Binary and Ternary Complexes of Chromium(III) involving Iminodiacetic Acid, L(+)-Aspartic Acid, L(+)-Glutamic Acid, or L(+)-Cysteine as Ligands

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Stability constants for binary complexes of the type MAH, MA, MA_2H_2, MA_2H, MA_2, or M_2A_3 in the Cr^{III}–iminodiacetic acid (H_2ida), –L(+)-aspartic acid (asp), –L(+)-glutamic acid (glu), and –L(+)-cysteine (cys) systems and for ternary complexes $MABH_2$, MABH, or MAB in the Cr^{III}–H_2ida(A)–asp or –glu(B) and Cr^{III}–cys(A)–asp or –glu(B) systems have been determined for the first time at 50 ± 0.1 °C and I = 0.1 mol dm^{-3} (Na[ClO_4]) by potentiometry using a computer iteration technique. The acid dissociation constants of these ligands have also been obtained under identical conditions. The pH dependence of the electronic spectra of the equilibrated solutions provides information on the nature and type of complexes formed. The results suggest that H_2ida and asp act as tridentate ligands in the binary complexes MA and MA_2, as well as in the ternary complex MAB. However, it appears that glu is tridentate in the MA complex, but bidentate in MA_2 and MAB. The potentially tridentate ligand cys has been established to behave as a bidentate ligand towards Cr^{III}. The probable site of protonation in the binary complexes MAH, MA_2H, and MA_2H_2 is discussed on the basis of the dissociation constant of the ligand and the stability-constant data for the complexes. Preferential formation and enhanced stability were observed for the ternary relative to the binary complexes. This has revealed factors such as ring size, ligand–ligand interactions, and steric effects which influence the stabilization of chromium(III) mixed-ligand complexes.

CHROMIUM was shown to be an essential trace element 15 years ago.[1,2] Since then substantial progress has been made in understanding its physiological role.[1-3] Recent observations led to the conclusion that the biological activity of Cr^{III} largely depends on the chemical nature of chromium(III) complexes. The hypothetical chromium(III) mixed-ligand complex occurring in brewer's yeast was found to have outstanding biological activity.[3] Its constituents were identified as Cr^{III}, nicotinic acid, glycine, glutamic acid, and cysteine. It has been assumed[4] that the transportation of Cr^{III} from the intestine to the reaction site occurs in the form of a stabilized low-molecular-weight diffusing mixed-ligand complex which is resistant to hydrolysis and polymerization, characteristics of Cr^{III}. Chromium(III) mixed-ligand complex formation is also involved in the stabilization of collagen with Cr^{III} as a chief mineral tanning agent. Of the various functional groups of collagen, the current theories favour[5] the direct co-ordination of side-chain carboxylic groups of aspartic and glutamic acids.

In spite of the immense biological and technological importance of chromium(III) mixed-ligand complexes, data on their stability constants are almost non-existent. Considering the difficulties in obtaining reliable stability constants for chromium(III) binary complexes,[6,7] it is not surprising that obtaining stability-constant data for chromium(III) ternary complexes is a most difficult task. A comprehensive review of earlier work on the equilibrium chemistry of chromium(III) complexes indicates the necessity to (*i*) set up adequate experimental conditions over wide concentration and pH ranges for ensuring complete equilibration, (*ii*) consider all relevant equilibria, including species of the type MAH, MA(OH), M(OH), $M_2A_2(OH)_2$, $M_2A_4(OH)_2$, M_2A_3, or M_3A_4 and ternary complexes of the type MAB, MABH, $MABH_2$, MA_2B, or MAB_2, in addition to the mononuclear binary species, using advanced computer techniques, and (*iii*) revise the estimates of auxiliary data such as the acid-dissociation constants of the ligands and binary stability constants under specified experimental conditions.

It is desirable to understand the factors which determine the net effect of competitive reactions giving rise to the most probable chemical species of Cr^{III} in the presence of ligands with a potential biological role. The study of the distribution of Cr^{III} among different species under simulated biological conditions will undoubtedly elucidate the mechanism of transportation, storage, and function of Cr^{III} as an essential trace element. We have previously carried out[8-14] extensive research on the equilibrium chemistry of metal complexes of biological interest. The present paper reports the first systematic study on chromium(III) binary and ternary complexes containing some potentially tridentate ligands, *viz.* iminodiacetic acid (H_2ida), L(+)-aspartic acid (asp), L(+)-glutamic acid (glu), and L(+)-cysteine (cys).

EXPERIMENTAL

A Radiometer pHM4d pH meter with ±0.01 mV accuracy was used together with a GK 2401C glass and calomel combination electrode for all pH measurements and titrations. This was standardised with buffers of pH 4.06 (0.05 mol dm^{-3} potassium hydrogenphthalate), 6.45 (Radiometer phosphate), and 8.88 (borate of Hindustan Precision Instruments Private, Ltd.) at 50 ± 0.1 °C. Standard $HClO_4$ with $I = 0.1$ mol dm^{-3} (Na[ClO_4]), corrected for hydrogen-ion activity, was used to calibrate the pH meter in the more acidic regions. A Pye-Unicam SP1800 u.v. spectrophotometer was used to record visible and u.v. spectra.

