20.87, 20.55, and 20.41 G, respectively. The close similarity may be fortuitous, as our measurements were made for two rather different solutions, and there may be some effect of the medium. The decrease in coupling with increasing ionization has been explained[12] by the increasing delocalization of the unpaired electron into the carboxylate group. Radical II^{3-} has been identified[13] in γ-irradiated single crystals of sodium citrate pentahydrate, and the isotropic α-hydrogen coupling which results from the reported principal values is 60.7 MHz. Our value in the same units is 56.9 MHz. The difference due to media seems rather large for an α hydrogen.

The three small proton couplings of II^{3-} must arise from the two γ protons and the OH proton. In order to make the proper assignments, a solution was made with D_2O in place of H_2O. The ratio of D atoms to exchangeable H atoms in this solution was 8.9. The high-field group of lines for this solution is shown in Figure 4b. Only the largest of the three small splittings can be seen, but the lines are broad enough that the smallest splitting (0.11 G) would not give resolved lines. Clearly the H atom giving the intermediate coupling of 0.27 G was replaced by D which caused pairs of lines with a splitting of 0.27 G to be replaced by triplets with a line to line spacing of 0.04 G. These triplets were not resolved, but they introduced enough width that the 0.11-G splitting was also not resolved. The OH proton is the exchangeable proton and has the coupling of 0.27 G.

The two γ protons have strikingly different couplings of 0.55 and 0.11 G. This is not surprising in II^{3-} where the energy barrier between conformations is expected to be large (larger than in II). It is interesting to compare with findings[7] for $C_2H_5\dot{C}H_2$ in liquid propane. At temperatures below $-145°$ the γ protons become nonequivalent showing that the γ coupling is strongly dependent upon orientation of the methyl group.

It is probable that most of the molecules of II^{3-} are not protonated in the measured solutions, otherwise pK_3 for II would have to be more than 3 units larger than pK_3 for citric acid. The epr spectrum of II^{3-} was also observed in solutions containing the same amount of H_2O_2 but progressively less sodium citrate. The concentration of sodium citrate was reduced by a factor of 2 for successive solutions starting with a 1.6 M solution. For each solution a high-resolution scan was made for one of the two widely spaced groups of eight lines. After the first dilution, the lines were much better resolved. With further dilution the lines which were separated by the smallest splitting became more poorly resolved. After the fourth dilution, there was no indication of the smallest splitting. It was not possible to tell whether the smallest coupling (0.11 G) was changing in the different solutions or whether there were changes in width or both. However, it was clear that very little change if any occurred in the two larger splittings. After a fifth dilution, the absorption abruptly became weaker by more than a factor of 100. It was also found that the addition of NaOH to a 1.7 M sodium citrate solution containing H_2O_2 made it unstable. With 0.05 M NaOH present, the spectrum very nearly disappeared, and gas formed in the solution.

(13) D. B. Russell, *J. Chem. Phys.*, **43**, 1969 (1965).

Relaxation Spectra of L-Phenylalanine– and L-Dopa (3,4-Dihydroxyphenylalanine)–Copper(II) Complexes[1]

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Abstract: Temperature-jump experiments of the Cu(II)–phenylalanine and Cu(II)–dopa (amino acid end) systems have been interpreted according to the reaction $CuL_{n-1} + L \rightleftharpoons CuL_n$ (k_n, forward; k_{-n}, reverse), where L is the anionic form of the ligand and charges have been neglected. The results, at 25° and $\mu = 0.1$ M, are: phenylalanine, $k_1 = 1.2 \times 10^9\ M^{-1}\ sec^{-1}$, $k_{-1} = 22\ sec^{-1}$, $k_2 = 3 \times 10^8\ M^{-1}\ sec^{-1}$, $k_{-2} = 30\ sec^{-1}$; dopa, $k_1 = 1.1 \times 10^8\ M^{-1}\ sec^{-1}$, $k_{-1} = 8.3\ sec^{-1}$, $k_2 = 4.2 \times 10^7\ M^{-1}\ sec^{-1}$, $k_{-2} = 22\ sec^{-1}$. The dissociative, water-loss mechanism appears to be operative, although the hydroxyl groups of the dopa may have a misorienting effect on this ligand's reactivity.

