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Articles

Selective, High-Affinity Binding of Ferric Ions by Glycine-Extended Gastrin$_{17}$[†]

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ABSTRACT: Uptake of dietary iron is essential for replenishment of body stores. A role for the hormone gastrin in iron uptake as a chelator of ferric ions in the gastric lumen has been proposed previously [Baldwin, G. S. (1992) *Med. Hypotheses 38*, 70−74]. Here, spectroscopic evidence of selective, high-affinity binding of ferric ions to progastrin-derived peptides in aqueous solution at low pH is provided. The maximum at 281 nm in the absorption spectrum of glycine-extended gastrin$_{17}$ at pH 4.0 increased (2.07 ± 0.30)-fold in the presence of ≥2 equiv of ferric ions. Titration of glycine-extended gastrin$_{17}$ with ferric ions under stoichiometric conditions indicated that the stoichiometry of binding was 2.00 ± 0.28 mol of Fe^{3+}/mol of peptide. Fluorescence quenching experiments yielded values for the stoichiometry and apparent dissociation constant of the ferric ion−glycine-extended gastrin$_{17}$ complex at pH 4.0 of 2.39 ± 0.17 mol of Fe^{3+}/mol and 0.62 ± 0.19 μM, respectively. No interaction was detected with Co^{2+}, Cu^{2+}, Mn^{2+}, or Cr^{3+}. Electron paramagnetic resonance spectroscopy suggested that the iron ligands were either oxygen or sulfur atoms. Fluorescence quenching experiments with peptides derived from the glycine-extended gastrin$_{17}$ sequence indicated that one or more of the five glutamic acid residues were necessary for iron binding. The binding of ferric ions by glycine-extended gastrin$_{17}$ at low pH is consistent with a role for progastrin-derived peptides in iron uptake from the lumen of the gastrointestinal tract.

More than 8% of the world's population suffers from iron deficiency (*1*). At least some of this group will have defective mechanisms of iron uptake. While there have been major recent advances in our understanding of the pathway of intestinal iron uptake with the cloning of the divalent metal ion transporters responsible for ferrous ion uptake into [divalent metal transporter 1 (DMT1)[1] (*2*)], and export from [ferriportin (*3*)/IREG1 (*4*)], duodenal enterocytes, significant gaps in knowledge still remain. In particular, the problem of the limitation of absorption by the insolubility of ferric and ferrous ions in the neutral milieu of the duodenal lumen has not been addressed. We have previously proposed that the peptide hormone gastrin bound ferric ions in the acidic environment of the gastric lumen, and prevented the formation of insoluble ferric hydroxides as the pH increased on entry into the duodenum (*5*).

Gastrin was identified originally as an hormonal stimulant of gastric acid secretion (*6*), but significant concentrations are also found in the gastric lumen (*7*). The minimum active

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[1] Abbreviations: CCK, cholecystokinin; DMT1, divalent metal transporter 1; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EPR, electron paramagnetic resonance.

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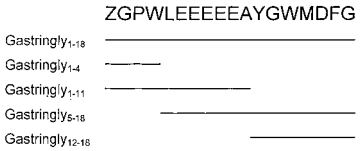

FIGURE 1: Structures of glycine-extended gastrin$_{17}$ and related peptides. The structures of N- and C-terminally truncated peptides derived from glycine-extended gastrin$_{17}$ (Gastringly$_{1-18}$) are shown. Amino acids are shown in one-letter code, and Z represents a pyroglutamic acid residue. Glycine-extended gastrin$_{17}$ corresponds to residues 55−72 of mature progastrin$_{1-80}$.

fragment is the C-terminal tetrapeptide amide, which is also present in the related hormone cholecystokinin (CCK). Like many other peptide hormones, gastrin is synthesized as a large precursor molecule of 101 amino acids, which is converted to progastrin (80 amino acids) by cleavage of the N-terminal signal peptide. Progastrin is processed further by endo- and carboxypeptidases and by C-terminal amidation to yield the final end products glycine-extended gastrin$_{17}$ and amidated gastrin$_{17}$ (*8*). The recognition that nonamidated C-terminally extended gastrins are able to stimulate growth of the colonic mucosa in vivo and colon-derived cell lines in vitro has renewed interest in the gastrin/CCK family of peptides (*9*).