Doubly distilled water was used for the preparation of all solutions. The ligands H_2ida, asp, glu (B.D.H. L.R., 98.5% pure), and cys (Merck, 99% pure) were estimated for acidity by potentiometric titrations of *ca.* 0.1 mol dm^{-3} solutions against 0.1 mol dm^{-3} Na[OH]. Hexa-aquachromium(III) perchlorate was prepared from chromium trioxide as described elsewhere.[15] The visible and u.v. spectra of the $Cr[ClO_4]_3 \cdot 6H_2O$ solution showed $\lambda_{max.}$ at 260, 408, and 578 nm with $\varepsilon_{max.}$ values of 5.50, 15.44, and 13.80 dm^3 mol^{-1} cm^{-1}.

Chromium was estimated by potentiometry. The free acid in $Cr[ClO_4]_3$ was estimated by an ion-exchange method. Solutions of other reagents, *e.g.* sodium hydroxide (E. Merck, G.R.), sodium perchlorate (Riedel, pure), and perchloric acid (E. Merck, G.R. 60%) were prepared and estimated as described elsewhere.[8-14]

Dissociation constants of all the ligands used were determined afresh at 50 ± 0.1 °C and $I = 0.1$ mol dm^{-3} ($Na[ClO_4]$) by conventional potentiometric methods. In general, the concentration of the ligands corresponded to that used in the metal complexation process. The titration cell was designed in such a way as to minimize solvent loss due to evaporation.

In order to ensure complete equilibration between Cr^{III} and complexing ligands, a modified approach with prolonged equilibration [16] was used at 50 ± 0.1 °C and $I = 0.1$ mol dm^{-3} ($Na[ClO_4]$). All necessary precautions were taken when conducting the experiments at higher temperatures.[17] Equimolar quantities of $Cr[ClO_4]_3 \cdot 6H_2O$, mineral acid ($HClO_4$), electrolyte ($Na[ClO_4]$) to maintain the ionic strength at $I = 0.1$ mol dm^{-3}, and the ligand or ligands, along with various molar quantities of CO_2-free $Na[OH]$ were combined in a 50-cm^3 standard flask. The solutions were made up to the mark and thermostatted at 50 ± 0.1 °C in a water-bath for *ca.* 20 d. Special precautions were taken for the systems involving cys. The solutions were deaerated after mixing using nitrogen and the standard flasks were sealed with Serum caps to maintain an inert atmosphere during equilibration. The equilibration process was monitored by periodically measuring the pH and optical density of selected flasks containing solutions of different pH ranges. It was assumed that equilibration was complete when the pH changes were within ±0.01 units for *ca.* 7 days at 50 ± 0.1 °C and the optical density was invariant over the same period. In one run, the solutions were kept for 1 month after the equilibration period in order to study the possible changes in pH and optical density. It was observed that the changes in the above parameters were within experimental error. The chelating agent alone, in the absence of $Cr[ClO_4]_3 \cdot 6H_2O$, was kept under similar conditions at different pH ranges in order to note the effect of prolonged equilibration on ligand decomposition, if any. It was found that pH values for this solution remained unchanged. After equilibration, the pH values of all the solutions were measured at 50 ± 0.1 °C under nitrogen.

A simulated titration curve was obtained by plotting the volume of $Na[OH]$ added to each flask against the corresponding pH measured after attaining equilibrium.

The average number of ligands bound per metal ion, $\bar{n}$, was obtained for various metal-to-ligand ratios in the binary systems by algebraic methods.[18] In calculating the hydrogen-ion concentration from pH measurements, the activity coefficient of the hydrogen ion [19] (0.85) and the value of pK_w[20] (13.05) at 50 ± 0.1 °C and $I = 0.1$ mol dm^{-3} were used. The ligand-dissociation constants and the gross stability constants of the binary and ternary complexes of Cr^{III} were evaluated by the SCOGS computer program [21-23] on an IBM-370 computer. Various chemical models have been tested for each system and the best-fit model chosen is based on the lowest residual consistent with the analyses of the spectroscopic and formation curves. In calculating the stability constants of the ternary complexes, the stability constants of protonated-ligand and binary complex species obtained under similar conditions were held constant as non-refinable parameters. The pH range selected for each system is based on the fact that the residuals increased sharply beyond this range, indicating the onset of other complicated species which could not be handled with precision. In general, the range of error associated with the residuals and the standard deviations in the equilibrium constants of the binary and ternary systems is understandable if the complexity of the systems is taken into consideration, with more than 10 species contributing.