Transition metal ion α-amino acid complexes are generally formed *via* a mechanism in which the rate-determining step is the loss of a water molecule from the metal ion's inner coordination sphere.[2,3] Ring closure does not appear to be rate determining for certain ligands forming five-membered chelate rings,[4] of which an α-amino acid is an example.

L-β-Phenylalanine forms five-membered chelates with metal ions through the carboxylate and α-amine groups. L-Dopa (3,4-dihydroxyphenylalanine) coordinates to

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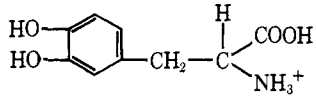
(2) M. Eigen and R. G. Wilkins, *Advan. Chem. Ser.*, **No. 49**, 55 (1965).
(3) K. Kustin and J. Swinehart, *Progr. Inorg. Chem.*, **13**, 107 (1970).
(4) K. Kustin, R. F. Pasternack, and E. M. Weinstock, *J. Amer. Chem. Soc.*, **88**, 4610 (1966).

Table I. Equilibrium Data at 25°

Ligand	pK_{A_1} (–COOH)	pK_{A_2} ($-NH_3^+$)	pK_{A_3} (–OH)	pK_{A_4} (–OH)	Ref
A. Protolytic Constants					
Phenylalanine	2.16	9.15			*a* (μ = 0.1 *M*)
Dopa	2.31	8.71	9.74	13.4	*b* (μ = 1.0 *M*)
B. Stability Constants of Copper(II) Complexes					
Ligand	Log K_1[d]	Log K_2[d]	Log K_1[e]	Log K_2[e]	
Phenylalanine	7.74	6.90			*c* (μ = 0.027 *M*)
Dopa	7.12	6.29	12.99	11.95	*b* (μ = 1.0 *M*)

[a] J. C. Nevenzel, W. E. Shelberg, and C. Niemann, *J. Amer. Chem. Soc.*, **71**, 30 (1949). [b] J. E. Gorton and R. F. Jameson, *J. Chem. Soc. A*, 2615 (1968). [c] J. Curchod, *J. Chim. Phys. Physicochim. Biol.*, **53**, 182 (1956). [d] Amino acid type complexes. [e] Catechol (3,4 dihydroxy) type complexes.

metal ions with these groups in neutral or acidic solutions; at higher pH's the catechol end of the molecule becomes available for complexation.[5] This study was



dopa, fully protonated

carried out to examine the effects of the β-phenyl group of L-phenylalanine and the β-dihydroxyphenyl group of L-dopa on the copper(II) complexation kinetics of these ligands.

Materials and Methods

$Cu(NO_3)_2 \cdot 3H_2O$ (Baker and Adamson), L-β-phenylalanine and L-dopa (Nutritional Biochemicals Corp.), bromochlorophenol blue (Matheson Coleman and Bell), and bromocresol green and methyl orange (Eastman) were used without further purification. Solutions to be studied were prepared by addition of the crystalline metal nitrate and the solid ligand to 100-ml volumetric flasks. Appropriate volumes of the indicator stock solutions were added, and the ionic strengths were adjusted to 0.1 *M* by addition of KNO_3. The solutions were then diluted to the mark with distilled water.

The pH was adjusted by dropwise addition of dilute NaOH and/or HNO_3. The final pH value was measured to ±0.01 unit on a Corning Model 12 pH meter. Hydrogen ion concentrations were obtained by dividing the measured hydrogen ion activity by $\gamma_{\pm} = 0.79$.[6] The temperature in all experiments was 25 ± 1°.

The temperature-jump apparatus described in a previous paper[7] was used to measure relaxation times. Blank experiments with metal ion and indicator and with ligand and indicator at μ = 0.1 *M* showed no relaxation effects within the sensitivity limit of the instrument. Thus, all observed relaxation spectra correspond to metal–ligand interaction. An acid–base indicator of pK_A close to the pH of the system was used since color changes, if any, due to complexation did not permit direct detection of the temperature jump.

Results and Treatment of Data

A Newton–Raphson iteration, employing the equilibrium constants in Table I and stoichiometric concentrations in Table II, was used on an IBM 1130 computer to calculate solution composition. The stability constants listed for the phenylalanine complexes are slightly different from the values of Izatt, *et al.*[8] However, the results of Izatt were calculated for infinite dilution; application of the Davies equation[6] indicates that the differences in the results can be ascribed to the ionic strength dependencies of the stability constants. The stability constants listed for dopa are the only values currently available for this ligand.

