rate-limiting step in this pathway, as well as in the dissociative pathway, is most likely the formation of the first cadmium–nitrogen bond.

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Absorption and Circular Dichroism Spectral Studies of Chelate Complexes of Praseodymium(III) with α-Amino Acids[1]

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Preparation is described of solutions of amino acid chelate complexes with Pr(III), in the neutral and alkaline pH range. Characteristic absorption and CD spectra are shown for the dominant 1:1 complexes. Differences between spectra for the simple amino acids (alanine, valine, leucine) and the potentially tridentate serine and asparagine are demonstrated. Stability and ease of formation are correlated with the pK of the α-amino group. Data are also given for the basic amino acids, ornithine and lysine. Aspartic and glutamic acids give CD spectra in acid solution which are interpreted as reflecting chelation through the two carboxyl groups. In alkaline solution, this may become chelation through the amino group and the carboxyl β to it, to give a six-membered ring.

The absorption and circular dichroism (CD) spectra of complexes of Pr(III) with a number of optically active hydroxy acids have been described.[2] In these systems, evidence was presented for formation of six- and seven-membered chelate rings. This report extends observations to chelate complexes of Pr(III) with the α-amino acids. Not only is coordination with an amino group introduced, but in these systems five-membered rings must be formed.

Experimental Section

Materials.—Stock solution 0.894 M in $PrCl_3$ was prepared from Pr_6O_{11} (99.9% pure) by digesting a weighed amount of oxide with a small excess of HCl and then diluting the solution to volume with water. The concentration calculated was verified by comparing the extinction values for the maxima at 444.0 and 482.5 nm with those given by Stewart and Kato.[3] In some early experiments, crystalline $PrCl_3$ of uncertain hydration was used, and the stock concentrations were determined from these extinction values alone.

Most of the amino acids were used as supplied by Nutritional Biochemicals Corp. An exception was proline, which was recrystallized from methanol to which ether was added. The other amino acids were glycine, alanine, valine, leucine, serine, ornithine, lysine, arginine, asparagine, aspartic acid, and glutamic acid.

Sample Preparation.—The following procedure was adopted for routine solution preparations after preliminary experiments. This operated smoothly for all of the monocarboxylic acids. A desired weight of the amino acid was dissolved in water, and 5 F ammonia was added to make the solution alkaline. The required volume of stock Pr(III) solution (usually 1–2 ml) was then added dropwise, with mixing. Additional 5 F NH_3 was then added, as required, to achieve the desired final pH, estimated with Hydrion paper. In cases where precipitation limited the pH which might be attained, ammonia was added only until hazy solution showed a trace of permanent precipitate to be formed. This was usually centrifuged down before spectral measurements were made. Occasionally precipitation which was slow in initiation interrupted a spectral scan. Final solutions were about 0.1 F in Pr(III).

Preliminary attempts to prepare samples by dissolving the freshly precipitated Pr(III) hydroxide with excess amino acid solution were only occasionally successful, and even then the stoichiometry of the solution resulting was uncertain. Addition of NaOH to mixtures of Pr(III) and amino acid also was unsatisfactory. Consistent results were obtainable only with the procedure outlined. Dicarboxylic acids presented problems of a different type; glutamic acid preparations in solution above pH 7 were not obtainable.

Spectral Measurements.—Absorption and CD spectra were plotted on the Durrum-Jasco ORD-UV-CD-5, as in previous work.[2] For the CD spectra a 30-mm cell was generally used, and for the absorption measurements, one of 5-mm length was used. No thermostating of the samples was attempted. When spectra were to be analyzed into gaussian components, the Du Pont Model 310 curve analyzer (10 channels) was used. As in the prior study,[2] attention was concentrated on the spectral region, 420–510 nm, in which the ^3P transitions of Pr(III) are found.