RESULTS AND DISCUSSION

Binary Complexes of Chromium(III) *with* H_2*ida, asp, glu, and cys*.—The acid-dissociation constants obtained for H_2ida, asp, glu, and cys (Table 1) are in good agreement with those reported in the literature [24] considering the variations in experimental conditions. From the potentiometric data for the binary systems, $\bar{n}$ the average number of ligands bound per metal ion, and pA, the negative logarithm of the free-ligand concentration, were calculated in the pH range under investigation. Formation curves obtained by plotting $\bar{n}$ against pA for solutions with different metal-to-ligand ratios are not superimposable, indicating the existence of some other species, in addition to the mononuclear binary complexes. The $\bar{n}$ hump observed in the lower pH regions of the Cr^{III}–glu and –cys systems, similar to that reported previously by Williams and co-workers,[17] indicates the presence of protonated metal complexes. In the Cr^{III}–H_2ida system, the $\bar{n}$ value, even at pH 1.6, was found to be *ca.* 0.8, suggesting that 80% of the total metal is to be found in the form of the MA complex. However, beyond pH 3.5 the $\bar{n}$ value of this system decreased, both at 1 : 3 and 1 : 5 metal-to-ligand ratios indicating the formation of appreciable amounts of stable 1 : 2 binary complexes. The maximum $\bar{n}$ value found in the Cr^{III}–cys system was only 1.0, even with a five-fold excess of ligand, demonstrating the restrictions imposed on the formation of MA_2.

TABLE 1

Stability constants for the proton and chromium(III) complexes of H_2ida, asp, glu, and cys at 50 ± 0.1 °C and $I = 0.1$ mol dm^{-3} ($Na[ClO_4]$). Standard deviations are given in parentheses

	Ligand A			
Parameter	H_2ida	asp	glu	cys
log β_{HA}	8.97(1)	9.23(3)	9.115(8)	10.36(1)
log β_{H_2A}	11.75(2)	13.00(4)	13.34(1)	18.27(2)
log β_{H_3A}	13.44(2)	14.89(4)	15.66(1)	20.17(3)
log β_{MAH}			14.04(5)	18.33(3)
log β_{MA}	12.07(18)	12.15(3)	11.39(3)	
log $\beta_{MA_2H_2}$				35.90(3)
log β_{MA_2H}	23.51(19)	24.07(7)	23.91(5)	31.83(6)
log β_{MA_2}	21.78(17)	21.13(4)	18.96(19)	
log $\beta_{M_2A_2}$				44.49(9)
pK_{a_1}	8.97	9.23	9.12	10.36
pK_{a_2}	2.78	3.77	4.22	7.91
pK_{a_3}	1.69	1.89	2.32	1.90
log K^{M}_{MA}	12.07	12.15	11.39	
log $K^{MA}_{MA_2}$	9.71	8.98	7.57	
log $(K^{M}_{MA}/K^{MA}_{MA_2})$	2.36	3.17	3.82	
pK^{H}_{MAH}			2.65	
$pK^{H}_{MA_2H}$	1.73	2.94	4.95	
pH range	1.70—4.00	1.70—4.00	2.00—4.30	1.90—4.20

A similar situation was apparent in the Cr^{III}–glu system where the maximum $\bar{n}$ value was 1.3. However, generally, the family of formation curves are not equidistant and hence it is not possible to interpret the experimental data quantitatively by graphical analysis alone.

More than 10 different chemical models were tested by the SCOGS computer program for each binary system. In the Cr^{III}–H_2ida system, that consisting of MA, MA_2, and MA_2H gives the best fit with the lowest standard deviation in the titre, $\sigma(V) = 0.027$, and species like MA_3, M(OH), MA(OH), $M_2A_2(OH)_2$, MA_2H_2, and MA_3H_3 are eliminated. This is consistent with the analysis of the formation curve. In the Cr^{III}–asp system, this model also converged successfully with $\sigma(V) = 0.019$. In the Cr^{III}–glu system, however, convergence was obtained with the model containing MAH, MA, MA_2H, and MA_2, with $\sigma(V) = 0.016$. In the Cr^{III}–cys system, only the protonated metal complexes MAH, MA_2H, and MA_2H_2 in addition to the dimer, M_2A_3, are found to converge successfully [$\sigma(V) = 0.017$]. In all cases, the best-fit models accord with the information obtained from formation plots.

Values of log β_{MA} obtained for the Cr^{III}–H_2ida, –asp, and –glu systems (Table 1) are substantially larger than that[25] of 8.70 for the Cr^{III}–glycine (gly) system indicating tridentate binding of H_2ida, asp, and glu in their respective MA complexes. The log β_{MA} values in these three systems are in the order H_2ida $\sim$ asp $>$ glu, whereas the overall basicity is in the order glu $>$ asp $>$ H_2ida. This is readily explained by considering the size of the chelate rings involved in the MA complexes: five and five, five and six, and five and seven respectively when A = H_2ida, asp, and glu. The five- and seven-membered chelate rings in the Cr^{III}–glu system are least preferred due to well known steric factors. Thus, the effect of increased basicity is overcompensated for by ring-size effects. Again, it should be noted that the values of log $K^{MA}_{MA_2}$ for the Cr^{III}–H_2ida and –asp systems are higher than that of 7.63 for the Cr^{III}–gly[25] system, indicating bonding of the second H_2ida or asp ligand to MA through all three donor groups. However, in the Cr^{III}–glu system, the log $K^{MA}_{MA_2}$ value is similar to that in the Cr^{III}–gly system, thereby revealing a possible bidentate binding of the second glu molecule to MA. The difference in successive equilibrium constants, log $(K^{M}_{MA}/K^{MA}_{MA_2})$ (Table 1), for the Cr^{III}–H_2ida, –asp, and –glu systems decrease in the order glu $>$ asp $>$ H_2ida, all values being higher than the statistically expected value of 0.68. It is interesting that a linear correlation between log $(K^{M}_{MA}/K^{MA}_{MA_2})$ and chelate ring size is obtained for these three systems (Figure 1), giving a direct measure of the geometrical factor (ligand) which has a major role in the successive equilibration processes.

