Solution Visible Difference Spectral Properties of Fe^{3+}–L-Amino Acid Complexes at pH 6.60

I. Mohan Reddy* and Arthur W. Mahoney[†]

Western Center for Dairy Protein Research and Technology, Department of Nutrition and Food Sciences, Utah State University, Logan, Utah 84322-8700

Solution visible difference absorption spectral properties of the complexes of Fe^{3+} with different L-amino acids, N-acetyl-L-amino acids, L-amino acid methyl esters, O-phospho-L-serine, phosvitin, and conalbumin were studied at pH 6.60 in the range 350–750 nm. There were extensive similarities in the spectral properties of various Fe^{3+}–amino acid complexes but considerable variation in the differential molar absorptivities ($\Delta\epsilon$, M^{-1} cm^{-1}) of the absorption bands (λ_{max}, nm) calculated for different Fe^{3+}–amino acid complexes. In general, N-acetyl-L-amino acid derivatives showed much higher affinity to complex with Fe^{3+} than either L-amino acids or their methyl ester derivatives. The spectral data indicated that coordination of Fe^{3+} to an oxygen is more strongly favored than that to the nitrogen group of the amino acid. α-Amino nitrogens in amino acids, however, appear to lack an affinity for Fe^{3+} ions, as do hydroxyl groups of L-serine and L-threonine. The spectra of the complexes of Fe^{3+} with various amino acids and their derivatives were used to make the following spectral band assignments: The positive peaks at 422–424, 470–472, and 571–575 nm and the positive shoulder at 493–494 nm, as in the case of various L-amino acids (except L-tyrosine) and their N-acetylated derivatives, were assigned to yellow complexes of Fe^{3+} with carboxyl oxygens, although the ϵ-amino nitrogen of lysine, the guanidino nitrogen of arginine, and the imidazole nitrogen of hisitidine may also be involved in the Fe^{3+}–ligand bonding. The single positive peak at 485–493 nm as in the case of L-tyrosine and N-acetyl-L-tyrosinamide was assigned to reddish brown complexes of Fe^{3+} with phenolate oxygens. The negative absorption band at 416–420 nm as in the case of O-phospho-L-serine and phosvitin was assigned to rust colored complexes of Fe^{3+} with phosphoseryl groups. The complexes of Fe^{3+} involving both carboxyl and phenolate oxygens as in the case of N-acetyl-L-tyrosine and conalbumin, however, had a characteristic spectrum with positive peaks at 473 and 493 nm and positive shoulders at 430 and 570 nm.

INTRODUCTION

The study of iron(III)–amino acid complexes not only aids in elucidating the nature of nonheme iron(III)–protein interactions but also increases our understanding of the nature of binding of iron(III) to amino acids in view of (i) their beneficial effect on iron absorption (Green et al., 1947; Kroe et al., 1963; Martinez-Torres and Layrisse, 1970; Van Campen, 1979) and (ii) their possible use in clinical applications as chelating agents for the treatment of genetic disorders, such as Cooley's anemia and Wilson's disease (Cartwright et al., 1954; McAuliffe and Murray, 1972; Winston et al., 1985). To this end, the crystalline complexes of iron(III) perchlorate with glycine, L-alanine, L-valine, L-leucine, L-isoleucine, and L-proline have been isolated, and their electronic, infrared, and Mössbauer spectral properties, magnetic properties, and three-dimensional X-ray data have been determined (Holt et al., 1974; Tucker et al., 1975). However, the properties of solution complexes of iron(III) with amino acids have not been studied, except with glutathione, cysteine, and some related thiols and lysine (Bell et al., 1971; Hamed et al., 1982, 1983a,b; Hamed and Silver, 1983; Flynn et al., 1984; McAuliffe and Murray, 1972; Terzian et al., 1981). We

report here the solution difference absorption spectral properties of various L-amino acids (except L-cysteine), N-acetyl-L-amino acids, L-amino acid methyl esters, and O-phospho-L-serine with iron(III)–nitrilotriacetic acid in piperazine-N,N'-bis[2-ethanesulfonic acid] (PIPES) (10 mM)–NaCl (100 mM) buffer, pH 6.60. We noted the similarities among the spectra of different iron(III)–amino acid complexes and compared these with the spectra of iron(III)–phosvitin and iron(III)–conalbumin complexes. This information was then used to make spectral band assignments to the specific complexes of iron(III) with carboxyl oxygen, phenolate oxygen of tyrosine, and phosphoseryl groups; these assignments should be useful in determining the nature of nonheme iron(III)–protein interactions. A subsequent paper describes the determination of the nature of groups involved in the binding of iron(III) to bovine α_{s1}-, β-, and κ-caseins, bovine serum albumin, β-lactoglobulin, and α-lactalbumin (Reddy and Mahoney, 1995).

MATERIALS AND METHODS

Materials. L-Alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamine, L-glutamic acid, glycine, L-histidine hydrochloride, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-valine, L-alanine methyl ester hydrochloride, L-arginine methyl ester dihydrochloride, L-aspartic acid dimethyl ester hydrochloride, L-glutamic acid dimethyl ester hydrochloride, L-histidine methyl ester dihydrochloride, L-lysine methyl ester dihydrochloride, L-phenylalaninamide, L-serine methyl ester hydrochloride, L-threonine methyl ester hydrochloride, L-

* Address correspondence to this author at Archer Daniels Midland Co., Protein Specialties Division, 1001 Brush College Rd., Decatur, IL 62521-1656 [telephone (217) 424-4224; fax (217) 424-2457].
[†] Deceased.

tryptophanamide, *N*-acetyl-L-alanine, N^α-acetyl-L-arginine, *N*-acetyl-L-aspartic acid, *N*-acetyl-L-glutamic acid, N^α-acetyl-L-histidine, N^α-acetyl-L-lysine, N^ϵ-acetyl-L-lysine, *N*-acetyl-L-phenylalanine, *N*-acetyl-L-tryptophan, *N*-acetyl-L-tyrosinamide, *N*-acetyl-L-tyrosine, *O*-phospho-L-serine, phosvitin, iron-free conalbumin, ferric chloride, and disodium salt of piperazine-*N*,*N*'-bis[2-ethanesulfonic acid] (PIPES) were obtained from Sigma Chemical Co., St. Louis, MO. Trisodium hydrogen nitrilotriacetate (NTA), [5064-31-3] and iron atomic absorption standard solution were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. All other chemicals were of analytical grade. The glassware was acid washed, and double-deionized water was used for the preparation of buffers.