The sequences of glycine-extended gastrin$_{17}$ (Figure 1) and amidated gastrin$_{17}$ contain five consecutive glutamic acid residues. The possibility that this negatively charged sequence might bind metal ions was first investigated some twenty years ago (*10*−*12*). Binding of Mg^{2+} and Ca^{2+} to both [Nle^{11}]gastrin$_{13}$ and [Nle^{15}]gastrin$_{17}$ was detected by spectrophotometric titration and by circular dichroism in trifluoroethanol. The stoichiometry of binding was 3 mol/mol, and the dissociation constants for binding of Mg^{2+} to the three sites were as follows: $K_1 = 0.17$ μM, $K_2 = 0.5$ μM, and $K_3 = 2.0$ μM. Because binding of Ca^{2+} was not detected in aqueous solution by circular dichroism (*10*) or by nuclear magnetic resonance spectroscopy (*13*), there was little further interest in the field.

We subsequently observed an interaction between gastrin$_{17}$ and ferric ions at pH 5.0 in sedimentation velocity experiments in the ultracentrifuge (*14*). To obtain further information about the complex of ferric ions and gastrins, a detailed spectroscopic study has been undertaken. Because of the limited solubility of amidated gastrin$_{17}$ at low pH, the glycine-extended form of the peptide was chosen for investigation, since the extra C-terminal carboxyl group was expected to increase the peptide's solubility. In this paper, we report the results of an investigation of the stoichiometry, affinity, and selectivity of the binding of paramagnetic metal ions to glycine-extended gastrin$_{17}$.

MATERIALS AND METHODS

Chemicals. Gastrin$_{17}$gly was from Auspep (Melbourne, Australia). All other peptides were from Chiron (Melbourne, Australia). Peptide concentrations were calculated from their absorbance at 280 nm using the molar extinction coefficients of the component amino acids as described by Gill and Von Hippel (*15*). The molar extinction coefficient for gastrin$_{17}$-gly calculated by this method (12 660 M^{-1} cm^{-1}) was not significantly different from the experimentally determined value of the molar extinction coefficient for [Nle^{11}]gastrin$_{13}$ at 278 nm (12 320 M^{-1} cm^{-1}) reported by Wunsch and coworkers (*10*). Absorbance values were determined on a Cary 5 spectrophotometer (Varian, Mulgrave, Australia) at 25 °C.

Absorption Spectroscopy. Absorption spectra of peptides in the presence of increasing concentrations of metal ions were measured, in 3 mL quartz cuvettes thermostated at 25 °C, in a Cary 5 spectrophotometer (Varian).

Fluorescence Spectroscopy. The tryptophan fluorescence of peptide solutions was measured, in 3 mL quartz cuvettes thermostated at 25 °C, with a Spex Fluorolog-τ2 spectrofluorimeter (Spex Industries, Edison, NJ), with the excitation and emission wavelengths set at 290 and 345 nm, respectively. The quenching of tryptophan fluorescence induced by the binding of metal ions was used to calculate the fraction of binding sites occupied, f_a:

$$f_a = (y_f - y)/(y_f - y_b)$$

where y is the fluorescence signal at a given concentration of metal ions and y_b and y_f are the signals when the binding sites are fully occupied and unoccupied, respectively (*16*). The stoichiometry, p, and apparent dissociation constant, K_d, were then obtained, using the program Sigmastat (Jandel Scientific, San Rafael, CA), from the intercept and slope of a linear regression of the data transformed as described by Winzor and Sawyer (*16*) in terms of the equation

$$C_s/f_a = pC_a + K_d/(1 - f_a)$$

where C_s is the total concentration of metal ions and C_a is the total concentration of glycine-extended gastrin$_{17}$.

Electron Paramagnetic Resonance Spectroscopy. X-band electron paramagnetic resonance (EPR) spectra of the Fe^{3+}−peptide complex were obtained using a Bruker EC106 spectrometer. Samples were loaded into glass hematocrit capillary tubes, which had previously been checked for the absence of iron and manganese. Similarly, blank samples of buffer were run at the same frequency and magnetic field range to exclude the presence of paramagnetic contaminants. The sample temperature was maintained at 110 K using a flow-through cryostat. The microwave frequency was measured using a Bruker EIP 548B frequency counter, and the magnetic field was calibrated with a DPPH sample.

Statistics. Results are expressed as the mean ± the standard error of the mean, except where otherwise stated.