The pH dependence of the electronic spectra for the Cr^{III}–H_2ida, –asp, and –glu binary systems recorded at 50 ± 0.1 °C provides valuable evidence as to the donor groups of the complexes formed. In the first two systems, as the pH is increased, $\lambda_{max.}$ shifts to higher frequency indicating increased co-ordination of nitrogen to Cr^{III}. At *ca.* pH 4.0, the Cr^{III}–H_2ida spectrum corresponds to the co-ordinatively saturated $Cr(H_2ida)_2$ complex with $\lambda_{1max.} = 524$ and $\lambda_{2max.}$ 390 nm as reported in the literature.[26] The Cr^{III}–asp spectrum is similar with $\lambda_{1max.} = 520$ and $\lambda_{2max.} = 390$ nm at *ca.* pH 4.8 indicating a similar mode of co-ordination. The shift in $\lambda_{max.}$ to 560 and 420 nm in the Cr^{III}–glu system resembles the spectra for the Cr^{III}–gly system[25] ($\lambda_{1max.} = 555$, $\lambda_{2max.} = 420$ nm). This demonstrates that in the Cr^{III}–glu system the species with the glycine mode of co-ordination predominates.

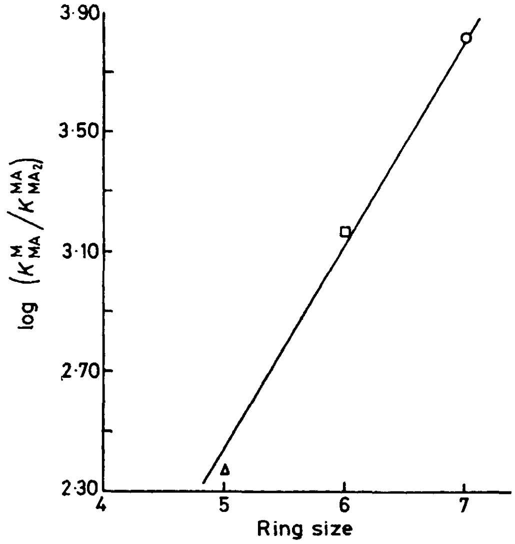

FIGURE 1 Plot of $\log(K^{M}_{MA}/K^{MA}_{MA_2})$ as a function of ring size in the Cr^{III}–H_2ida (△), –asp (□), and –glu (○) binary systems

The site of protonation in the MAH, A = glu, complex may be assigned to the γ-carboxyl group. The lower protonation constant pK^{H}_{MAH} 2.65 for the process $CrA + H \rightleftharpoons CrAH$ in this system (Table 1) compared to pK γ-carboxyl of 4.22 may be attributed to the fact that competition of the α- and γ-carboxyl groups for co-ordination considerably reduces the pK_a. Similarly, for the process $MA_2 + H \rightleftharpoons MA_2H$ (A = H_2ida, asp), the p$K^{H}_{MA_2H}$ values (Table 1) are lower than the respective pK_a values for the less acidic carboxyl groups of these ligands, 2.78 and 3.77 (Table 1). Thus, from this trend it may be concluded that the protonation site for the second ligand co-ordinated in the MA_2H, A = H_2ida and asp, complexes is the less acidic carboxyl group of the respective free ligand. It is surprising that the protonation constant, p$K^{H}_{MA_2H}$, of the Cr^{III}–glu system is greater than pK γ-carboxyl (Table 1). This indicates that the third binding site is not competing for the metal ion.

In the Cr^{III}–cys system, the protonated complexes were more readily formed than in the corresponding H_2ida, asp, and glu systems investigated. The significant decrease in the standard deviation of the titre when the mononuclear species were removed from the model indicates that the third donor group is too weak to co-ordinate with the metal and will instead join to a proton.