Ferric Nitrilotriacetate (NTA). A 0.3 M stock solution of Fe(III)–NTA chelate was prepared (Carmichael et al., 1975) by dissolving FeCl$_3$ · 6H$_2$O (0.3 M) and trisodium NTA (0.6 M) in deionized water and diluting to 500 mL. The solution was filtered through 0.45 μm filter paper (Millipore). Since >99.5% of the chelate typically passes an Amicon PM-10 ultrafiltration membrane, iron was predominantly in mononuclear form. A working solution of Fe(III)–NTA (2 × 10^{-2} M) was prepared in PIPES (10 mM)–NaCl (100 mM) buffer, pH 6.60. Buffer salts were dissolved in 230 mL of deionized water, and the pH was adjusted to about 7.60. The stock solution (16.7 mL) of Fe(III)–NTA (0.3 M) was then added with constant stirring; the pH was adjusted to 6.60 with 0.1 N HCl and the solution brought to a final volume of 250 mL. Sodium hydroxide was not used to adjust the pH of Fe(III)–NTA solution to avoid localized formation of ferric hydroxide polymers. The concentration of [Fe] in Fe(III)–NTA solution was determined by atomic absorption (Instrumentation Laboratory Model 457 atomic absorption spectrophotometer, Wilmington, MA).

Preparation of Iron(III)–Amino Acid and Iron(III)–Protein Complexes. Stock solutions of amino acids (6 × 10^{-2} M), iron-free conalbumin (10 mg/mL), and phosvitin (10 mg/mL) were prepared in PIPES (10 mM)–NaCl (100 mM) buffer, and the pH was adjusted to 6.60. Amino acid and protein solutions were filtered through 0.2 μm Acrodisc LC13 PVDF syringe filters (Gelman Sciences, Ann Arbor, MI). Stock solutions of amino acids (6 × 10^{-2} M) and proteins (10 mg/mL) were mixed with Fe(III)–NTA (2 × 10^{-2} M) working solution in 1:1 (v/v) ratio and incubated at 24 ± 1 °C for 2 h before the spectra were recorded.
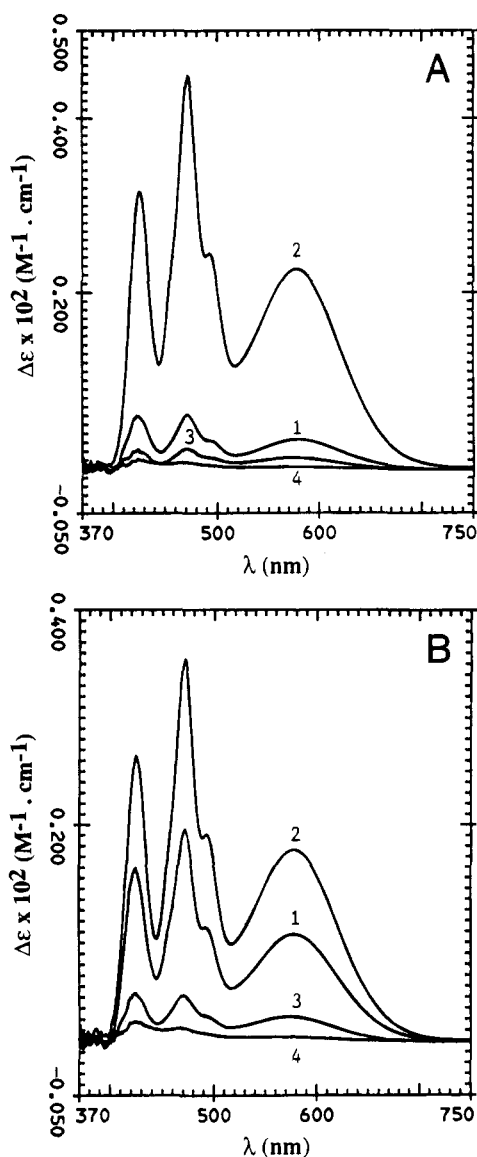
Difference Absorption Spectra. Difference absorption spectra of iron(III)–amino acid complexes were measured from 350 to 750 nm in a Shimadzu Model UV2100U UV–vis double-beam recording spectrophotometer (Shimadzu Corp., Kyoto, Japan) interfaced to an IBM-PC computer. Quartz cells of 1 cm path length were used. Spectra of iron(III)–NTA–amino acid mixtures in the sample cell were recorded against corresponding concentrations of iron(III)–NTA solution in the reference cell. Computer analysis of the difference spectra was carried out to obtain λ_{max} (nm) and corresponding difference absorbance (ΔA) values of different absorption bands. All differential molar absorptivities ($\Delta\epsilon$, M^{-1} cm^{-1}) for various iron(III)–amino acid complexes at different wavelengths were calculated on the basis of total iron content (Stadtherr and Martin, 1972). All experiments were performed at room temperature, 24 ± 1 °C.