RESULTS

The majority of the experiments described in this paper were conducted at pH 4.0. This pH was chosen as a balance to maintain both the solubility of gastrin, which decreases rapidly at pH <5.5 (*14*), and the solubility of ferric ions, which decreases rapidly at pH >4.0 with formation of insoluble hydroxides.

Affinity and Stoichiometry of Binding of Ferric Ions by Glycine-Extended Gastrin$_{17}$. Clear evidence for the formation of a complex of glycine-extended gastrin$_{17}$ and two ferric ions in aqueous solution at low pH was provided by absorption spectroscopy. Binding of ferric ions profoundly affected the absorption spectrum of glycine-extended gastrin$_{17}$ in 10 mM sodium acetate (pH 4.0) containing 100 mM NaCl and 0.005% Tween 20 (Figure 2A). The maximum absorbance at 281 nm increased (2.07 ± 0.30)-fold ($n = 3$) in the presence of ≥2 equiv of ferric ions. Titration of glycine-

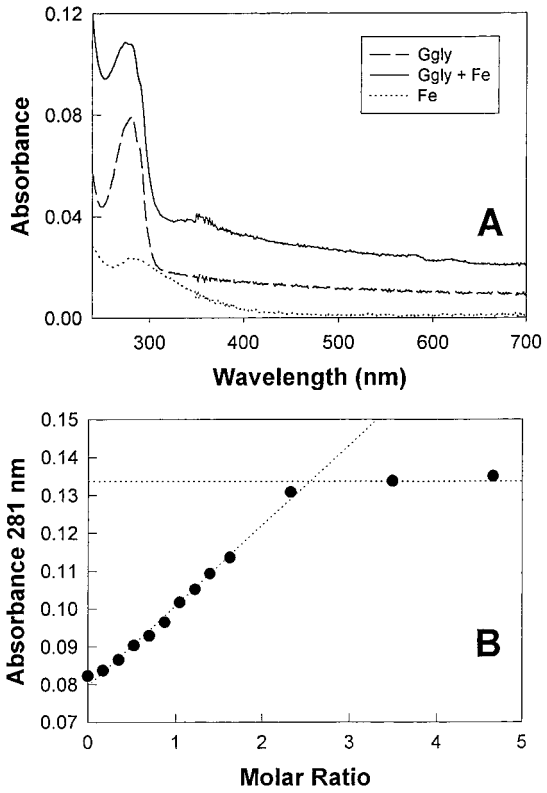

FIGURE 2: Binding of metal ions by glycine-extended gastrin$_{17}$. (A) The absorption spectrum of 6.9 μM glycine-extended gastrin$_{17}$ (dashed line), in 10 mM sodium acetate (pH 4.0) containing 100 mM NaCl and 0.005% Tween 20, was measured against a buffer blank at 25 °C in a Cary 5 spectrophotometer as described in Materials and Methods. Addition of 2.2 mol of ferric chloride/mol resulted in a 1.4-fold increase in the absorption maximum at 281 nm (solid line), after correction for the change observed when ferric chloride was added to buffer alone (dotted line). (B) In a separate experiment, addition of aliquots of ferric chloride to 6.9 μM glycine-extended gastrin$_{17}$ in the same buffer resulted in a linear increase up to a molar ratio of 2 in the absorption maximum at 281 nm. Curve fitting similar data from three independent experiments gave values of 2.00 ± 0.28 mol of Fe^{3+}/mol of peptide for the stoichiometry and (2.07 ± 0.30)-fold for the maximum increase in absorption at 281 nm.

