The Relative Copper(I) Ion Affinities of Amino Acids in the Gas Phase

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Abstract: The relative Cu(I) ion affinities of amino acids (A.A.) are determined in the gas phase based on the unimolecular dissociations of their Cu^+-bound heterodimers, $A.A._1-Cu^+-A.A._2$ (kinetic method). For the 20 common α-amino acids, the Cu^+ affinities increase in the order Gly < Ala < Ser < Val < Leu < Ile < Thr < Pro < Asp < Asn < Glu < Phe < Tyr < Cys < Gln < Met < Trp < His < Lys < Arg and their values fall within <20 kcal/mol. For comparison, the proton affinities of amino acids cover a range of >33 kcal/mol. Correlation of the experimentally derived Cu^+ affinity order to the reported proton affinity order of amino acids points out that the $Cu^+-A.A.$ bond is longer and less covalent in nature than the $H^+-A.A.$ bond. Increasing the alkyl side chain of the amino acid, and hence inductive effects, augments the proton affinity substantially more than the Cu(I) affinity. Further, soft donor groups, such as SH of cysteine, SCH_3 of methionine, or the aromatic π-electrons of phenylalanine, stabilize $Cu^+-A.A.$ bonds more than $H^+-A.A.$ bonds.

Introduction

Copper ions (Cu^+ and Cu^{2+}) play an essential role in several biological processes, including oxidation, dioxygen transport, and electron transfer.[1] These functions are effected in conjunction with proteins, which also are responsible for the *in vivo* storage and transport of the ions.[1] One important factor that influences the location of copper ions in the hydrophobic interior of a protein is the relative intrinsic bond strength between the ionized metal and the various possible binding sites. This important thermochemical property can be obtained in the gaseous state where complicating or interfering solvent effects are avoided. In the present study, the so far unknown relative Cu(I) (i.e. Cu^+) affinities of the 20 common α-amino acids, the simplest building blocks of peptides and proteins, are determined in the gas phase by tandem mass spectrometry (MS/MS).[2]

MS/MS methods have successfully been used for the elucidation of the structures of amino acids and peptides cationized by alkali, alkaline earth, and transition metal cations.[3-5] Studies by Gross,[3] Adams,[4] and other researchers[5] have demonstrated that, in many cases, the gas phase binding in these complexes parallels that encountered inside proteins in aqueous solution chemistry. For example, alkali metal ions coordinate at the carbonyl oxygens in the gas phase as well as in the aqueous environment.[3a] Similarly, Ca^{2+} prefers attachment to C-terminal carboxylates in both phases.[4d] Gaseous and solution behavior may, however, deviate due to solvent effects.[3-5] Thermodynamic data obtained in the gas phase are of particular value both for understanding the nature of metal ion−protein interactions in condensed media and for explaining solvent phenomena.

Despite their importance in biochemistry, only very few Cu(I) complexes of amino acids (A.A.) and peptides have been studied in the gas phase.[3a,6-8] Cu^+ attachment ions of such molecules have been produced by fast atom bombardment (FAB),[3a,6] matrix assisted laser desorption ionization (MALDI),[7] and plasma desorption (PD).[8] In all instances, the source of Cu^+ was a Cu^{2+} salt (e.g., $CuCl_2$ or $CuSO_4$), showing that redox reactions yielding Cu^+ (and Cu^+ adducts) take place during ion formation irrespective of the ionization mode used. Gross et al. presented the MS/MS spectrum of $[GlyGlyLeu]Cu^+$ formed by FAB;[3a] the fragmentations observed from this cation were different from those occurring with $[GlyGlyLeu]Na^+$, possibly owing to a distinct binding location for Cu^+ vs Na^+. Nelson and Hutchens acquired MALDI mass spectra of glycine-, phenylalanine-, and histidine-containing oligopeptides which were pretreated with aqueous copper sulfate;[7] the number of Cu^+ ions attached was found to depend on the number of histidine residues, suggesting histidine as the most probable binding site. Most recently, Hoppilliard et al. documented that $[A.A.]Cu^+$ ions produced by PD may decompose to yield both organometallic and organic fragments.[8] In this study, we interrogate by MS/MS the structures and unimolecular chemistry of selected $[A.A._1 + A.A._2]Cu^+$ complexes, before proceeding with the determination of the Cu^+ affinity order of the 20 mammalian amino acids.[9]

$$[A.A.]Cu^+ \rightarrow A.A. + Cu^+ \qquad \Delta H^\circ = Cu^+A \qquad (1)$$

The Cu(I) affinity (Cu^+A) of an amino acid is defined as the enthalpy change of reaction 1 and corresponds to the dissociation