RESULTS

The difference absorption spectra of 1:3 molar complexes of iron(III) with different L-amino acids, *N*-acetyl-L-amino acids, L-amino acid methyl esters, *O*-phospho-L-serine, conalbumin, and phosvitin at pH 6.60 are shown in Figures 1–5, and the λ_{max} (nm) of absorption bands and corresponding $\Delta\epsilon$ (M^{-1} cm^{-1}) are presented in Table 1. In Figures 1–5, the peaks above zero $\Delta\epsilon$ (M^{-1} cm^{-1}) or ΔA represent features that have higher intensities in the spectrum of amino acids or proteins containing iron(III)–NTA than in the one with iron-(III)–NTA only. Peaks below zero represent absorption bands whose height and area are greater in the spec-
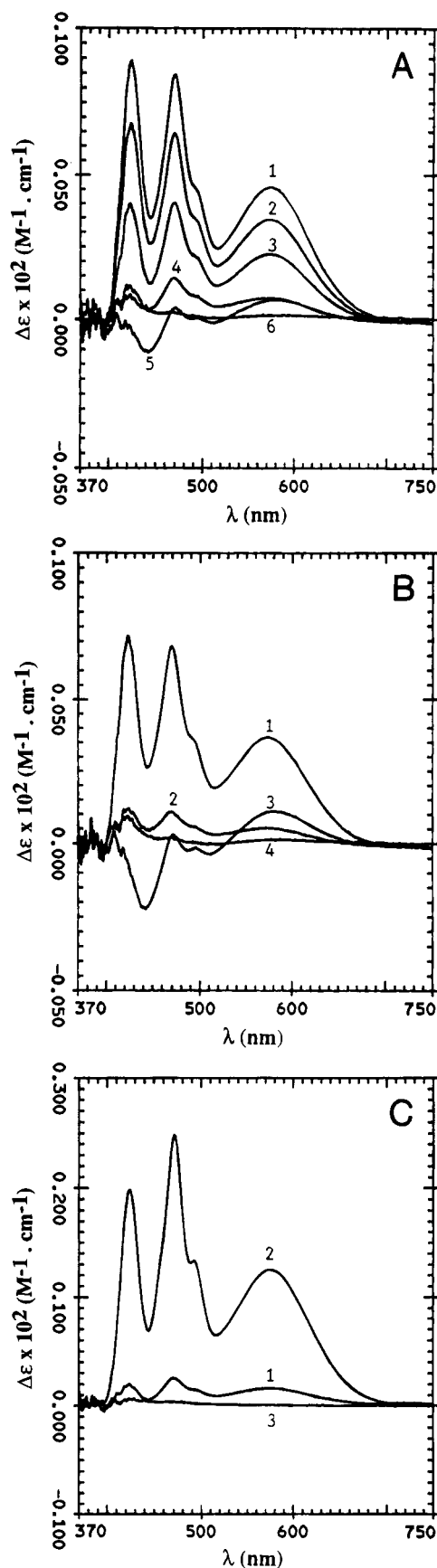
Figure 1. Difference absorption spectra of the complexes of iron(III) (1 × 10^{-2} M) with L-amino acids (3 × 10^{-2} M) containing hydrophobic and polar (neutral) side-chain groups at pH 6.60: (A) (1) L-Ile, (2) L-Leu, (3) L-Met, (4) Gly, (5) L-Ser, (6) L-Ser methyl ester; (B) (1) L-Val, (2) L-Pro, (3) L-Thr, (4) L-Thr methyl ester; (C) (1) L-Ala, (2) *N*-acetyl-L-Ala, (3) L-Ala methyl ester.

Figure 2. Difference absorption spectra of the complexes of iron(III) (1 × 10⁻² M) with L-amino acids (3 × 10⁻² M) containing acidic side-chain groups at pH 6.60: (A) (1) L-Asp, (2) N-acetyl-L-Asp, (3) L-Asn, (4) L-Asp dimethyl ester; (B) (1) L-Glu, (2) N-acetyl-L-Glu, (3) L-Gln, (4) L-Glu dimethyl ester.

trum of iron(III)−NTA. Where the spectra of both iron-(III)−NTA and amino acids or proteins containing iron(III)−NTA have no absorption bands or where each has a peak at the same wavelength position, with identical shapes, intensities, and widths, the difference spectrum will have only a horizontal line at zero absorbance. Therefore, the peaks above zero or below zero in Figures 1−5 represent the specific absorbances contributed by different iron(III)−amino acid or iron-(III)−protein complexes.

Difference Spectra of Iron(III)−L-Amino Acid and Iron(III)−Protein Complexes. The difference spectra of various iron(III)−L-amino acid complexes (except L-tyrosine) had positive peaks at 422−424, 470−472, and 571−575 nm (580 nm for L-serine and L-threonine) and a positive shoulder at 493−494 nm (Figures 1−5; Table 1). Iron(III) complexes of L-tyrosine had a single positive absorption band at 485 nm (Figure 3C; Table 1). The complexes of iron(III) with O-phospho-L-serine had a negative band at 420 nm and positive peaks at 467, 485, and 552 nm (Figure 5; Table 1). However, there was considerable variation in Δε

