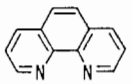
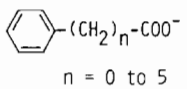
The formation of the species $M(ATP)(Trp)_{cl}^{3-}$ was first shown [5] in 1974; its occurrence was subsequently confirmed by studies in several laboratories using different methods [6].

To learn more about the factors which govern the position of the intramolecular equilibrium (1), we have now used the following simple systems, since with them structural alterations are easily achieved:



Phen

Cu^{2+}



$n = 0$ to 5

$R-(CH_2)_n-COO^-$

In 50% aqueous dioxane (I = 0.1, 25 °C) the stacking interaction is most pronounced for n = 1; *i.e.* for the ternary complex $Cu(Phen)(C_6H_5-CH_2-COO)^+$ about 60 percent exists in the closed form. If phenylacetate is replaced by 2-(β-naphthyl)acetate the concentration of the closed isomer increases to about 80 percent. Ligands $R-(CH_2)_n-COO^-$ with n = 0 or n > 1 form ternary complexes with a less pronounced intramolecular stacking interaction. Variation of the solvent composition also leads to a change in the percentage of $[Cu(Phen)(C_6H_5-CH_2-COO)^+]_{cl}$:

solvent	H_2O	30% diox.	60% diox.	90% diox.
% closed isomer:	48	56	64	48

The observation of a maximal degree of formation for the closed isomer in about 60% aqueous dioxane is very surprising, because the stability of binary adducts like $(Phen)(R-COO^-)$ decreases with increasing dioxane concentration. Hence, two opposite effects must be operating in the presence of metal ions. This puzzling result is of interest for biological systems, because in these the water activity may also be altered, *e.g.*, at the surface or in grooves of proteins. Hydrophobic interactions are important, *e.g.*, in adduct formation between carboxypeptidase A and the inhibitor β-phenylpropionate [7]. Presently we are repeating the experiments in several ethanol/water mixtures to provide a broader generalization.

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1 E. Frieden, *J. Chem. Educ.*, *52*, 754 (1975).
2 C. F. Naumann and H. Sigel, *J. Am. Chem. Soc.*, *96*, 2750 (1974).
3 H. Sigel, *Coordination Chemistry – 20* (IUPAC), p. 27 (1980).
4 H. Sigel, B. E. Fischer and E. Farkas, *Inorg. Chem.*, *22*, 925 (1983).
5 C. F. Naumann and H. Sigel, *FEBS Letters*, *47*, 122 (1974).
6 (a) H. Sigel and C. F. Naumann, *J. Am. Chem. Soc.*, *98*, 730 (1976). (b) R. Basosi, E. Gaggelli and E. Tiezzi, *J. Chem. Research* (S), 278 (1977). (c) J.-J. Toulmé, *Bioinorg. Chem.*, *8*, 319 (1978). (d) P. R. Mitchell, B. Prijs and H. Sigel, *Helv. Chim. Acta*, *65*, 1723 (1979). (e) G. Arena, R. Calì, V. Cucinotta, S. Musumeci and E. Rizzarelli, submitted for publication (personal communication *via* a preprint, August 1982).
7 I. Bertini and C. Luchinat, *Met. Ions Biol. Syst.*, *15*, 101 (1983).

U24

Intramolecular Ionic Interactions in Ternary Amino Acid Complexes [1]

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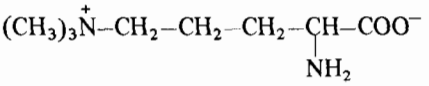
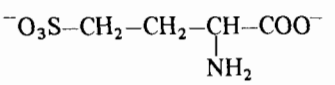
The so-called 'noncovalent' interactions [4] between biomolecules are not only crucial for the structural organization of high molecular weight biological systems, but also determine to a large extent the structure of amino acid and nucleotide containing low molecular weight metal ion complexes in solution. For example, the *intra*molecular equilibria between different isomers of mixed ligand complexes are well established for aromatic-ring stacking and hydrophobic interactions [3, 5, 6]. Ionic interactions between oppositely charged side-chains of two amino acids coordinated to the same metal ion are also known [7], but the extent of this interaction has so far hardly been characterized [6]. Therefore, the ionized forms of the following two amino acids were selected for such a study:



DL-δN-Trimethylornithine (=TMO)



DL-Homocysteic acid (=HC)

In the $(CH_3)_3\overset{+}{N}$-residue the positive charge is somewhat shielded, but the advantage of this residue is that no hydrogen bonds can be formed with ^-O_3S-, as would have been the case with the more common $H_3\overset{+}{N}$- group. In addition, the latter group and the use of a carboxylate group (instead of the sulfonate residue in HC) would have led to additional

proton equilibria, thus complicating the systems significantly. For comparison *DL*-alanine (Ala) with its non-reactive side chain was also employed. As a representative metal ion, Cu^{2+} was used, because it forms rather stable complexes with the glycinate-like structural unit. That ionic interactions between the $(CH_3)_3\overset{+}{N}$- and the ^-O_3S-residues are possible was proven by ^{1}H-NMR shift experiments with benzenesulfonate and the tetramethylammonium ion; the binary adduct has a stability constant of 0.7 M^{-1} in aqueous solution at 34 °C (I = 0.1, $NaNO_3$).