In the binding of cysteine with $\mathrm{Cr^{III}}$, there are four modes available for co-ordination: (*i*) one five-membered ring through $\mathrm{NH_2}$ and $\mathrm{COO^-}$ groups; (*ii*) one six-membered ring through $\mathrm{S^-}$ and $\mathrm{COO^-}$ groups; (*iii*) one five-membered ring through $\mathrm{S^-}$ and $\mathrm{NH_2}$; and (*iv*) two five-membered rings through $\mathrm{COO^-}$, $\mathrm{NH_2}$, and $\mathrm{S^-}$ groups. Information regarding the donor groups may be obtained from the electronic spectrum of the $\mathrm{Cr^{III}}$–cys system, shown in Figure 2. The high-intensity u.v. peak at $\lambda_{\mathrm{max.}}$ = 260 nm may be due to a $\mathrm{S \rightarrow Cr^{III}}$ charge-transfer transition.[27] At pH 3.0, the spectrum exhibits $\lambda_{\mathrm{max.}}$ at 550 and 408 nm in the visible range (Figure 2) indicating the possible co-ordination of nitrogen in addition to sulphur. The intensity of the u.v. band increased rapidly with pH indicating the increased participation of sulphur in co-ordination. Thus, spectral studies show that cys is co-ordinated to $\mathrm{Cr^{III}}$ through $\mathrm{NH_2}$ and $\mathrm{S^-}$. Since the existence of mononuclear species of the type MA, $\mathrm{MA_2}$, and $\mathrm{MA_3}$ is shown to be insignificant by means of computer analysis, it is difficult to determine the mode of co-ordination from the stability-constant values alone. However, the log β values of 18.33, 35.90, and 31.83 respectively for MAH, $\mathrm{MA_2H_2}$, and $\mathrm{MA_2H}$ complexes in the $\mathrm{Cr^{III}}$–cys system (Table 1) are comparable to the values of 18.46, 35.74, and 31.78 for the corresponding complexes in the $\mathrm{In^{III}}$–cys system.[28] Likewise in the case of $\mathrm{In^{III}}$, it was found that under these experimental conditions, protonated complexes predominate. It appears that in the $\mathrm{Cr^{III}}$–cys system the formation of the protonated complexes is more readily observed than in the other tridentate amino-acids investigated. Hence, clearly the geometry of the cys ligand favours its acting in a bi- rather than a tri-dentate manner.

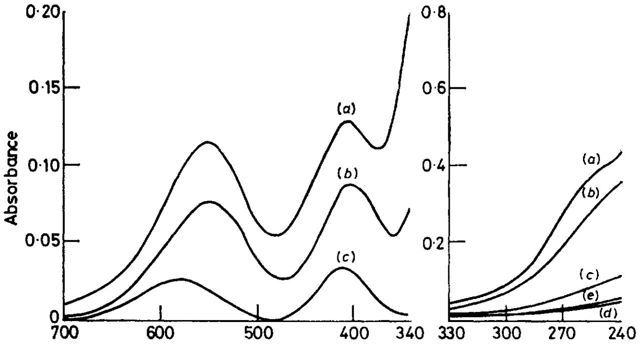

FIGURE 2 pH Dependence of the electronic spectra of the $\mathrm{Cr^{III}}$–cys(A) binary system with $C_{\mathrm{M}} = 2.0 \times 10^{-3}$ mol dm^{-3} and $C_{\mathrm{A}} = 9.9 \times 10^{-3}$ mol dm^{-3}. pH = 3.89 (*a*), 2.96 (*b*), 1.94 (*c*), 3.00 (*d*) (metal alone), and 4.04 (*e*) (ligand alone)

The distribution of species as percentages of total metal have been plotted against pH for different metal-to-ligand ratios for all the systems. A typical plot, for $\mathrm{Cr^{III}}$–cys, is given in Figure 3. As anticipated from analysis of the formation curves, the protonated metal complexes, MAH, $\mathrm{MA_2H_2}$, and $\mathrm{MA_2H}$, predominate in the lower pH regions. In all four binary systems studied, the free-metal-ion concentration was negligibly small beyond pH 3.50, demonstrating that there is an insignificant contribution from metal hydrolysis to the equilibrium. Previously, Chaberek and Martell[29] also reported that metal hydrolysis is inhibited in the presence of an excess of strongly chelating tridentate ligands. The percentage concentrations of the $\mathrm{MA_2}$ (A = $\mathrm{H_2}$ida, asp, glu) complexes reached their maxima of *ca.* 99, 96, and 10 respectively at pH 4.0. This conforms with the electronic spectra of these solutions where $\lambda_{\mathrm{1max.}}$ and $\lambda_{\mathrm{2max.}}$ values in the first two cases at pH 4.0 resemble those reported[26,30] for the $\mathrm{Cr(H_2ida)_2}$ complex with $\lambda_{\mathrm{1max.}} = 524$ and $\lambda_{\mathrm{2max.}} = 390$ nm and the $\mathrm{Cr(asp)_2}$ complex with $\lambda_{\mathrm{1max.}} = 520$ and $\lambda_{\mathrm{2max.}} = 390$ nm. The $\mathrm{MA_2H}$ complexes predominated in the $\mathrm{Cr^{III}}$–glu and –cys systems whereas these complexes were found in relatively low concentrations in the $\mathrm{Cr^{III}}$–asp and –$\mathrm{H_2}$ida systems, the respective percentages being 78, 42, 24, and 16. This underlines the differences between these four ligands as regards their ability to act in a bidentate manner in chromium(III) complexes.