(M⁻¹ cm⁻¹) of the absorption bands (λ_{max}, nm) calculated for different iron(III)−amino acid complexes (Table 1). The iron(III)−L-amino acid complexes were yellow, and the iron(III)−O-phospho-L-serine complexes were rust colored.
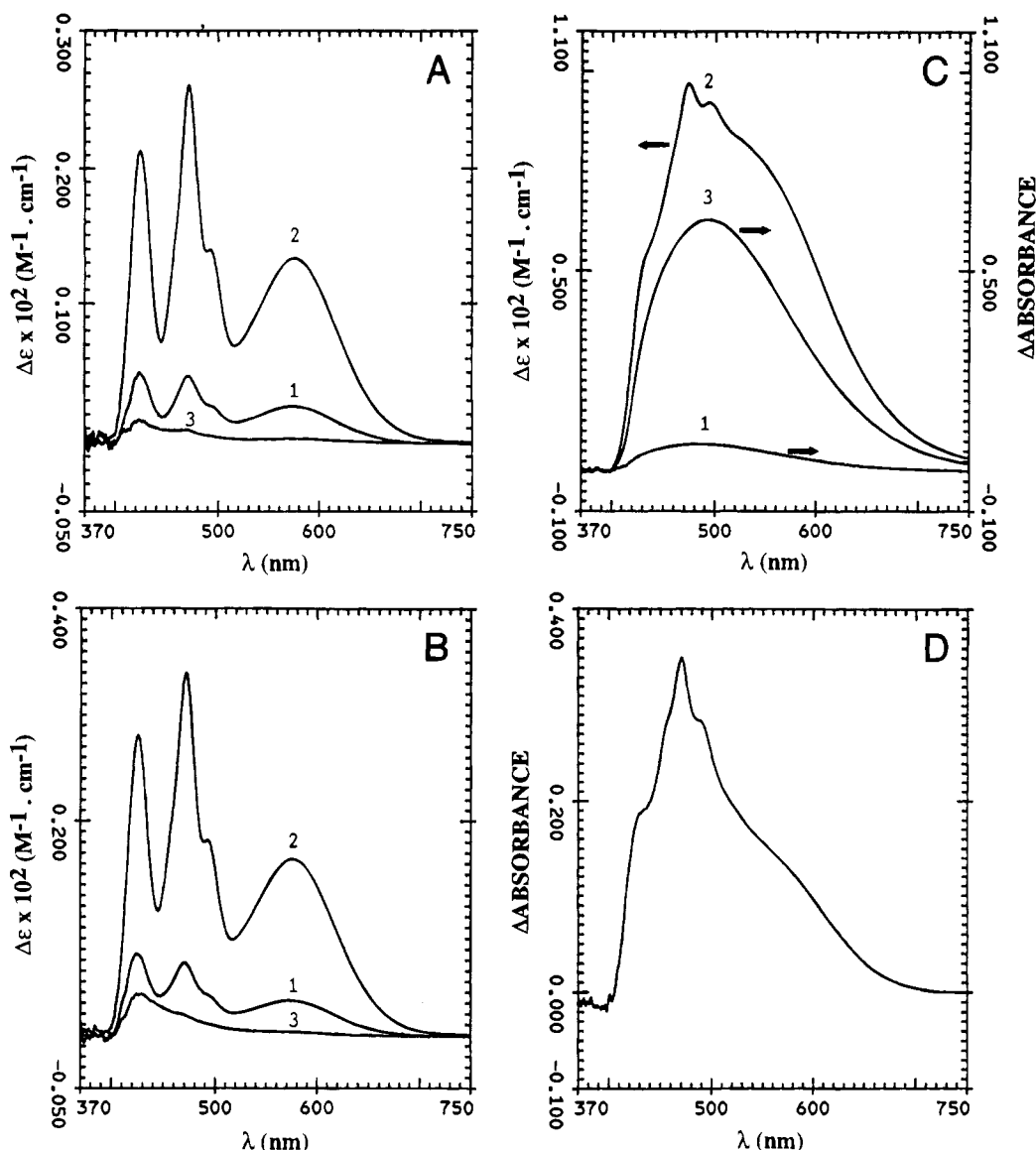
The difference spectra of the complexes of iron(III) with phosvitin had a single intense negative absorption band at 416 nm (Figure 5; Table 1), and conalbumin (ovotransferrin) had a positive peak at 470 nm and positive shoulders at 428, 491, and 578 nm (Figure 3D; Table 1).

Effect of Modification of α-Amino Groups by Acetylation. Difference spectra were generated with N-acetyl-L-amino acids to elucidate the role of α-amino groups in the complex formation with iron(III). The resulting difference spectra (except N-acetyl-L-tyrosine) were similar to that of L-amino acids, i.e., positive peaks at 423−425, 471, and 572−577 nm and a positive shoulder at 492−494 nm; the peaks and shoulders were more intense as shown by higher Δε (M⁻¹ cm⁻¹) values (Figures 1−4; Table 1). In contrast to iron(III)−L-tyrosine complexes, which had a single positive peak at 485 nm, the complexes of iron(III) with N-acetyl-L-tyrosine had positive peaks at 473 and 493 nm and positive shoulders at 430 and 570 nm (Figure 3C; Table 1). The different iron(III)−N-acetyl-L-amino acid complexes (except N-acetyl-L-tyrosine) were dark yellow and the iron(III)−N-acetyl-L-tyrosine complexes were reddish brown.

Effect of Modification of Carboxyl Groups by Esterification or Amidation. To elucidate the role of α-carboxyl groups in complex formation with iron-(III), we generated the difference spectra with either esterified or amidated L-amino acids. Modification of α-carboxyl groups decreased the affinity of amino acids to complex with iron(III), as indicated by the disappearance of absorption bands and a decrease in Δε (M⁻¹ cm⁻¹) values (Figures 1−4; Table 1). The difference absorption bands completely disappeared with methyl esters of L-alanine (Figure 1C), L-serine (Figure 1A), and L-threonine (Figure 1B) and with dimethyl esters of L-aspartic acid (Figure 2A) and L-glutamic acid (Figure 2B). The complexes of L-phenylalaninamide and L-tryptophanamide had only one positive peak at 423−424 nm with low Δε (M⁻¹ cm⁻¹) values (Figure 3A,B; Table 1). The complexes of iron(III) with methyl esters of L-lysine, L-arginine, and L-histidine, on the other hand, had less intense positive bands at 423−424, 469−471, 492−493, and 570−573 nm than the spectra of their respective L-amino acids (Figure 4; Table 1).
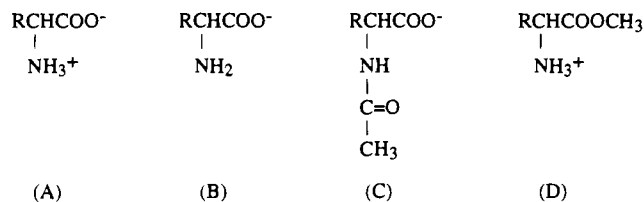
DISCUSSION