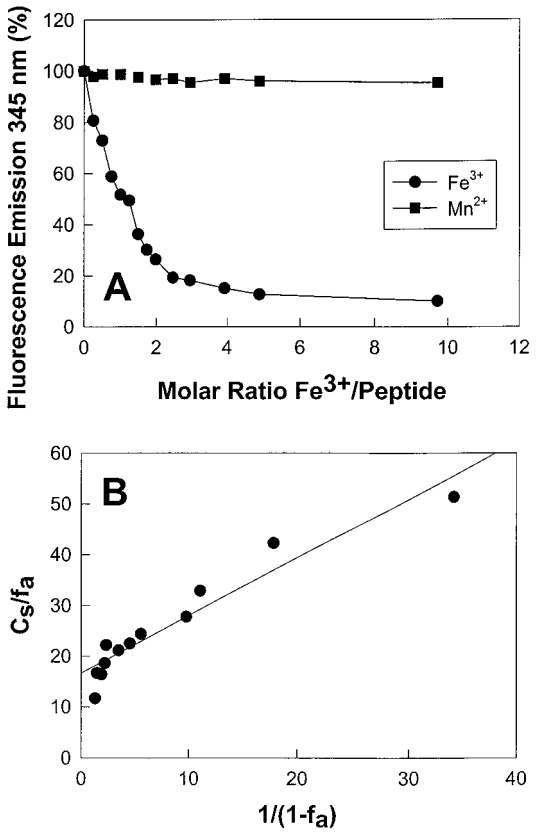

FIGURE 3: Selectivity of metal ion binding by glycine-extended gastrin$_{17}$. (A) The quenching by ferric (●) or manganous ions (■) of the tryptophan fluorescence of 10 μM glycine-extended gastrin$_{17}$ in 10 mM sodium acetate (pH 4.0), containing 100 mM NaCl and 0.005% Tween 20, was measured as described in Materials and Methods. (B) The experimental data were linearly transformed as described in Materials and Methods, and the line indicates the best fit obtained by least-squares fitting with the program Sigmastat. The values of the stoichiometry and the apparent dissociation constant obtained from the slope and intercept in three independent experiments were combined to obtain the mean values presented in Table 1.

extended gastrin$_{17}$ with ferric ions under stoichiometric conditions indicated that the stoichiometry of binding was 2.00 ± 0.28 mol of Fe^{3+}/mol of peptide (n = 3) (Figure 2B). A similar increase in absorption was also observed on addition of ferric ions to amidated gastrin$_{17}$. However, the glycine-extended form of gastrin$_{17}$ was chosen over amidated gastrin$_{17}$ for further experiments because the extra C-terminal carboxyl group was expected to increase peptide solubility.

Additional evidence for the formation of a ferric ion−glycine-extended gastrin$_{17}$ complex was provided by fluorescence quenching experiments. The tryptophan fluorescence of glycine-extended gastrin$_{17}$ in 10 mM sodium acetate (pH 4.0) containing 100 mM NaCl and 0.005% Tween 20 was quenched by added ferric ions (Figure 3A). The maximum concentration of glycine-extended gastrin$_{17}$ in fluorescence experiments was restricted to 10 μM (A_{290} = 0.060) to minimize possible complications from inner filter effects. Analysis of the data (Figure 3B) from 12 independent experiments as described by Winzor and Sawyer (*16*) yielded mean values for the stoichiometry and apparent dissociation constant of 2.39 ± 0.17 and 0.62 ± 0.19 μM, respectively (Table 1). Quenching of tryptophan fluorescence was also observed on addition of ferric ions to amidated gastrin$_{17}$ (data not shown).

Table 1: Stoichiometry and Affinity of Metal Ion Binding by Glycine-Extended Gastrin$_{17}$ and Related Peptides[a]

peptide	metal ion	pH	stoichiometry	K_d (μM)	no. of replicates
Gastringly$_{1-18}$	Fe^{3+}	4.0	2.4 ± 0.2	0.6 ± 0.2	12
Gastringly$_{1-18}$	Fe^{3+}	2.8	2.2 ± 0.3	6.1 ± 0.6	3
Gastringly$_{1-11}$	Fe^{3+}	4.0	2.0 ± 0.3	1.20 ± 0.04	3
Gastringly$_{5-18}$	Fe^{3+}	4.0	2.2 ± 0.2	0.6 ± 0.2	3

[a] The tryptophan fluorescence of glycine-extended gastrin$_{17}$ and related peptides was measured in the presence of increasing concentrations of ferric ions at the indicated pH values as described in the legends of Figures 2−4. Values of the stoichiometry and the apparent dissociation constant (K_d) were obtained from linear transformations of the data by least-squares fitting with the program Sigmastat as described in Materials and Methods. Values from the indicated number of independent experiments were combined to obtain the mean values (±standard error) presented above.

Selectivity of Metal Ion Binding by Glycine-Extended Gastrin$_{17}$. Comparison of the effect of several paramagnetic metal ions on the tryptophan fluorescence of glycine-extended gastrin$_{17}$ indicated that only ferric ions quenched appreciably (Figure 3A). No quenching was observed in the presence of cobaltous, cupric, manganous, or chromic ions.