Results

The spectral behavior of the monocarboxylic acid–praseodymium(III) mixtures with pH was fairly uniform. As pH increased toward about pH 6, the absorption peaks at 443.5, 468.5, and 482 nm decreased a little in intensity and broadened slightly toward the long-wavelength side. As with the hydroxy acids,[2] the decrease was least in the $^3P_1$ band (468.5 nm). At pH 6–7, depending on the amino acid, the broadening to the long-wavelength side became sharply accentuated, and, in the case of the $^3P_0$ band (482 nm), a definite new peak at 484 nm was initiated. At lower pH values, it

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.
(2) L. I. Katzin, *Inorg. Chem.*, **7**, 1183 (1968).
(3) D. C. Stewart and D. Kato, *Anal. Chem.*, **30**, 164 (1958).

TABLE I

COMPONENT RESOLUTION OF AMINO ACID–PRASEODYMIUM COMPLEX ABSORPTION AND CD SPECTRA

Pr(III) (aq)	Alanine-Pr(III) (Figure 1a)					Asparagine-Pr(III) (Figure 1b)					Valine-Pr(III) (Figure 2d)				
	Absorpn		CD			Absorpn		CD			Absorpn		CD		
λ, nm	λ, nm	A^a	λ, nm	$10^3\Delta A^a$	$10^3\lvert\Delta\epsilon/\epsilon\rvert$	λ, nm	A^a	λ, nm	$10^3\Delta A^a$	$10^3\lvert\Delta\epsilon/\epsilon\rvert$	λ, nm	A^a	λ, nm	$10^3\Delta A^a$	$10^3\lvert\Delta\epsilon/\epsilon\rvert$
	432	0.04									432	0.04			
						434	0.04				435	0.07			
	437	0.06	437	(Neg)		438.5	0.10	438.5	+0.02	0.2	438	0.13			
443.5 ($^3P_2$)	441	0.24	441	+0.02	0.08	441	0.29	441	+0.10	0.35	441	0.37			
	444	0.65	444	(−0.1)	(0.15)	444	0.78	444	(−0.10)	0.13	445	1.25			
	446	1.26	446	(+0.24)	(0.19)	446	0.97	446	(+0.24)	(0.25)	447	0.19			
	450	0.75	450	−0.27	0.36	449	0.85	449	−0.27	0.32	450	0.70			
	454	0.23	454	−0.22	0.96	452	0.37	452	(−0.1)	0.3	454	0.34	453	+0.07	0.2
	456	0.20	457	+0.06	0.3	455.5	0.23	454	(−0.03)	0.12	458	0.13	457	−0.02	0.15
	460[b]	0.18	459	+0.07	0.4	459	0.18	457.5	+0.02	0.11	460	0.14	460	−0.03	0.2
462 ($^1I_6$)	463	0.15	462	+0.03	0.2	462	0.18	462	−0.06	0.3	462	0.15	462	−0.02	0.13
			465.5	−0.04	⩾0.13[c]			466	−0.18	⩾0.75[c]	464	0.24	464	±	0
	467[b]	0.30				466[b]	0.24				467	0.28	467	+0.07	0.25
468.8 ($^3P_1$)			468	−0.20	⩾0.67[c]			468	−0.03	⩾0.16[c]					
	470	0.33	470	−0.07	0.2	470	0.24	470	−0.18	0.75	470	0.30	470	+0.14	0.47
	473	0.50	472	+0.29	0.58	472	0.50	472	+0.24	0.48	472	0.47	472	−0.18	0.38
	476	0.29	476	+0.30	1.03	476	0.27	475	+0.14	0.52	475.5	0.34	475.5	−0.24	0.70
			478	−0.20	⩾1.6[c]	478	0.12								
	480[b]	0.12				481	0.08	480	+0.07	⩾0.8[c]	480	0.08	479	+0.09	1.2
								482.5	−0.04	⩾0.5[c]					
482.0 ($^3P_0$)	483	0.10	482	−0.09	0.9	484	0.18				482	0.16	482	+0.10	0.6
													484	+0.67	⩾1.0[c]
	485	0.55	485	−0.59	1.07	485.5	0.52	484.5	−0.54	1.04	485	0.68			
													487	−0.67	⩾1.0[c]
	488	0.29	488	0.29	1.0	488	0.24	488	+0.22	0.88	488	0.25	488	−0.26	1.04
	492	0.06	491.5	+0.26	4.3	491.5	0.07	491	+0.10	1.4	491	0.06	491	−0.18	3.0
			(493)	(+0.09)											
	495		(495)	(−0.03)		495	0.03	494	+0.04	1.3	493.5	0.05	493.5	(Neg)	
								(498)	(Pos)		497	0.03	496	(Pos)	
								(502)	(Pos)		501		500	(Pos)	