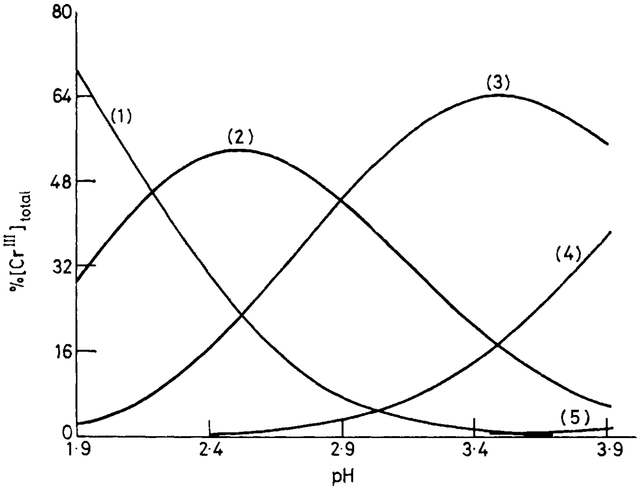

FIGURE 3 Distribution of various species as a function of pH in the $\mathrm{Cr^{III}}$–cys(A) binary system at a M : A ratio of 1 : 5. Unbound metal (1), MAH (2), $\mathrm{MA_2H_2}$ (3), $\mathrm{MA_2H}$ (4), and $\mathrm{M_2A_3}$ (5)

Ternary Systems of Chromium(III).—Four ternary systems, (*i*) $\mathrm{Cr^{III}}$–$\mathrm{H_2}$ida(A)–asp(B), (*ii*) $\mathrm{Cr^{III}}$–$\mathrm{H_2}$ida(A)–glu(B), (*iii*) $\mathrm{Cr^{III}}$–cys(A)–asp(B), and (*iv*) $\mathrm{Cr^{III}}$–cys(A)–glu(B), were studied. In addition to the various binary species due to ligands A and B (Table 1), the best-fit model contains the ternary complexes MABH and MAB in systems (*i*), (*ii*), and (*iii*) and $\mathrm{MABH_2}$, MABH, and MAB in system (*iv*). The standard deviations in the titre, $\sigma(V)$, in the best-fit model for systems (*i*), (*ii*), (*iii*), and (*iv*) are respectively 0.022, 0.017, 0.014, and 0.015. It should be noted that the $\sigma(V)$ values obtained for the binary and ternary systems of $\mathrm{Cr^{III}}$ investigated are comparable to those obtained while computing ligand-protonation constants. Hence, the complex species obtained in the best-fit models are valid under the experimental conditions employed.

In order to assess the significance of the stability of the ternary complex species in relation to those of the parent binary complexes, the parameter, Δ log K, the difference in stability between the binary and ternary complexes, and log X, the disproportionation constant, were calculated for all the ternary complexes detected. For MAB complexes, Δ log K and log X are defined by equations (1)—(4). For the other types of ternary complexes, the appropriate equations defining Δ log K and log X are given in Table 2. The Δ log β stabilization constants included in Table 2 for the various ternary complexes result from the difference between the measured stabilities and those calculated on statistical grounds. For statistical reasons,[31] values of Δ log $K > -0.4$, log $X > 0.6$, and positive values of Δ log β demonstrate the enhanced stability of the regular octahedral chromium(III) ternary complexes.

$$\mathrm{MA} + \mathrm{MB} \rightleftharpoons \mathrm{MAB} + \mathrm{M} \qquad (1)$$

$$\Delta \log K_{\mathrm{MAB}} = \log\beta_{\mathrm{MAB}} - (\log\beta_{\mathrm{MA}} + \log\beta_{\mathrm{MB}}) \qquad (2)$$

$$\mathrm{MA_2} + \mathrm{MB_2} \rightleftharpoons 2\,\mathrm{MAB},\ X_{\mathrm{MAB}} = [\mathrm{MAB}]^2/[\mathrm{MA_2}][\mathrm{MB_2}] \qquad (3)$$

$$\log X_{\mathrm{MAB}} = 2\log\beta_{\mathrm{MAB}} - (\log\beta_{\mathrm{MA_2}} + \log\beta_{\mathrm{MB_2}}) \qquad (4)$$

TABLE 2

Stability constants for the $\mathrm{Cr^{III}}$–$\mathrm{H_2}$ida or –cys(A)–asp or –glu(B) ternary systems at 50 ± 0.1 °C and $I = 0.1$ mol dm^{-3} (Na[ClO$_4$]). Standard deviations are given in parentheses

	$\mathrm{H_2}$ida (A)		cys (A)	
Parameter	asp (B)	glu (B)	asp (B)	glu (B)
log $\beta_{\mathrm{MABH_2}}$				32.55(3)
log β_{MABH}	24.74(30)	25.52(7)	29.74(4)	29.53(3)
log β_{MAB}	22.78(6)	22.22(5)	26.03(3)	24.82(7)
log $K^{\mathrm{MA}}_{\mathrm{MAB}}$	10.71	10.15		
log $K^{\mathrm{MB}}_{\mathrm{MAB}}$	10.63	10.83	13.88	13.43
p$K^{\mathrm{H}}_{\mathrm{MABH}}$	1.96	3.30	3.71	4.71
Δ log K_{MAB}	−1.44	−1.24		
Δ log K_{MABH}		−0.59 [a]	−0.74 [b]	−0.19 [b]
Δ log $K_{\mathrm{MABH_2}}$				+0.18 [c]
log X_{MAB}	2.65	3.70		
log X_{MABH}	1.90 [d]	3.62 [d]	3.58 [d]	3.32 [d]
Δ log β_{MAB}	1.03	1.55		
Δ log β_{MABH}	0.65	1.51	1.49	1.36
pH range	1.70—4.10	1.80—4.30	2.20—4.20	1.80—4.20

[a] $\mathrm{MA} + \mathrm{MBH} \rightleftharpoons \mathrm{MABH} + \mathrm{M}$. [b] $\mathrm{MAH} + \mathrm{MB} \rightleftharpoons \mathrm{MABH} + \mathrm{M}$. [c] $\mathrm{MAH} + \mathrm{MBH} \rightleftharpoons \mathrm{MABH_2} + \mathrm{M}$. [d] $\mathrm{MA_2H} + \mathrm{MB_2H} \rightleftharpoons 2\,\mathrm{MABH}$.