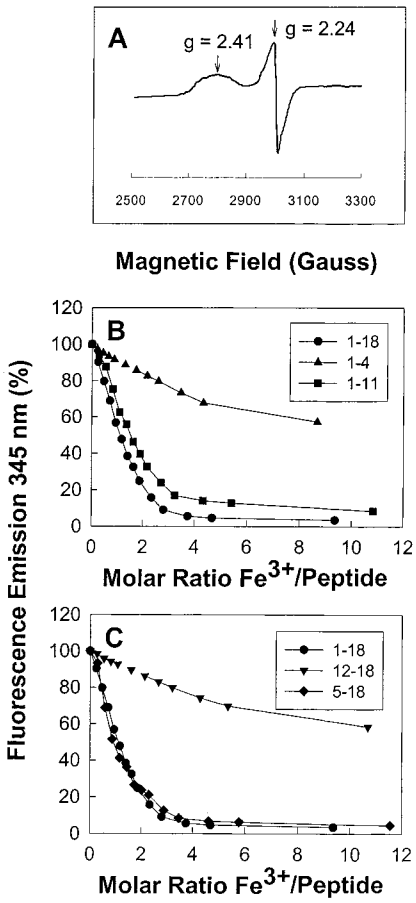

FIGURE 4: Glutamate residues 6−10 act as metal ion ligands. (A) The X-band (9.135 GHz) EPR spectrum of the complex of glycine-extended gastrin$_{17}$ and ferric ions was obtained at 110 K as described in Materials and Methods. The two observed peaks (*g* values shown on plot) were consistent with a low-spin Fe^{3+} ($S = {}^1/_2$) and either O or S atoms acting as ligands. The quenching by ferric ions of the tryptophan fluorescence of C-terminally (B) or N-terminally (C) truncated peptides derived from glycine-extended gastrin$_{17}$ was measured as described in Materials and Methods. The peptides were as follows: glycine-extended gastrin$_{17}$ (Gastringly$_{1-18}$, ●), residues 1−4 of glycine-extended gastrin$_{17}$ (Gastringly$_{1-4}$, ▲), residues 1−11 of glycine-extended gastrin$_{17}$ (Gastringly$_{1-11}$, ■), residues 12−18 of glycine-extended gastrin$_{17}$ (Gastringly$_{12-18}$, ▼), and residues 5−18 of glycine-extended gastrin$_{17}$ (Gastringly$_{5-18}$, ◆). The buffer was 10 mM sodium acetate (pH 4.0) containing 100 mM NaCl and 0.005% Tween 20. Values of the stoichiometry and the apparent dissociation constant were obtained from linear transformations of the data by least-squares fitting with the program Sigmastat. The values from three independent experiments were combined to obtain the mean values presented in Table 1. Only those peptides containing the five glutamic acid residues at positions 6−10 of glycine-extended gastrin$_{17}$ bound ferric ions.

Identification of Metal Ion Ligands. The identities of the side chains of glycine-extended gastrin$_{17}$ which act as ferric ion ligands were next investigated. A preliminary study of mixtures of glycine-extended gastrin$_{17}$ and ferric ions by electron paramagnetic resonance (EPR) spectroscopy at 110 K suggested that the Fe center was liganded to either oxygen or sulfur atoms (Figure 4A). To narrow the candidate ligands, the effect of ferric ions on the fluorescence of various peptides derived from the glycine-extended gastrin$_{17}$ sequence was investigated. The quenching of fluorescence of a peptide comprised of residues 1−11 of the glycine-extended gastrin$_{17}$ sequence by ferric ions was similar to that observed with glycine-extended gastrin$_{17}$ (Figure 4B). In contrast, the quenching of fluorescence of a peptide comprised of residues 1−4 of the glycine-extended gastrin$_{17}$ sequence by ferric ions was markedly reduced. Similarly, the quenching of fluorescence of a peptide comprised of residues 5−18 of the glycine-extended gastrin$_{17}$ sequence by ferric ions was similar to that observed with glycine-extended gastrin$_{17}$ (Figure 4C), while the quenching of fluorescence of a peptide comprised of residues 12−18 of the glycine-extended gastrin$_{17}$ sequence by ferric ions was markedly reduced. The affinities and stoichiometries of binding of ferric ions to the above peptides are presented in Table 1. These observations suggest that the major binding determinant is within residues 5−11, in the region which encompasses the five glutamic acids at positions 6−10, and are consistent with the involvement of one or more of the five glutamic acids (E6−E10) in iron binding.