[a] Adjusted to 10-mm path length; absorbance units. [b] Broad component, probably composed of two unresolved members. [c] Minimum value, on the assumption that the total absorption intensity is due solely to this component.

is questionable whether something comparable to the acid complex CD of the hydroxy acids can be distinguished. With the change in absorption, however, a definite detailed CD pattern starts to take shape.

Solutions with serine:praseodymium ratios of 1:1 showed only the new spectrum and CD pattern at about pH 7 and were stable. Asparagine–praseodymium mixtures at the 1:1 ratio were stable even to appreciably higher ammonia concentrations, such as pH 9. With other amino acids, higher ratios of ligand to Pr(III) were required for stability of solution at pH 7. Valine required about 1.5:1; leucine, 1.5–2:1; proline, 2.5:1 or more. For most of the amino acids, solutions with high ratios of ligand, such as 5:1, might be stable to pH values as high as 10 or 11. Difficulty in fixing the limits is related to a long induction period before a metastable solution finally (and often suddenly) precipitates praseodymium hydroxide. Frequently spectral measurements might be completed before precipitation occurred.

The full CD pattern was developed between pH 7 and 8. An efficient chelating agent like serine or asparagine, at a given ligand ratio, seemed to give the maximum pattern at the lower pH and a less efficient one at higher pH. Because the pattern is obtained with a 1:1 ligand:praseodymium ratio for the efficient agents, it will be designated as referring to this composition even when higher ratios are required to give a stable solution. In some instances, at low pH for 1:1 solution composition and with only partial conversion, the pattern can be seen clearly. Likewise, in some instances, spectral measurements showing this pattern in solutions with 1:1 ratio can essentially be completed before precipitation takes place. There seems little doubt, therefore, about the correctness of the indicated ratio in the composition of the complex.

There are, in fact, two slightly different and definite versions of the 1:1 CD and absorption spectra. The simple amino acids with no secondary basic groups (alanine, valine, leucine) have CD spectra which are recognizable primarily by the appearance of the $^3P_0$ band. This appears to have only three components, with the positive long-wavelength one dropping sharply to the base line (Figure 1). In the cases of alanine and leucine, where the spectra are uncomplicated, it is also seen that the extremum at 482 nm characteristically is negative. The second spectral form is found with serine and asparagine. The $^3P_0$ CD extends to longer wavelengths in a manner suggesting two or more additional components exist past the 492-nm CD (Figure 1). Also, the CD extremum at 481–482 nm is positive, and there are other differences in the $^3P_1$ band. The results of component analyses of both CD and absorption spectra appear in Table I.

Two types of CD spectra have been observed in addition to those characteristic of the 1:1 amino acid–praseodymium complex. One of these is seen in solutions with valine:praseodymium(III) ratios of 2:1 and higher. This minimal ratio may not give a stable solution through the full pH range, but 3:1 does. The