Table II. Relaxation Spectra of Copper(II) Complexes[a,b]

$[Cu]_0 \times 10^3$ *M*	[L-Phenylalanine]$_0 \times 10^3$ *M*	$[H^+] \times 10^4$ *M*	τ_{exptl}, msec	τ_{calcd}, msec
10.8	0.981	1.15	3.6[c]	4.2
7.07	0.902	1.29	6.3[c]	6.1
5.58	0.617	1.38	9.0[c]	8.9
2.51	0.466	1.45	17[c]	16
5.43	0.478	0.309	2.3[d]	2.2
3.95	0.157	0.275	4.2[d]	4.3
1.03	7.73	0.263	1.4[d]	1.4
0.539	11.3	0.275	2.2[d]	2.4
	[L-Dopa]$_0$, $\times 10^3$ *M*			
1.61	0.761	1.74	45[e]	48
0.931	1.52	1.87	43[e]	46
1.02	2.03	1.78	52[e]	52
1.10	1.04	1.66	47[e]	47
1.45	0.903	1.91	52[e]	47
1.45	1.01	0.708	21[e]	21
1.47	1.14	5.63	140[e]	100
5.00	1.01	5.45	43[e]	48
1.03	5.07	6.73	44[e]	47

[a] The subscript 0 refers to total stoichiometric concentration. [b] Temperature = 25°, μ = 0.1 *M* (KNO_3). [c] Monitored with bromochlorophenol blue, 1.0×10^{-5} *M*. [d] Monitored with bromocresol green, 1.7×10^{-5} *M*. [e] Monitored with methyl orange, 1.0×10^{-5} *M*.

Owing to the hydrolysis of the copper(II) ion, the pH range of the experiments was necessarily limited to mildly acidic solutions. Complexation occurs at the catechol end of dopa only at high pH's.[5] The protons of the hydroxyl groups are replaced by the metal ion in these complexes.[5] Since the pK_A's of the –OH's are roughly 10 and 13 (*vide* Table I), it was assumed that only α-amino acid type complexes were formed in the dopa experiments.

The concentration and pH variation of the experiments with dopa were further restricted by the observed interactions of this ligand with a number of

(5) J. E. Gorton and R. F. Jameson, *J. Chem. Soc. A*, 2615 (1968).
(6) C. W. Davies, *ibid.*, 2093 (1938).
(7) P. Hurwitz and K. Kustin, *Inorg. Chem.*, **3**, 823 (1964).
(8) R. M. Izatt, J. W. Wrathall, and K. P. Anderson, *J. Phys. Chem.*, **65**, 1914 (1961).

indicators. Blank experiments of methyl orange and dopa did not show any relaxation effects, and this indicator was thus used exclusively to monitor the Cu(II) reactions with this ligand.

For both ligands, mono and bis amino acid type complexes are present in solution.[5,9] As in the case of previous studies, it was assumed that only the anionic amino acid species are attacking forms. The data were thus analyzed according to the scheme

$$Cu^{2+} + L^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CuL^+ \quad (1a)$$

$$CuL^+ + L^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} CuL_2 \quad (1b)$$

where L^- is the anionic form of the ligand. The relaxation expression for this scheme is given by[10]

$$(1/\tau)^2 - (a_{11} + a_{22})(1/\tau) + (a_{11}a_{22} - a_{12}a_{21}) = 0 \quad (2)$$

or

$$(1/\tau_\pm) = {}^1/_2\{(a_{11} + a_{22}) \pm [(a_{11} + a_{22})^2 - 4(a_{11}a_{22} - a_{12}a_{21})]^{1/2}\} \quad (3)$$

τ is the relaxation time and the a_{ij}'s are functions of the rate constants and equilibrium concentrations of the species in solution.[10] Measured relaxation times are listed in Table II.

A computerized nonlinear least-squares routine based on eq 2 was used to calculate the forward and reverse rate constants tabulated in Table III. The relative

Table III. Rate Constants

Ligand	$k_1 \times 10^{-9}$ M^{-1} sec^{-1}	$k_2 \times 10^{-8}$ M^{-1} sec^{-1}	k_{-1}, sec^{-1}	k_{-2}, sec^{-1}
Phenylalanine	1.2	3	22	30
Dopa	0.11	0.42	8.3	22

error in the rate constants is ±20%, except for k_2 and k_{-2} of phenylalanine, for which it is ±50%.