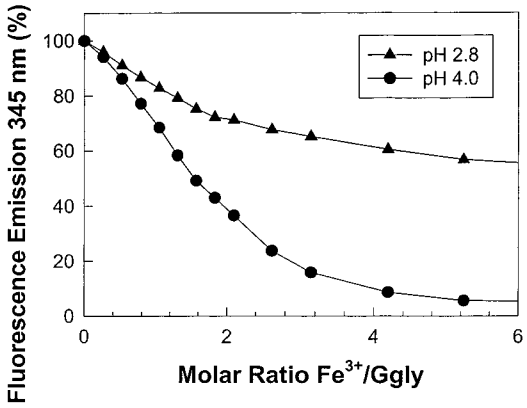

FIGURE 5: pH dependence of the binding of ferric ions by glycine-extended gastrin$_{17}$. The quenching by ferric ions of the tryptophan fluorescence of 10 μM glycine-extended gastrin$_{17}$ in 10 mM sodium acetate (pH 4.0) (●) or 10 mM sodium formate (pH 2.8) (▲) was measured as described in Materials and Methods. Both buffers contained 100 mM NaCl and 0.005% Tween 20. The values of the stoichiometry and the apparent dissociation constant obtained by least-squares fitting of linear transformations of the data from three independent experiments were combined to obtain the mean values presented in Table 1. Binding of ferric ions was still observed at pH 2.8, but the affinity was 10-fold lower than at pH 4.0.

pH Dependence of Binding of Ferric Ions by Glycine-Extended Gastrin$_{17}$. The ability of ferric ions to quench the tryptophan fluorescence of glycine-extended gastrin$_{17}$ was measured at different pH values (Figure 5). Quenching was maximal at pH 4.0, but was still detected at pH 2.8. The apparent dissociation constant ($K_d = 6.1$ μM) was 10-fold higher than the value at pH 4.0 (Table 1).

DISCUSSION

This paper provides clear evidence for the formation in aqueous solution of a complex of glycine-extended gastrin$_{17}$ and two ferric ions. The two dissociation constants for the $(Fe^{3+})_2$−glycine−extended gastrin$_{17}$ and Fe^{3+}−glycine-extended gastrin$_{17}$ complexes must be similar, since the secondary plot shown in Figure 3B does not show a major deviation from linearity at low abscissa values. A similar phenomenon was observed for the binding of Mg^{2+} and Ca^{2+} to both [Nle^{11}]gastrin$_{13}$ and [Nle^{15}]gastrin$_{17}$ in trifluoroethanol. The dissociation constants for binding of Mg^{2+} to the three sites differed by only 1 order of magnitude ($K_1 = 0.17$ μM, $K_2 = 0.5$ μM, and $K_3 = 2.0$ μM). Since the coordination number of the ferric ion is usually 6, the observed stoichiometry is 2, and glycine-extended gastrin$_{17}$ contains seven

acidic residues (E6−E10, D16, and the C-terminal carboxyl group), the complex may contain as many as five water molecules. Alternatively, the carboxyl groups or water molecules may form Fe−O−Fe bridges. In any case, the complex will have a net charge of −1 when all the carboxyl groups are unprotonated.

The data presented herein are consistent with the involvement of one or more of the five glutamic acids (E6−E10) in iron binding. The two peaks with g values of 2.24 and 2.41 observed in the EPR spectra (Figure 4A) of the complex of glycine-extended gastrin$_{17}$ and ferric ions were consistent with the presence of a low-spin Fe^{3+} center ($S = {}^1/_2$) with either oxygen or sulfur atoms as ligands (*17*). The inhomogeneous broadening of the $g = 2.41$ peak prevented measurement of g anisotropy. Further resolution of the liganding would require collection of spectra at <12 K, and possibly the use of electron-spin-echo techniques. Candidates for the oxygen ligand include the carboxyl groups of the five glutamic acid residues (E6−E10), of the single aspartic acid residue (D16), and of the C-terminal glycine (G18) (Figure 1). On the other hand, the sulfur atom of the single methionine (S15) might also contribute to the binding. However, the fact that a peptide consisting of residues 1−11 of glycine-extended gastrin$_{17}$, and hence lacking the methionine (S15), the aspartic acid (D16), and the C-terminal glycine (G18), had an affinity for ferric ions similar to that of glycine-extended gastrin$_{17}$ itself indicates that none of these three residues contributes significantly to binding. These fluorescence quenching experiments further suggest that the major binding determinant is within residues 5−11, in the region which encompasses the five glutamic acids at positions 6−10, and are consistent with the involvement of one or more of the five glutamic acid (E6−E10) in iron binding. Since four of the five glutamic acid residues of human gastrin are conserved across all mammalian species except the horse (*18*), similar binding should be observed with most mammalian gastrins.