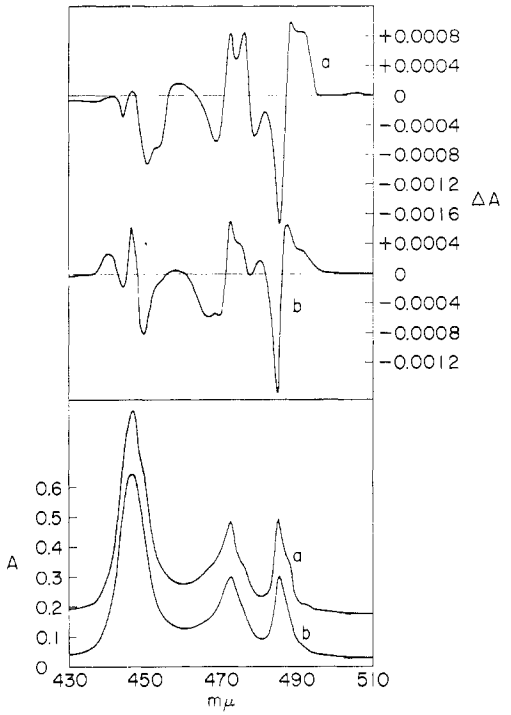

Figure 1.—CD and absorption spectra in the 430–510-nm spectral region for 1:1 complexes of Pr(III) with (a) alanine and (b) asparagine. CD path lengths, 30 mm; absorption, 5 mm; [Pr(III)] = *ca.* 0.1 *F*.

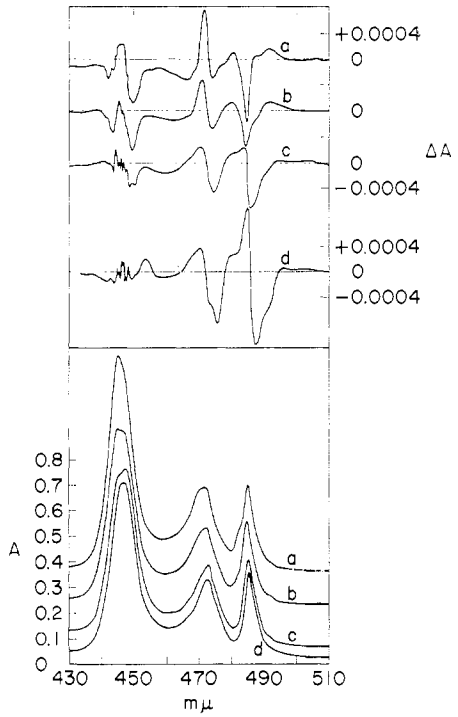

Figure 2.—Alterations of CD and absorption spectra for 3:1 valine–praseodymium(III) mixture, pH increasing from pH 7+ (a) to pH 8+ (d). At about pH 7, spectra would be those of Figure 1a. CD path lengths, 30 mm; absorption, 5 mm.

change from the spectra characteristic of the 1:1 complex species can be traced as a function of ammonia addition, going from pH 7 to 8, or slightly higher, as in Figure 2. Some of the spectra seen with the basic amino acids, at higher ligand ratios (*e.g.*, 5:1) and pH 8–9, resemble intermediate stages in the above series, implying a tendency for the same change.

A second unmistakable deviation from the 1:1 pattern is encountered with asparagine, ligand:praseodymium ratio 5:1, at pH 9–10 (Figure 3). Below pH 9, essentially the standard 1:1 pattern is seen. Transition to the alkaline pattern might involve a time lag as well as higher ammonia concentration, but this is not defined. Somewhat similar patterns arise with proline, under the same conditions (Figure 3).

Aspartic acid differs markedly from the monocarboxylic acids in its effects with Pr(III). Essentially the same weak CD is seen at pH 6, with ligand ratios of 2–5:1, and the absorption spectrum also does not vary. The solution with a 2:1 ratio cannot, under our standard concentration conditions, be raised in pH, even to pH 7, before a heavy permanent precipitate forms. With a ligand:praseodymium ratio of 3:1, this pH region can be passed and the solution maintained even to pH 10 or so. To about pH 8, spectra are independent of ligand ratio, 3–5:1, and are illustrated in Figure 4. For pH 8–10, the spectra for the 3:1 ratio are essentially invariant. In this range the spectra for the 5:1 ligand ratio show a small difference from the 3:1 ratio, primarily in the relative intensity of the negative CD at about 487 nm.