Chromium(III)–$\mathrm{H_2}$*ida*(*A*)–*asp or* –*glu*(*B*) *systems.* The Δ log $K_{\mathrm{Cr^{III}AB}}$ values (Table 2) for the above two systems indicate that the binary complexes, MA and MB, are preferred to the ternary complex, MAB. A more negative Δ log K_{MAB} value for the B = asp system than for the B = glu system suggests that asp preferentially adds on to the hexa-aquometal ion due to the relative ring-size effects. The Δ log K_{MABH} value also exhibits a similar tendency, the equilibrium shifting towards the left. In general the factors responsible for the destabilization of ternary complexes relative to the 1 : 1 binary complex may arise from the absence of charge neutralization in the former.

In log X_{MAB}, the stability of the mixed complex MAB is compared to that of the complexes $\mathrm{MA_2}$ and $\mathrm{MB_2}$. Although the Δ log K_{MAB} values are negative, signifying little preference for MAB over MA and MB, the log X_{MAB} values in Table 2 for both the ternary systems with B = asp or glu are higher than the statistical value of 0.6 indicating stabilization of the ternary complexes relative to the 1 : 2 binary complexes. The higher log X_{MAB} value in the B = glu system may be due to the fact that steric hindrance in the $\mathrm{MB_2}$, B = glu, complex is greater than in $\mathrm{MB_2}$, B = asp. The log X_{MABH} values in Table 2 exhibit the same general trends as log X_{MAB}. Ternary is perhaps preferred over binary complexation due to the fact that destabilization caused by ligand repulsion and steric hindrance is smaller in the former. The stabilization constants, Δ log β, for the MAB and MABH complexes in the $\mathrm{Cr^{III}}$–$\mathrm{H_2}$ida(A)–asp and –glu (B) systems are positive indicating the enhanced stabilities of the ternary complexes compared to the statistical case, which is in agreement with the conclusion reached from log X values.

The log $K^{\mathrm{MA}}_{\mathrm{MAB}}$ values (Table 2) for the $\mathrm{Cr^{III}}$–$\mathrm{H_2}$ida(A)–asp or –glu(B) systems are higher than the corresponding log $K^{\mathrm{MB}}_{\mathrm{MB_2}}$ values (Table 1). This indicates that asp or glu (B) ligands preferentially co-ordinate to the 1 : 1 $\mathrm{Cr^{III}}$–$\mathrm{H_2}$ida(A) complex to form MAB rather than to the 1 : 1 MB complex to form the corresponding $\mathrm{MB_2}$ complexes. Similarly, the value of log $K^{\mathrm{MB}}_{\mathrm{MAB}}$ is higher than that of log $K^{\mathrm{MA}}_{\mathrm{MA_2}}$ for both systems, suggesting that $\mathrm{H_2}$ida-(A) adds to 1 : 1 complexes of asp and glu(B) to form MAB rather than to MA to form $\mathrm{MA_2}$. Further, $\mathrm{H_2}$ida-(A) adds preferentially to the 1 : 1 complex of glu(B) rather than to that of asp(B). This may be due to the fact that a co-ordinated molecule of glu binds in a bidentate manner in a 1 : 2 complex, leaving the third binding site free. This may possibly also be involved in the stabilization of the ternary complex. By comparing the successive equilibrium constants of protonated binary and ternary complexes in Tables 1 and 2, it may be concluded that the MABH complexes are preferred over the corresponding binary complexes, as is the case with the MAB complexes.

Chromium(III)–*cys*(*A*)–*asp or* –*glu*(*B*) *systems.* The Δ log K_{MAB} and log X_{MAB} [equations (1)—(4)] values could not be calculated for these systems. Hence, the extent of stabilization of ternary over binary complexes is assessed by comparing the stability constants of the corresponding protonated complexes. The negative Δ log K_{MABH} values obtained for both these systems (Table 2) indicate that the equilibrium is shifted to the left. A positive Δ log $K_{\mathrm{MABH_2}}$ value of 0.18 in the $\mathrm{Cr^{III}}$–cys(A)–glu(B) system suggests that the ternary complex is preferred over the corresponding binary complexes MAH and MBH. The stabilization of ternary complexes MABH over the binary $\mathrm{MA_2H}$ and $\mathrm{MB_2H}$ species in these systems is confirmed by the log X_{MABH} values in Table 2, which are far higher than the statistically expected value of 0.6. The slightly higher log X_{MABH} value for the B = asp system than for B = glu (Table 2) may be rationalized if the differences in log $\beta_{\mathrm{MA_2}}$ and log $\beta_{\mathrm{MA_2H}}$

for asp and glu (Table 1) are taken into account, despite the fact that the steric hindrance in 1 : 2 binary species for B = glu is greater. The Δ log β_{MABH} values in Table 2 for both these systems are positive. This also suggests enhanced stability of the ternary complex, MABH, compared to the corresponding binary complexes.