In this manner, prior to the calculation of the rate constants, no assumptions were made as to the sign of the observed relaxation times, *i.e.*, whether a particular τ_{obsd} corresponded to a τ_+ or a τ_-. The calculated k's were then fed into a program employing eq 3, and τ_+'s and τ_-'s were obtained. The calculated relaxation times listed in Table II are generally the reciprocals of the larger roots of eq 3. Only in two cases ($[dopa]_0 = 2.03 \times 10^{-3}$ and 1.14×10^{-3} M) did calculated τ_-'s correspond to observed τ's. It should be emphasized that the identification of a calculated τ_+ or τ_- with an observed τ was made *after* the computation of the rate constants.

Discussion

The kinetic results (*vide* Table III) of the copper(II)–phenylalanine system are consistent with a dissociative (SN1) substitution. The similarity of k_1 with the results of the Cu(II)–serine (β-hydroxyalanine),[11] –α-alanine,[12] and –glycine[13] systems lends support of this mechanism. A characteristic of copper complex formation is that $k_2 < k_1$;[11–13] this feature is also exhibited for phenylalanine. Unlike the forward rate constants, the reverse rate constants should reflect the ability of the ligand to coordinate to the metal ion. Substitution of an electron-withdrawing group should make phenylalanine a poorer ligand than unsubstituted α-alanine. A comparison of k_{-1} constants for α-alanine,[12] phenylalanine, and serine[11] shows this trend with decreasing basicity of the α-amine group; *viz.*, 10, 22, 32 sec^{-1}, respectively. This trend is not exhibited for the k_{-2} rate constants. The reverse rate constant of the CuL_2 complex of phenylalanine is unusually small and reflects the relatively large stability constant of the bis complex.[8]

Each rate constant for the formation of copper(II)–dopa complexes is an order of magnitude less than the corresponding phenylalanine constant; the trend $k_2 > k_1$ is preserved. If the dissociative mechanism is operative, this variation should be ascribed to differences in the ion-pair formation constants.[2,10] Although it is conceivable, and likely, that ion pairing will vary somewhat from ligand to ligand of the same charge, change of an order of magnitude in the forward rate constants cannot be explained solely in terms of ion-pairing differences.

The dopa-α-amine basicity is low in comparison to phenylalanine's (*cf.* Table III). In an associative (SN2) mechanism, a decrease in ligand basicity would lead to a decrease in formation rate constant. At the same time the reverse rate constant should increase. Since the forward rate constants for dopa are consistent with this mechanism, but the reverse rate constants are not, it does not seem likely that an SN2 substitution mechanism is operative.

If the waters bound to the copper(II) ion are hydrogen bonded to the hydroxyl group on the ligand, the dopa molecule would be unfavorably oriented for chelation. The effect would reduce the available solid angle of approach leading to reactive encounters. A similar effect was not observed for the Cu(II)–serine system, although in this case the ligand's smaller size might minimize the misorientation.

In conclusion, the complexation kinetics of phenylalanine appear to be normal. The large substitution rate constant typical of copper(II) is also found for this metal ion's reaction with dopa. However, the values of both forward and reverse rate constants for this ligand are lower than normal. It should also be mentioned that Gorton and Jameson encountered difficulties in their experiments and data analyses for determination of the equilibrium constants for copper(II)–dopa.[5] These constants may not be as accurate as comparable values for other less complicated ligands, which could influence the quantitative interpretation of the relaxation data.

(9) J. Curchod, *J. Chim. Phys. Physicochim. Biol.*, **53**, 182 (1956).
(10) G. G. Hammes and J. I. Steinfeld, *J. Amer. Chem. Soc.*, **84**, 4639 (1962).
(11) R. L. Karpel, K. Kustin, and R. F. Pasternack, *Biochim. Biophys. Acta*, **177**, 434 (1969).
(12) W. B. Makinen, A. F. Pearlmutter, and J. E. Stuehr, *J. Amer. Chem. Soc.*, **91**, 4610 (1969).
(13) A. F. Pearlmutter and J. E. Stuehr, *ibid.*, **90**, 858 (1968).