Glutamic acid, at 1:1 ratio to Pr(III), gives Pr precipitates between pH 4 and 5. Between pH 3 and 4, a weak CD can be detected, reminiscent of the acid complexes[2] of the hydroxy acids. Even with ligand ratio 5:1, precipitation occurs below pH 7. Prior to precipitation the CD seen is not notably different from that of the 1:1 solution below pH 4.

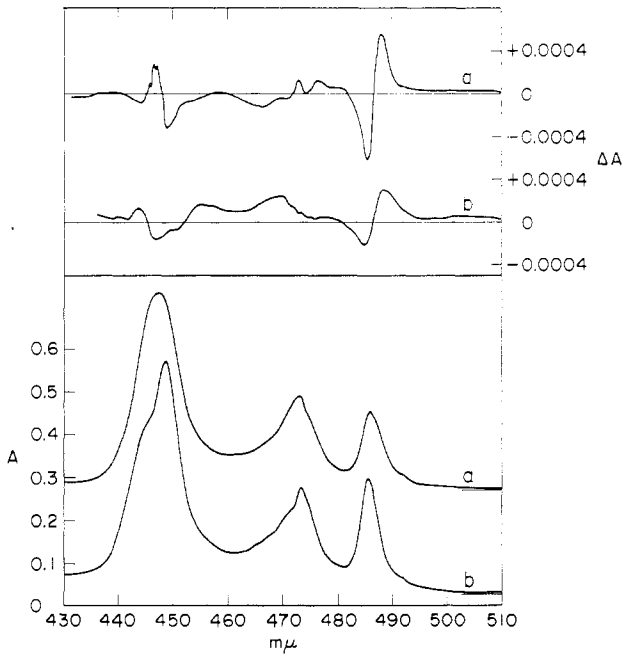

Figure 3.—CD and absorption spectra for 5:1 amino acid–praseodymium(III) mixtures, at pH 9–10: (a) asparagine; (b) proline. CD path lengths, 30 mm; absorption, 5 mm.

Glycine–praseodymium mixtures have absorption spectra whose changes with pH parallel those seen with

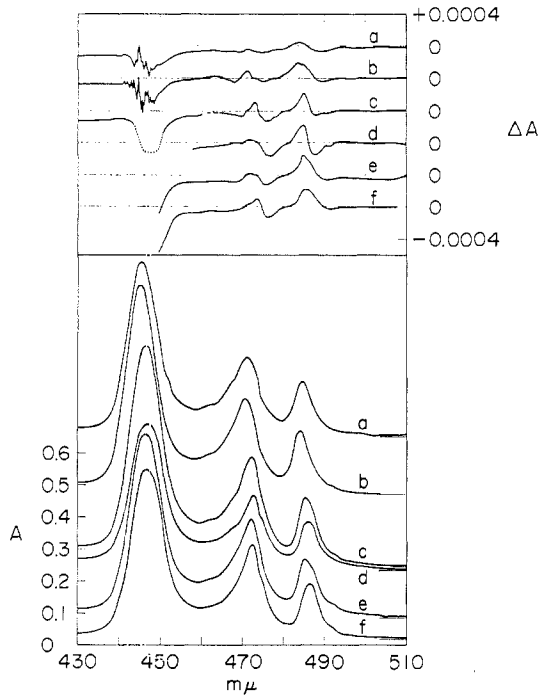

Figure 4.—CD and absorption spectra for aspartic acid–praseodymium(III) mixtures, with increasing pH: (a) 2:1, pH 6; (b)–(d) 3:1, pH 6, 8, 9, respectively; (e) and (f) 5:1, pH 8 and 9, respectively. CD path lengths, 30 mm; absorption, 5 mm.

the optically active monocarboxylic amino acids. There is indication that solutions with less than 1:1 ligand:praseodymium ratios may not precipitate as readily as corresponding mixtures with the heavier acids.