Again, the unco-ordinated binding site of glu in its 1 : 2 complex may be involved in the stabilization of the ternary complex due to interligand interaction through hydrogen bonding. This is in accord with the ternary complex proposed by Mertz[3] for the biologically active material extracted from brewer's yeast. It appears that the Cr^{III}–cys protonated 1 : 1 complex prefers to co-ordinate with glu than with asp even though both these ligands are available in blood serum.

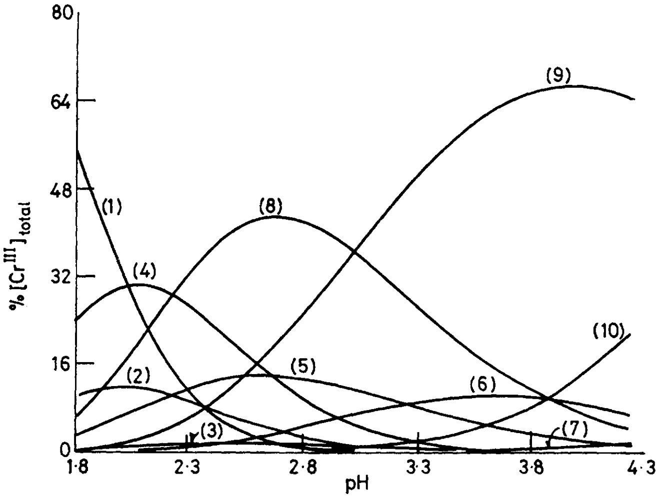

FIGURE 4 Distribution of various species as a function of pH in the Cr^{III}–cys(A)–glu(B) ternary system at a M : A : B ratio of 1 : 3 : 3. Unbound metal (1), MAH (2), MA_2H_2 (3), MBH (4), MB (5), MB_2H (6), MB_2 (7), $MABH_2$ (8), MABH (9), and MAB (10). The species MA_2H and M_2A_3 are not represented because of their very low concentrations

Distribution of species as a function of pH in the chromium(III)*-*H_2*ida or cys(A)-asp or -glu*(B) *systems.* The distribution of various species in terms of the percentage of bound Cr^{III} as a function of pH was calculated for all four systems. A typical example is given in Figure 4 for the Cr^{III}–cys(A)–glu(B) ternary system. The percentage of the protonated ternary species, MABH, is relatively higher in the Cr^{III}–H_2ida(A)–glu(B) (32%) than in the Cr^{III}–H_2ida(A)–asp(B) system (11%), whereas the non-protonated ternary species, MAB, increases from 60% in the former to 80% in the latter. This indicates the relative tendency of glu or asp (B) ligands to act in a bidentate manner. A similar trend was also observed in the Cr^{III}–cys(A)–glu or –asp(B) systems. The concentration of the ternary species, MAB, reaches 52% in the Cr^{III}–cys(A)–asp(B) system, whereas only protonated metal complexes were obtained in the Cr^{III}–cys binary system. This may be explained on the basis of the presence of free additional donor groups even in ternary complexes consistent with the fact that a considerable amount of MA_2H occurs in the Cr^{III}–cys system. As in Figure 4, in all four ternary systems, the concentrations of the ternary complexes (which appear to be more resistant to hydrolytic aggregation) increase with pH thus, possibly making Cr^{III} biologically available at physiological pH.

Comparison of the chromium(III)*-*H_2*ida(A)-B systems with chromium*(III)*-cys(A)-B systems.* The Δ log K_{MAB} and Δ log K_{MABH} values in Table 2 for the Cr^{III}–H_2ida–B and Cr^{III}–cys–B (B = asp or glu) systems suggest that 1 : 1 complexes of both Cr^{III}–H_2ida and –cys(A) prefer to co-ordinate with the ligand B. Furthermore, these Δ log K values indicate that glu is preferred by 1 : 1 Cr^{III}–H_2ida or –cys(A) complexes rather than asp. This can be explained by the fact that glu is bidentate in its 1 : 2 binary complex which leaves the third donor site free to contribute extra stability to the ternary complex, possibly through interligand interactions. Such stabilization is more evident in the case of the Cr^{III}–cys(A)–glu(B) system than in the Cr^{III}–H_2ida(A)–glu(B) system. This is understandable since free donor atoms are present in both ligands A and B in the former system. The values of Δ log K_{MABH} (-0.19) and Δ log K_{MABH_2} ($+0.18$) for the Cr^{III}–cys–glu system also indicate similar enhancement in the stabilization of $MABH_2$ ternary species relative to the MABH species.

We thank Dr. T. Ramasami of this Institute for his help in the experimental design, computations, and in preparation of the manuscript.

[1/1082 *Received, 8th July,* 1981]

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