The fluorescence of both tryptophan residues (W4 and W14) of glycine-extended gastrin$_{17}$ appears to be quenched in the presence of ferric ions. Thus, the fluorescence emission of glycine-extended gastrin$_{17}$ in the presence of a 10-fold molar ratio of ferric ions is less than 5% of the emission in the absence of ferric ions (Figure 3A). In contrast, circular dichroism experiments have suggested that the binding of calcium ions to [Nle^{15}]gastrin$_{17}$ in trifluoroethanol affects only one of the two tryptophan residues (*12*). This difference may be attributed to either the different metal ion, the different solvent, or both. The fact that the fluorescence of two peptides related to glycine-extended gastrin$_{17}$, in which each tryptophan had been separately replaced with glycine, was completely quenched by added ferric ions (data not shown) confirmed that the fluorescence of both tryptophan residues of glycine-extended gastrin$_{17}$ was quenched by ferric ions in aqueous solution.

High-affinity binding ($K_d = 6.1\ \mu M$) of ferric ions to glycine-extended gastrin$_{17}$ was still observed at pH 2.8. Interestingly, comparison with the value [$K_d = 26\ \mu M$ (*19*)] calculated for the commonly used ferric ion chelator nitrilotriacetic acid indicates that glycine-extended gastrin$_{17}$ is actually the better chelator at pH 2.8. Moreover, the interaction may be physiologically relevant, since the resting pH of the gastric lumen is approximately 2, and may rise above 4 after a meal (*20*). Conversely, binding of ferric ions to glycine-extended gastrin$_{17}$ will presumably be stronger at neutral pH than at pH 4.0, because of the reduced competition by H^+ ions for binding to glutamate residues at the higher pH. For comparison, the apparent first dissociation constants calculated for the complex of ferric ions and nitrilotriacetic acid are 0.19 and 830 nM at pH 7.6 and 4.0, respectively (*19*). Similar calculations could not be made for glycine-extended or amidated gastrin$_{17}$, because attempts to determine the pK_a values of the glutamate residues were hampered by precipitation of the peptide at low pH. Estimates of pK_4, pK_5, and pK_6 for amidated gastrin$_{17}$ were ∼5.0, 6.6, and ∼9.2, respectively, at an ionic strength of 0.15 M and 25 °C (E. P. Serjeant and G. S. Baldwin, unpublished data). In addition, precipitation of ferric hydroxides interfered with attempts to measure the apparent dissociation constant of the complex of ferric ions and glycine-extended gastrin$_{17}$ by fluorescence spectroscopy at pH >4.0. However, dialysis experiments with the complex formed at pH 4.0 between recombinant human progastrin$_{6-80}$ (*21*) and $^{59}Fe^{3+}$ ions indicate that its stability is greater at pH 7.6 than at pH 4.0, with half-lives at 25 °C of 16 and 2800 h, respectively (G. S. Baldwin, unpublished data). Binding of ferric ions to glycine-extended gastrin$_{17}$ at pH 7.6 was weaker than binding of ferric ions to nitrilotriacetic acid [$K_{d1} = 2$ pM (*19*)], since no change in the fluorescence spectrum of glycine-extended gastrin$_{17}$ was observed on addition of the ferric−nitrilotriacetic acid (1/1) complex at pH 7.6 (data not shown).

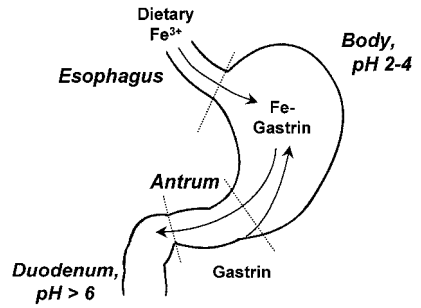

FIGURE 6: Model for a possible role of gastrin in iron uptake. Some gastrin is released from the mucosa of the gastric antrum into the lumen of the stomach (*7*). The model proposes that the binding of ferric ions by gastrin in the acidic environment (pH 2−4) of the gastric lumen prevents the formation of insoluble ferric hydroxides as the pH increases on entry into the duodenum (pH >6). A greater concentration of ferric ions would then be available, either for reduction to ferrous ions with subsequent uptake by the divalent metal ion transporter DMT1 (*2*) or for transfer to luminal apotransferrin with subsequent uptake of the iron-loaded transferrin by the duodenal mucosa (*22*).