Discussion

The apparent ordering of stability and ease of formation of the 1:1 amino acid complexes of Pr(III) are approximately correlated to the ordering of the pK values[4] for acid dissociation of the protonated α-amino groups. Thus, we have the ordering: asparagine (8.80) $\gtrsim$ serine (9.15) > valine (9.62) ≈ leucine (9.60) ≈ alanine (9.69) > proline (10.60), where the number in parentheses is the pK value. This seems further confirmation that the CD and absorption changes which occur in the pH region 6.5–8 are related to formation of a 1:1 chelate. Formation of a chelate with this stoichiometry apparently suffices to stabilize against hydrolytic precipitation of the Pr(III) which otherwise would occur at even lower pH. The glycine results suggest a possibility that some precipitates might contain some constitutional amino acid.

The corresponding pK values for the basic amino acids, ornithine and lysine, for example, lie below 9, in the asparagine–serine range. It might be inferred that the positive proton charge on the second amino group, of much higher pK, acts as a destabilizing factor, to the point that higher ligand:praseodymium(III) ratios are required for stability.

The second CD spectrum shown by valine, in the ligand:praseodymium ratio region 2:1 and higher, may tentatively be ascribed to a 2:1 complex. The apparent tendency for the basic amino acids, under high ligand ratio conditions, to show initial stages of the same transformation at higher pH is not unreasonable. The failure of serine and asparagine, which so readily form the 1:1 complex, to form the same 2:1 species might be ascribed to interference from tridentate chelation (see below). The meaning of the fact that alanine, which has only a bidentate structure, fails to show any strong tendency toward this complex either is not certain. Leucine has too low a solubility to test the hypothesis adequately. The impression gained from the prior work on the hydroxy acids is reinforced: it is relatively difficult to form a 2:1 complex, and, when formed, it probably has a weaker and less sharply structured CD. Sign reversals in the CD on going to 2:1 also seem characteristic.

The changed spectra of the high ligand ratio solutions of asparagine and proline, above pH 9, probably reflect a different sort of alteration. We would favor the interpretation that it involves some hydrolytic equilibrium of the Pr(III) itself, perhaps analogous to that suggested for the alkaline form of the praseodymium–tartrate complex.[2] If it were related to a 2:1 complex, but of a sort different from the valine one, it would be expected that serine should give the same form as asparagine, and proline, one like valine. The high pH needed to produce the changed spectra is additional differentiation.

The two spectral forms of the 1:1 complexes correlate with the capability, or lack of it, for tridentate chelation. Thus the apparent additional splittings of the ground state of the complex with asparagine or serine, which are apparent in the CD spectrum of the $^3P_0$ band, would be related to the coordination of the terminal hydroxyl or amido group in addition to the carboxyl and α-amino groups. These are much too weak bases to allow the postulate that their effect is rather due to acquired protonic charge, in neutral and alkaline mediums. Further, they should then behave like ornithine and lysine. This tridentate chelation may, of course, lend additional stabilization to the complex, over that already ascribed to the relatively low value of the pK for the protonated amino group.

From the component analysis for the two absorption spectra, one sees some basis for the slight differences in the over-all spectral envelopes. In the $^3P_0$ band, these appear as a greater relative intensity of the 488-nm component relative to the 485-nm component determining the peak and a lesser relative intensity of the 483-nm component, comparing the alanine against the asparagine. There might also be a small difference in component breadth, to give the greater sculpturing of the absorption profile in the alanine case (this applies to the $^3P_1$ band also). Whether the total number of components in the CD is actually more in the asparagine complex than in the alanine one is not clear-cut. In order to duplicate the shape of the apparent 492-nm CD component of the alanine spectrum, it is necessary to divide it into two positive components and to intro-

(4) D. S. Greenberg, "Amino Acids and Proteins," Charles C Thomas, Springfield, Ill., 1951.

duce a third, a small negative CD component, as appears in Table I. Likewise, the spacing of the components appears fractionally less in the bidentate complex spectrum. The definite shift of the negative 484.5-nm component of the asparagine complex from the 485.5-nm position of its absorption component and from the 485-nm position of the equivalent component in the alanine complex must reflect the influence of an additional, unresolved component of like sign at 484 nm.