The results for the binary amino acid parent systems are given in Table I [8]. There are indications that the sulfonate group of HC interacts with an apical position of the Cu^{2+} coordination sphere and that decreasing ionic strength and the addition of dioxane favor this interaction. Such apical interactions are also known for related ligands [10].

The percentage of the 'closed' isomer of the ternary Cu(TMO)(HC) complex, *i.e.* of the isomer with an *intra*molecular ionic ligand-ligand interaction, was calculated [5, 6, 11] using the results obtained for the $Ala^-/Cu^{2+}/HC^{2-}$ system as a basis. From Table II it is evident that the 'intensity' of the ionic interaction increases with decreasing ionic strength and also with the decreasing polarity of the solvent; in other words, with the decreasing water activity. This result is exactly the behavior expected for ionic interactions, and it also holds if the optically pure ligand systems are used. The described ionic interactions are relatively weak ($\Delta G^\circ \simeq -3$ kJ/mol), but they are strong enough to allow 'flexible structural organizations' in biological systems.

Acknowledgement. Research supported by the Swiss National Science Foundation.

1 Part 43 of the series 'Ternary Complexes in Solution'; for parts 41 and 42 see [2] and [3], respectively.
2 N. Saha and H. Sigel, *J. Am. Chem. Soc., 104*, 4100 (1982).
3 H. Sigel, B. E. Fischer and E. Farkas, *Inorg. Chem., 22*, 925 (1983).
4 E. Frieden, *J. Chem. Educ., 52*, 754 (1975).
5 B. E. Fischer and H. Sigel, *J. Am. Chem. Soc., 102*, 2998 (1980).
6 H. Sigel, *Coordination Chemistry – 20* (IUPAC), p. 27–45 (1980).
7 (a) T. Sakurai, O. Yamauchi and A. Nakahara, *Bull. Chem. Soc. Jpn, 51*, 3203 (1978).
(b) L. D. Pettit and R. J. W. Hefford, *Met. Ions Biol. Syst., 9*, 173 (1979).

TABLE I. Negative Logarithms of the Acidity Constants for the Protonated Ligands and Logarithms of the Stability Constants for the Binary Amino Acid Cu^{2+} Complexes (25 °C).

Solvent	Ligand (A)	$pK^H_{H_2A}$	pK^H_{HA}	$\log K^{Cu}_{CuA}$	$\log K^{CuA}_{CuA_2}$
H_2O/I = 0.1	Ala^-	2.39	9.81	8.23	6.82
	TMO	1.94	8.81	7.41	6.29
	HC^{2-}	2.22	9.04	8.03	6.47
H_2O/I = 0.01	Ala^-	2.36	9.86	8.42	6.95
	TMO	1.88[a]	8.71	7.25	6.16
	HC^{2-}	2.33	9.19	8.36	6.47
60% dioxane (40% H_2O; v/v) I = 0.01	Ala^-	3.49	10.31	10.84	8.81
	TMO	2.58	8.84	9.20	7.69
	HC^{2-}	3.65	10.06	11.30	8.18

[a]This value was measured for experimental reasons at I = 0.02, $NaClO_4$.

TABLE II. Logarithms of the Stability Constants for the Ternary Amino Acid Cu^{2+} Complexes and Percentage of the Isomer with an Intramolecular Ionic Ligand-Ligand Interaction (25 °C).

Solvent	Ligands A/B	$\log \beta^{Cu}_{CuAB}$	$\Delta \log K_{Cu}$[a]	% $[CuAB]_{cl}$
H_2O/I = 0.1	HC^{2-}/Ala^-	15.22 ± 0.01	−1.04	
	HC^{2-}/TMO	14.57 ± 0.01	−0.87	32
H_2O/I = 0.01	HC^{2-}/Ala^-	15.57 ± 0.01	−1.21	
	HC^{2-}/TMO	14.77 ± 0.01	−0.84	57
60% dioxane (40% H_2O; v/v) I = 0.01	HC^{2-}/Ala^-	20.03 ± 0.02	−2.11	
	HC^{2-}/TMO	18.99 ± 0.02	−1.51	75

[a]$\Delta \log K_{Cu} = \log \beta^{Cu}_{CuAB} - (\log K^{Cu}_{CuA} + \log K^{Cu}_{CuB}) = \log K^{CuA}_{CuAB} - \log K^{Cu}_{CuB}$ (see [2, 3, 5, 6, 11]).