All amino acids exist as zwitterions (A) at physiological pH, and the complex-forming species with iron(III) is the anion (B) (Marsh and Donohue, 1967; McAuliffe and Murray, 1972; Voet and Voet, 1990). For example, in a solution of glycine at the physiological pH, the proportion of anionic form (B) is only 0.25% (Albert, 1950). The remainder of the glycine is present as the zwitterion (A), a species that is not capable of chelation but yields further quantities of anionic form (B) when the latter is largely removed by chelation until a new equilibrium is reached (Albert, 1950). In zwitterion structure (A), the ammonium group (−NH₃⁺) is an excellent hydrogen-bond donor and the carboxylate ion (−COO⁻) an excellent acceptor; accordingly, strong intramolecular hydrogen bonds are invariably formed between these groups (Marsh and Donohue, 1967).

Difference Spectra of Fe³⁺–Amino Acid Complexes

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Figure 3. Difference absorption spectra of the complexes of iron(III) (1×10^{-2} M) with L-amino acids (3×10^{-2} M) containing aromatic side-chain groups and conalbumin (1.3×10^{-4} M) at pH 6.60: (A) (1) L-Phe, (2) N-acetyl-L-Phe, (3) L-Phe amide; (B) (1) L-Trp, (2) N-acetyl-L-Trp, (3) L-Trp amide; (C) (1) L-Tyr, (2) N-acetyl-L-Tyr, (3) N-acetyl-L-Tyr amide; (D) conalbumin.
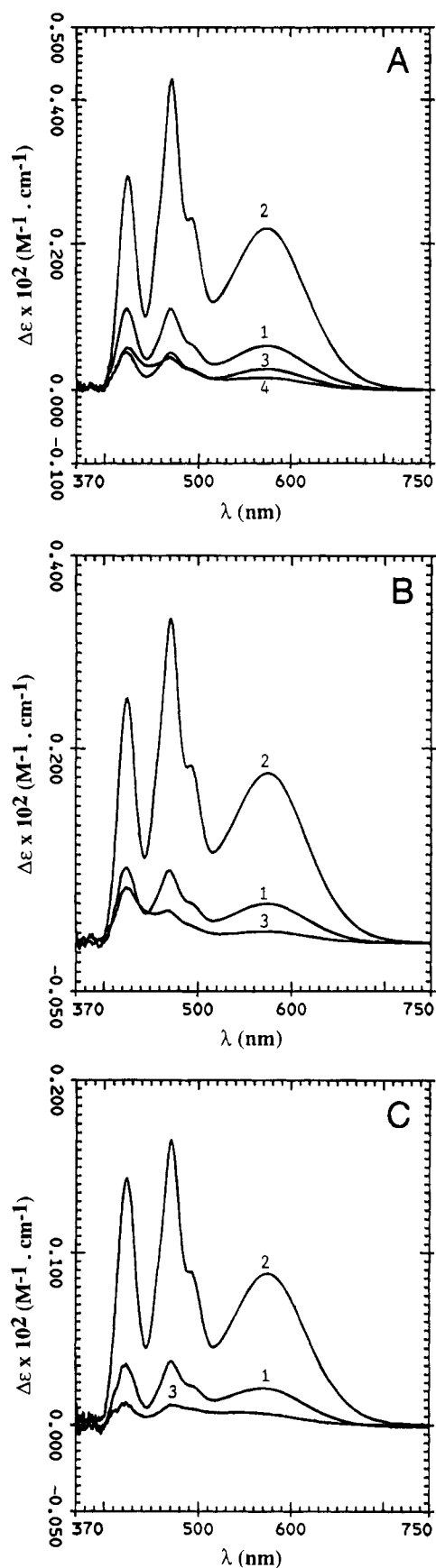
Hence, the zwitterionic form (A) has less net attraction for iron(III) than does anionic form (B), which lacks this hydrogen bond. This explains why the affinity of L-amino acids for iron(III) is much less than the affinity of N-acetyl-L-amino acids (C), which are similar to

RCHCOO⁻   RCHCOO⁻   RCHCOO⁻   RCHCOOCH₃
| | | |
NH₃⁺   NH₂   NH   NH₃⁺
                                  |
                                  C=O
                                  |
                                  CH₃

(A)   (B)   (C)   (D)

anionic form (B) (Figures 1−4; Table 1). L-Amino acid methyl esters, where α-carboxyl groups are modified by esterification (D), had the least or no affinity to complex with iron(III) (Figures 1−4; Table 1), indicating that the α-amino nitrogens lacked affinity for iron(III)−ligand bonding and carboxyl oxygens were essentially involved in chelating the iron(III). This agrees with the work of Tucker et al. (1975) and Fitzsimmons et al. (1985), whose measurements of magnetic behavior, optical

spectra, and Mössbauer parameters of crystalline complexes of ferric iron with aliphatic amino acids and ferrous iron with 15 different amino acids, respectively, also showed that the interaction between the iron and the amino acids involved the carboxyl group. In contrast, other transition metals interact with amino acids through both α-amino and α-carboxyl groups (Albert, 1950, 1952; Freeman, 1967).