Does the Ferric Ion−Gastrin Complex Play a Role in Dietary Iron Uptake? The complex of ferric ions and gastrin has previously been postulated to play a role in the uptake of ferric ions from the gastrointestinal tract (*5*). The model proposed that the binding of ferric ions by gastrin in the acidic environment of the gastric lumen prevented the formation of insoluble ferric hydroxides as the pH increased on entry into the duodenum (Figure 6). The observed affinities for binding of ferric ions by gastrin at different pH values (Figure 5) are exactly those required for efficient iron uptake. On one hand, moderate-affinity binding should be maintained at very low pH to ensure chelation of ferric ions under the acidic conditions of the gastric lumen. The

wide range of pK_a values resulting from the close proximity of the five consecutive glutamic acid residues of gastrins ensures that some of the glutamate carboxyl groups remain unprotonated, and hence able to contribute to ferric ion binding, even at low pH. On the other hand, binding at neutral pH should not be of excessively high affinity, since transfer of ferric ions to the proteins responsible for uptake of iron by the duodenal mucosa would then be impaired. The experimental evidence in the previous paragraph indicates that gastrins also satisfy the second criterion, and hence might well be involved in iron uptake from the lumen of the gastrointestinal tract. Further tests of the hypothesis might include measurement of the concentrations of nonamidated progastrin-derived peptides in gastric juice, and isolation from the same source of a complex of ferric ions and a progastrin-derived peptide.

Several groups have proposed that transferrin might also be involved in gastrointestinal iron uptake (*5*, *22*, *23*). Our model (*5*) was based on the observation that gastrin bound to apotransferrin in covalent cross-linking experiments (*24*). The stoichiometry and dissociation constant for the gastrin−apotransferrin complex, determined at pH 7.4 from sedimentation equilibrium experiments in the ultracentrifuge, were 2 and 4.8 μM, respectively (*14*). In our original model (*5*), binding of the gastrin−ferric ion complex to luminal apotransferrin (*14*, *24*) was postulated to permit transfer of the metal ions to transferrin, which was then taken up by the duodenal mucosa. An essential role for transferrin in iron uptake now appears unlikely, since hypotransferrinaemic mice, in which serum transferrin levels are less than 2% of normal, show a 2-fold enhancement of $^{59}Fe^{3+}$ uptake by the duodenal mucosa when compared to wild-type mice (*25*). However, the possibility remains that transferrin, either acting alone (*22*, *23*) or together with gastrin (*5*), may contribute to iron uptake.

Do Ferric Ions Influence Gastrin Receptor Binding? Although this study clearly defines an interaction between ferric ions and gastrins, the influence of ferric ions on receptor binding of gastrins is yet to be determined. Gastrin/CCK-B receptors, which mediate the release of acid from gastric parietal cells, are selective for amidated members of the gastrin/CCK peptide family (*26*). The observation that gastrin/CCK-B receptors recognize $gastrin_{17}$ and CCK_8, which lacks the pentaglutamate sequence implicated in the binding of ferric ions to gastrin (Figure 4), with similar affinities (*26*) suggests that complex formation with ferric ions may not affect binding to this receptor. High-affinity receptors selective for glycine-extended $gastrin_{17}$ were first reported for the rat pancreatic carcinoma cell line AR4-2J (*27*), and have subsequently been described for several colon-derived cell lines (*28*−*30*). These receptors are likely to mediate the mitogenic effects of glycine-extended $gastrin_{17}$ on cell lines in vitro (*27*−*30*), and may be responsible for the hyperplasia observed in the normal colonic mucosa of transgenic mice overexpressing glycine-extended $gastrin_{17}$ (*31*). However, cDNAs encoding high-affinity receptors for glycine-extended $gastrin_{17}$ have not yet been isolated, at least in part because of difficulties with the reproducibility of the binding assay. One exciting possibility is that the natural ligand for such receptors may not be glycine-extended $gastrin_{17}$ itself, but the complex of glycine-extended $gastrin_{17}$ and ferric ions.

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