone may also be involved in the above mechanism for iron uptake. A strong interaction between gastrin and trivalent metal ions is indicated by the precipitation observed when Eu^{3+} [5] or Fe^{3+} (Baldwin, G.S., unpublished) is added to concentrated (1–2 mg/ml) solutions of gastrin. The presence of significant amounts of gastrin in the gastric juice of many species [14,15] suggests that complexes between gastrin and trivalent metal ions may also exist in the gastric contents. Since efficient loading of transferrin in vitro requires an anionic chelating agent such as nitriliotriacetic acid [16], it is possible that the unusual pentaglutamic acid sequence present in gastrin may fulfil a similar catalytic role in vivo. Thus, gastrin and gastric transferrin are postulated to act synergistically in the uptake of dietary iron by the gastrointestinal tract.

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