The similarities in absorption spectral properties of various iron(III)−L-amino acid complexes (except for L-tyrosine) indicate that their structures are also similar, i.e., all of the complexes are suggested to contain iron(III) atom in similar environments. However, the differences in net charge, length of the hydrocarbon chain, and functional group of the side chain seem to affect the extent of complex formation with iron(III), as was evident in the differences in $\Delta\epsilon$ (M^{-1} cm^{-1}) of the absorption bands (λ_{max}, nm) for various iron(III)−amino acid complexes (Table 1). Crystalline complexes of iron-(III) with glycine, L-alanine, L-valine, L-leucine, L-isoleucine, and L-proline possess similar electronic and infrared spectral properties, Mössbauer parameters, and magnetic behavior and, hence, similar structures (Tucker

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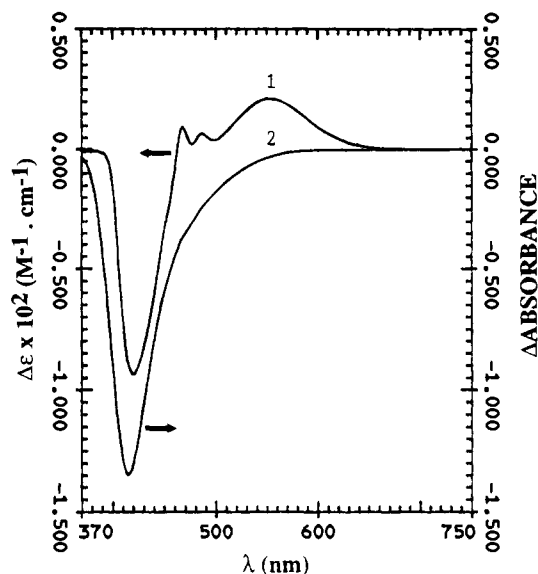
Figure 5. Difference absorption spectra of the complexes of iron(III) (1×10^{-2} M) with O-phospho-L-serine (3×10^{-2} M) and phosvitin (2.8×10^{-4} M) at pH 6.60: (1) O-phospho-L-Ser, (2) phosvitin.

et al., 1975). The overall structure of these molecules consists of a central oxygen bonded to three iron atoms, which are in turn bonded to an oxygen of the carboxyl group of each of four amino acid residues; the sixth coordination site is occupied by the oxygen of a water molecule (Holt et al., 1974; Tucker et al., 1975). Thus, the lack of affinity of α-amino nitrogens in L-amino acid methyl esters and increased affinity of α-carboxyl oxygens in N-acetyl-L-amino acids for iron(III)−ligand bonding suggest that a similar structure exists even in solution complexes of iron(III) with L-amino acids.

Amino Acids with Hydrophobic, Polar (Neutral), and Acidic Side-Chain Groups. The complexes of iron(III) with glycine, L-alanine, N-acetyl-L-alanine, L-valine, L-leucine, L-isoleucine, L-proline, L-methionine, L-aspartic acid, N-acetyl-L-aspartic acid, L-asparagine, L-glutamic acid, N-acetyl-L-glutamic acid, and L-glutamine exhibited characteristic positive peaks at 422−425, 470−472, and 572−577 nm and a positive shoulder at 492−494 nm (Figures 1 and 2; Table 1). N-Acetylation of α-amino groups in L-alanine, L-aspartic acid, and L-glutamic acid increased their complex-forming ability with iron(III), indicating that α-carboxyl oxygens were involved in iron(III)−ligand bonding (Figures 1 and 2; Table 1). The Δε (M^{-1} cm^{-1}) values for L-aspartic acid and L-glutamic acid complexes were higher than those for L-asparagine and L-glutamine complexes, indicating that γ- and δ-carboxyl groups, respectively, were involved in the complex formation with iron(III). L-Alanine methyl ester, with esterified α-carboxyl groups, and dimethyl esters of L-aspartic and L-glutamic acids, with esterified α-, γ-, and δ-carboxyl groups, did not complex with iron(III) due to the lack of affinity of α-amino nitrogens for iron(III) (Figures 1C and 2; Table 1). Methyl esters of L-serine and L-threonine also lost their ability to complex with iron(III), due to the lack of affinity of seryl and threonyl hydroxyl groups for iron-(III) (Figure 1A,B; Table 1). Thus, the characteristic positive difference absorption peaks at 422−425, 470−472, and 572−577 nm and the positive shoulder at 492−494 nm could be assigned to the complexes of iron(III) with carboxyl oxygens.

Amino Acids with Aromatic Side-Chain Groups and Conalbumin. The complexes of L-phenylalanine,

Figure 4. Difference absorption spectra of the complexes of iron(III) (1×10^{-2} M) with L-amino acids (3×10^{-2} M) containing basic side-chain groups at pH 6.60: (A) (1) L-Lys, (2) N^α-acetyl-L-Lys, (3) N^ϵ-acetyl-L-Lys, (4) L-Lys methyl ester; (B) (1) L-Arg, (2) N-acetyl-L-Arg, (3) L-Arg methyl ester; (C) (1) L-His, (2) N-acetyl-L-His, (3) L-His methyl ester.

Table 1. Difference Absorption Spectral Data of Iron(III)–Amino Acid and Iron(III)–Protein Complexes at pH 6.60 (Molar Ratio of Iron to Amino Acid, 1:3)[a]