The apparent differences in absorption in the $^3P_1$ band between alanine and asparagine complexes seem ascribable primarily to greater absorption for the alanine complex in the 466–470-nm region, using the 472-nm component intensity as reference. There may also be a slight width difference between the two 472-nm components. The CD is more differentiating. The alanine complex has a strongly negative component at 454 nm, where the asparagine is weak; the 472- and 476-nm components are more strongly positive for the alanine complex, yet the neighboring 480-nm component is strongly negative, though that for the asparagine complex is still positive. There is a difference at 466 and 468 nm also—the former is weakly negative, and the latter, strongly so, in the alanine complex, whereas in the asparagine complex the intensity relations are reversed. With this, the asparagine complex has a negative 462-nm component, in contrast to a positive one for the alanine complex. The strongly negative 470-nm component of the asparagine trace shown is due to the fact that this spectrum is for pH 9 (still higher pH is similar); spectra at pH 7–8 lack this strong component. CD spectra at these and intermediate pH values suggest that this results from an additional splitting of the 472-nm component. The $\Delta\epsilon/\epsilon$ values for the components of the $^3P_0$ band are comparable with those of the tartrate complex of Pr(III) in neutral solution;[2] those in the $^3P_1$ band may be smaller than those in the corresponding tartrate bands.

In the case of the hydroxy acids, formation of seven- and six-membered rings was indicated. With the simple amino acids, five-membered rings are the only possibility. There, therefore, seems to be no definitive steric requirement of the Pr(III) ion which restricts the chelate geometry (within these limits).

Around pH 6, the aspartic acid–praseodymium complex has a CD that is stronger than that shown by the monocarboxylic acids at the same pH, though still quite weak, and probably weaker than that shown by the acid complex of hydroxy acids. At pH 7, the chemical solubility evidence suggests that the aspartic acid complex is weaker than the 1:1 serine or asparagine complexes, or even than those with the simple amino acids. The stability of the latter is obviously involved with removal of charge from the amino group, allowing chelation. It seems likely, therefore, that the titration measurements[5] which show aspartic acid to form stronger complexes than do the simple amino acids, and even than the hydroxy acids, below pH 6, are dealing with bidentate chelation through the carboxyl groups, and not with tridentate chelation as the authors infer in this[5] and as has been done in other[6–8] systems. Where as many as three stages of complexation are reported for aspartic acid and tripositive Pr,[5] with the pK values for the latter two stages approximating those for the monocarboxylic acids, it seems possible that either between the first and the second stage or between the second and the third stage the chelation of the first stage may be reduced to monodentate attachment.

On bringing a solution with, say, a 3:1 ratio of aspartic acid to Pr(III) from pH 6 to 9, there is an alteration of the absorption spectrum, and there is a minor but probably real change in the CD at the $^3P_1$ band. If amine chelation has been achieved with the pH change, it cannot have existed at pH 6. Further, it has quite different CD effects than for the monocarboxylic acids. If it were purely tridentate chelation, it might be expected to show closer resemblance to the asparagine and serine CD spectra. Chemical considerations and the resemblance of the CD in the $^3P_0$ band to that of the alkaline solution of malic acid with Pr(III) suggest the possibility that, rather than a tridentate chelate, one may have a bidentate form involving the amino group and the carboxyl β to it, to give a six-membered ring. The solution composition does not rule out the possibility that two aspartic acid ions have been chelated in this higher pH region.

A report by others[9] on work with glycine and alanine complexes of Pr(III) also indicates the importance of 1:1 complexes and their stabilization above pH 7.

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