(c) O. Yamauchi and A. Odani, *J. Am. Chem. Soc., 103*, 391 (1981).
(d) A. Odani and O. Yamauchi, *Inorg. Chim. Acta, 66*, 163 (1982).
8 TMO was synthesized as described in [9]; the other materials were purchased from Fluka AG, Buchs, Switzerland, from Merck AG, Darmstadt, Germany, or from Sigma Chemical Co., St. Louis, USA. All constants were determined from potentiometric pH titrations; the ionic strength (I) was adjusted with $NaClO_4$; for further experimental details see, *e.g.*, [5]. Three times the standard error of the mean value for the listed constants is ≤ 0.02 log units.
9 T. Takemoto, K. Daigo and N. Takagi, *Yakugaku Zasshi, 84*, 1180 (1964).
10 H. Gampp, H. Sigel and A. D. Zuberbühler, *Inorg. Chem., 21*, 1190 (1982).
11 H. Sigel, *Angew., Internat. Ed. Engl., 21*, 389 (1982).

U25

Steady-state Kinetics of *Rhus* Laccase with Rapid Substrates

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Rhus laccase exhibits ping-pong kinetics [1]. Though reductants do not bind, an apparent K_m reflecting substrate-independent steps was reported with a DMPD, a rapid substrate. This work examines the effects of pH, D_2O and anions on reductant substrate-dependent (k_r) and substrate-independent (k_{cat}) steps.

Activity was measured with an O_2 electrode using DMPD as the reductant. The pH dependency of k_r is bell-shaped indicating contributions from at least two groups. The group required in its dissociated form has an apparent pk_a 7.55 ± 0.12 as reported previously [1], while the group required in its undissociated form has an apparent pk_a 8.43 ± 0.23. Anaerobic reduction data does not detect pH-dependencies consistent with these pk_a values and forms [2, 3]. In particular, no group with pk_a near 7.5 required in its undissociated form is detected. Therefore, the pH-dependency of k_r must involve enzymic states specific to catalytic turnover. Both pH-dependent steps are more likely associated with type 2 Cu than type 1 Cu reduction. Type 1 Cu(II) reduction in laccase which has been activated by a reduction-reoxidation cycle does not show these pH-dependencies [4]. A recently derived steady-state rate law implies that this also holds for type 1 reduction during turnover [4].

The pH dependence of k_{cat} is also bell-shaped. The implicated pk_a values were: pk_a 5.91 ± 0.035 for an acid catalyst and pk_a 8.99 ± 0.02 for a base catalyst. Residual activity (0.22 maximal) at high pH, which implies that the putative acid catalyst is not mandatory, was accounted for in the data fits. While k_r does not show a solvent isotope effect, k_{cat} does. In 50% D_2O, pH 7.40, k_H/k_D is 1.36, in 100%, 2.12 ± 0.038. The ratio of the pH independent k_{cat} is 1.48 in 50% D_2O. Thus, proton(s) transfers are implicated in a rate-limiting substrate-independent step. Analyses of the D_2O concentration dependence of k_{cat} at pH 7.4 are consistent with 2 proton transfers. The isotope-exchanging group is most likely functioning as the acid catalyst given the pk_a of the base catalyst and the magnitude of the effect at pH 7.40.

Both F^- and N_3^- inhibit laccase immediately when they are added during steady-state turnover. The inhibition patterns obtained indicate that both F^- and N_3^- inhibit both reductant-dependent and substrate-independent steps. Laccase exhibits partial activity for both the k_r and k_{cat} effects when saturated with F^-. ESR spectra of laccase at pH 6.0, 4 °C, show that both types 1 and 2 Cu are 30% reduced during steady-state turnover. The concentration of reduced type 1 and 2 are significantly increased when 40 mM F^- is added. Stopped-flow experiments show that F^- does not affect type 3 reoxidation of O_2 binding. Thus, the ESR results indicate that during steady-state turnover in the presence of F^-, the rate-limiting step is a substrate-independent step affecting type 1 and type 2 Cu reoxidation. These results also imply that F^- can remain bound to the reduced type 2 Cu.

1 L. C. Petersen and H. Degn, *Biochim. Biophys. Acta, 526*, 85 (1978).
2 B. Reinhammar and L.-E. Andréasson, *Biochim. Biophys. Acta, 445*, 579 (1976).
3 B. Reinhammar and L.-E. Andréasson, *Biochim. Biophys. Acta, 568*, 145 (1979).
4 F. B. Hansen, G. B. Koudelka, R. W. Noble and M. J. Ettinger, *J. Am. Chem. Soc.*, submitted.

U26

^{1}H-NMR Conformational Studies of Biomolecules and their Complexes with Diamagnetic Metal Ions: Solvent Exposure Delineations of Proton Nuclei by Using Stable Nitroxides

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Nitroxide induced perturbations of proton relaxation rates of compounds of established solution structure has been shown to be mainly correlated to the hydrogen solvent exposure and, hence, to the molecular conformation [1]. The solution dynamics and relaxation mechanisms involved in the nitroxide-