amino acid complex	λ_{max} (nm) of absorption bands and their $\Delta\epsilon$ (M⁻¹ cm⁻¹) in parentheses				
	positive bands				negative bands
	I	II	III	IV	V
Amino Acids with Hydrophobic and Polar (Neutral) Side-Chain Groups					
Gly	572 (0.7)	494[sh][b] (0.8)	470 (1.4)	422 (1.1)	
L-Pro	572 (0.5)	493[sh] (0.5)	470 (1.0)	424 (1.1)	
L-Met	572 (2.2)	493[sh] (2.1)	470 (4.0)	423 (3.9)	
L-Leu	572 (3.5)	493[sh] (3.5)	470 (6.4)	424 (6.7)	
L-Val	572 (3.7)	493[sh] (3.6)	470 (6.7)	424 (7.1)	
L-Ile	573 (4.5)	493[sh] (4.5)	470 (8.4)	424 (8.8)	
L-Ala	572 (1.6)	493[sh] (1.4)	470 (2.5)	424 (2.0)	
N-acetyl-L-Ala	575 (12.5)	493[sh] (13.3)	471 (24.5)	424 (19.8)	
L-Ala methyl ester					
L-Ser	580 (0.7)	493[sh] (0.1)	470 (0.4)		
L-Ser methyl ester					
L-Thr	580 (1.1)	.	470 (0.3)		
L-Thr methyl ester					
Amino Acids with Acidic Side-Chain Groups					
L-Asp	575 (3.3)	494[sh] (3.1)	471 (6.1)	423 (5.9)	
N-acetyl-L-Asp	576 (22.6)	492 (24.3)	471 (44.8)	425 (31.5)	
L-Asn	572 (1.3)	494[sh] (1.2)	470 (2.3)	424 (2.1)	
L-Asp dimethyl ester					
L-Glu	575 (9.7)	493[sh] (10.5)	472 (19.5)	424 (15.8)	
N-acetyl-L-Glu	577 (17.6)	493[sh] (19.0)	471 (35.3)	424 (26.4)	
L-Gln	573 (2.2)	493[sh] (2.3)	470 (4.1)	423 (4.3)	
L-Glu dimethyl ester					
Amino Acids with Aromatic Side-Chain Groups					
L-Phe	572 (2.6)	493[sh] (2.5)	471 (4.7)	424 (4.9)	
N-acetyl-L-Phe	573 (13.2)	493[sh] (13.8)	471 (26.1)	424 (21.2)	
L-Phe amide				423 (1.6)	
L-Trp	572 (3.2)	493[sh] (3.7)	470 (6.7)	424 (7.6)	
N-acetyl-L-Trp	576 (16.4)	493[sh] (18.0)	471 (33.9)	425 (28.0)	
L-Trp amide				424 (3.8)	
L-Tyr		485[c]			
N-acetyl-L-Tyr	570[sh] (68.5)	493 (92.4)	473 (96.9)	430[sh] (51.0)	
N-acetyl-L-Tyr amide		493[c]			
conalbumin	578[sh]	491[sh]	470	428[sh]	
Amino Acids with Basic Side-Chain Groups					
L-Lys	572 (6.0)	493[sh] (6.2)	470 (11.0)	424 (11.0)	
*N*ᵅ-acetyl-L-Lys	573 (22.1)	492 (23.5)	471 (42.7)	424 (29.3)	
*N*ᵋ-acetyl-L-Lys	573 (2.8)	492[sh] (2.8)	470 (5.0)	424 (5.1)	
L-Lys methyl ester	573 (1.6)	492[sh] (2.6)	469 (4.4)	424 (5.7)	
L-Arg	572 (4.0)	493[sh] (4.0)	470 (7.3)	424 (7.6)	
N-acetyl-L-Arg	573 (17.3)	493 (18.2)	471 (33.4)	425 (25.2)	
L-Arg methyl ester	572 (1.1)	493[sh] (1.7)	470 (3.2)	424 (5.6)	
L-His	571 (2.2)	493[sh] (2.3)	471 (3.7)	423 (3.5)	
N-acetyl-L-His	572 (8.7)	493[sh] (8.9)	471 (16.5)	423 (14.3)	
L-His methyl ester	570 (0.7)	493[sh] (0.9)	471 (1.2)	423 (1.2)	
O-phospho-L-Ser	552 (21.2)	485 (6.5)	467 (9.3)		420 (−93.6)
phosvitin					416

[a] Iron(III) concn = 1×10^{-2} M; amino acid concn = 3×10^{-2} M. [b] sh, shoulder. [c] L-Tyr and *N*-acetyl-L-Tyr amide were not completely soluble and hence $\Delta\epsilon$ was not calculated.

N-acetyl-L-phenylalanine, L-tryptophan, and *N*-acetyl-L-tryptophan exhibited positive difference absorption bands characteristic of the complexes of iron(III) with carboxyl oxygens (Figure 3A,B; Table 1). L-Tyrosine and *N*-acetyl-L-tyrosinamide complexes had a single positive peak at 485 and 493 nm, respectively (Figure 3C). The reddish brown color of the complexes (vs yellow for other amino acids) and the absence of positive peaks at 422, 470, and 572 nm contributed by carboxyl oxygens indicated that phenolate oxygens were involved in the complexes of iron(III) with L-tyrosine and *N*-acetyl-L-tyrosinamide. The complexes of *N*-acetyl-L-tyrosine

with positive peaks at 473 and 493 nm and positive shoulders at 430 and 570 nm (Figure 3C) indicated that both carboxyl oxygens as well as phenolate oxygens were involved in iron(III)–ligand bonding. The difference absorption spectrum of iron(III)–conalbumin complexes, in which the iron(III) chelate site involves two phenolate oxygens of tyrosine, one carboxyl oxygen of aspartic acid, one imidazole nitrogen of histidine, a bicarbonate anion, and a water molecule (Crichton, 1990), is similar to that of the difference spectrum of iron(III)–*N*-acetyl-L-tyrosine complexes (Figure 3C,D).

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Amino Acids with Basic Side-Chain Groups. The complexes of L-lysine, N^α-acetyl-L-lysine, N^ϵ-acetyl-L-lysine, L-arginine, N^α-acetyl-L-arginine, L-histidine, and N^α-acetyl-L-histidine had characteristic positive difference absorption bands contributed by the complexes of iron(III) with carboxyl oxygens (Figure 4; Table 1). N-Acetylation of α-amino groups of lysine increased the iron(III) binding affinity, indicating that α-carboxyl groups were involved in iron(III)−ligand bonding (Figure 4A; Table 1). N-Acetylation of ε-amino groups of lysine decreased the iron(III) binding affinity, indicating the involvement of ε-amino nitrogen in the iron(III)−ligand bonding (Figure 4A). These results are in agreement with the observations of Van Campen (1979) that removal of the ε-amino group of L-lysine eliminates its ability to chelate and thus enhance iron uptake from ligated in vivo segments of rat duodenum. The positive difference absorption bands of low intensity with methyl esters of L-lysine, L-arginine, and L-histidine further confirmed the involvement of the ε-amino nitrogen of lysine, the guanidino nitrogen of arginine, and the imidazole nitrogen of hisitidine in the iron(III)−ligand bonding (Figure 4; Table 1). The $\Delta\epsilon$ (M^{-1} cm^{-1}) values for the complexes of iron(III) with esterified L-lysine, L-arginine, and L-histidine were lower than for their respective L-amino acids (Table 1), indicating that the iron(III) binding affinity of the ε-amino nitrogen of lysine, the guanidino nitrogen of arginine, and the imidazole nitrogen of hisitidine was much less than that of carboxyl oxygens. This is consistent with the observations that decarboxylation of L-histidine resulted in a loss of its ability to chelate and enhance iron uptake (Van Campen, 1979).

O-Phospho-L-serine and Phosvitin. The iron-(III)−O-phospho-L-serine complexes had an intense negative difference absorption band at 420 nm and positive peaks at 467, 485, and 552 nm (Figure 5; Table 1). Positive absorption bands at 467, 485, and 557 nm contributed by iron(III)−carboxyl complexes may have been shifted to shorter wavelengths due to the intense negative absorption band at 420 nm. Also, the intense negative absorption band at 420 nm may have obscured the positive absorption band at 420−422 nm contributed by Fe(III)−carboxyl complexes (Figures 1−4; Table 1). Hegenaur et al. (1979a) reported that the absorption of iron(III)−phosphoseryl complexes in the visible region is weak compared to that of Fe(III); hence, the complexes could have contributed to a negative difference absorption band at 420 nm. In addition, the difference absorption spectrum of iron(III)−phosvitin complexes also gave a single intense negative difference absorption band at 416 nm (Figure 5). Phosvitin, a phosphoglycoprotein from egg yolk with 135 phosphoserines of a total of 225 amino acid residues in the protein, binds iron-(III) through phosphoseryl clusters (Hegenauer et al., 1979b; Shainkin and Perlmann, 1971; Webb et al., 1973). The presence of a single intense negative difference absorption band at 416 nm and the absence of characteristic positive peaks at 470, 490, and 570 nm in the spectrum of iron(III)−phosvitin complexes (Figure 5) confirmed the observations of Hegenauer et al. (1979b) and Webb et al. (1973) that phosvitin binds iron-(III) essentially through phosphoseryl clusters and that carboxyl groups are not involved in the binding. Thus, the negative absorption band at 416−420 nm could be assigned to iron(III)−phosphoseryl complexes. These results suggest that Fe(III) forms two types of complexes with O-phospho-L-serine—one involving phosphoseryl groups and the other involving α-carboxyl oxygens. We have observed that Fe(III)−α_{s1}-casein and Fe(III)−β-casein complexes have difference spectra similar to that of Fe(III)−O-phospho-L-serine complexes (Reddy and Mahoney, 1995).

CONCLUSIONS

The similarities in spectral properties of various iron-(III)−L-amino acid complexes (except L-tyrosine) strongly suggested that their structures are similar. The differences in net charge, length of the side chain, and functional group of the side chain seemed to influence the extent of complex formation with iron(III). Amino acids (except L-tyrosine) appeared to form complexes with iron(III) through carboxyl oxygens, although the ε-amino nitrogen of lysine, the guanidino nitrogen of arginine, and the imidazole nitrogen of hisitidine may also have been involved in the iron(III)−ligand bonding. However, α-amino nitrogens in the amino acids appeared to lack affinity for iron(III). L-Tyrosine appeared to form complexes with iron(III) through phenolate oxygens. At neutral pH L-cysteine reduces iron(III) and complexes with iron(II) through sulfur bonds (Hamed and Silver, 1983). Our results suggested that iron(III) had a much stronger affinity for oxygen than for the nitrogen of either the amino or guanidino or imidazole group.

It is important to indicate, at least to some degree, the potential biological implications of this work. These are threefold. First is the higher affinity of N-acetyl-L-amino acid derivatives to complex with iron(III) as compared with either L-amino acids or their methyl ester derivatives. Second, this study may explain the reported differences in the ability of different amino acids to enhance iron uptake from the gastrointestinal tract (Kroe et al., 1963; Van Campen, 1979). Third, the observations reported in this paper appear to have a bearing on some clinical applications of chelating agents for the treatment of genetic disorders, such as Cooley's anemia and Wilson's disease. Cartwright et al. (1954) reported on the intravenous use of protein hydrolysates to treat patients with Wilson's disease, where the excessive copper accumulated in the tissues is chelated by amino acids and excreted in the urine. Desferrioxamine is currently used as a subcutaneous/intravenous iron chelating agent in the treatment of Cooley's anemia (Cohen et al., 1990; Giardina et al., 1990). However, there is a continued research effort to develop safe and inexpensive chelating agents that effectively promote the excretion of iron after either intravenous or oral administration (Anderson and Hiller, 1975; Brittenham, 1990; Brown, 1981; Hider et al., 1990; Pitt et al., 1979; Rosenkrantz et al., 1986; Winston et al., 1985). Steglink et al. (1981) reported that the intravenous infusion of parenteral solutions containing Maillard reaction products resulted in an increase in urinary excretion of iron, copper, and zinc. Maillard reaction products are formed as a result of a heat-induced interaction between a reducing sugar and an amino group of an amino acid and may be similar to N-acetyl-L-amino acids in complexing with iron(III). Thus, N-acetyl-L-amino acid derivatives, with their high affinity to complex with iron(III), may hold promise as iron chelating agents in the treatment of Cooley's anemia.

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