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energy of the A.A.$-Cu^+$ bond. Although no information is available for the Cu(I) affinities of amino acids, the bond energies of complexes of copper(I) ions with simpler ligands, derived from small organic or inorganic molecules, have intensively been investigated[10,11] by both ion beam techniques[12] and equilibrium methods.[13] In such experiments, the organometallic complex of interest is formed by an ion−molecule reaction between the metal ion and the appropriate organic compound or by ligand exchange.[10−14] These approaches require an appreciable vapor pressure for the reactant molecule supplying the ligand[12−14] and, therefore, are difficult to implement with biomolecules (like most amino acids or peptides), due to the very low volatility of the latter. Moreover, polar or labile biomolecules may contain impurities which influence the ion−molecule reaction outcome.

The kinetic method, developed by Cooks and co-workers,[15] offers a suitable alternative for thermochemical determinations in cases involving non-volatile or impure samples. It is an approximate method using rate measurements to access thermodynamic properties. According to this technique,[15] the relative Cu^+ affinities of amino acids A.A.$_1$ and A.A.$_2$ can be obtained by comparing the rates of dissociation of the Cu^+-bound heterodimer [A.A.$_1$ + A.A.$_2$]Cu^+ to each of the individual Cu^+-attached monomers (eq 2):

$$[\text{A.A.}_1 + \text{A.A.}_2]\text{Cu}^+ \xrightarrow{k_1} [\text{A.A.}_1]\text{Cu}^+ + \text{A.A.}_2 \quad (2a)$$

$$[\text{A.A.}_1 + \text{A.A.}_2]\text{Cu}^+ \xrightarrow{k_2} \text{A.A.}_1 + [\text{A.A.}_2]\text{Cu}^+ \quad (2b)$$

From transition state theory,[16] the rate constant of such a dissociation is given by eq 3, in which T_{eff} is the effective temperature of the dimer ion undergoing dissociation, Q and Q^* are the partition functions for the ion and the activated

$$k = (RT_{eff}/h)(Q^*/Q)e^{-\epsilon_0/RT_{eff}} \quad (3)$$

complex, respectively, and ϵ_0 is the activation energy. Application of eq 3 to the competing reactions 2a and 2b leads to

$$\ln(k_1/k_2) = \ln(Q_1^*Q_2/Q_2^*Q_1) + \Delta\epsilon_0/RT_{eff} \quad (4)$$

where $\Delta\epsilon_0$ is the difference in activation energies for pathways 2a and 2b. Since both these pathways originate from the same reactant ion, namely [A.A.$_1$ + A.A.$_2$]Cu^+, Q_1 and Q_2 are identical. If fragmentations 2a and 2b proceed by simple bond cleavages from the loosely bound complex A.A.$_1-Cu^+-$A.A.$_2$ (as will be corroborated by MS/MS data), the reverse activation energies for channels 2a and 2b should be negligible.[2] In such a case, $\Delta\epsilon_0$ can be approximated by ΔCu^+A, i.e. the difference in copper ion affinities between the two amino acids of interest:[15]

$$\ln(k_1/k_2) = \ln(Q_1^*/Q_2^*) + \Delta Cu^+A/RT_{eff} \quad (5)$$

The term $\ln(Q_1^*/Q_2^*)$ in eq 5 corresponds to $T_{eff}\Delta(\Delta S°_{Cu^+})$, with $\Delta(\Delta S°_{Cu^+})$ being the difference in entropy change between reactions 2a and 2b. If A.A.$_1$ and A.A.$_2$ are chemically similar species, as expected with most amino acids, $\Delta(\Delta S°_{Cu^+})$ should be close to zero and $Q_1^* \approx Q_2^*$ (this assumption is tested by MS/MS and the behavior of [A.A.$_1$ + A.A.$_2$]Cu^+ dimers in which A.A.$_1$ is kept constant and A.A.$_2$ is varied, *vide infra*).[15] With $Q_1^* \approx Q_2^*$, eq 5 is simplified further to

$$\ln(k_1/k_2) \approx \Delta Cu^+A/RT_{eff} \quad (6)$$

now directly relating the difference in copper(I) ion affinities between amino acids A.A.$_1$ and A.A.$_2$ to the ratio of the rate constants of the competitive reactions 2a and 2b. The latter (i.e. k_1/k_2) is equal to the experimentally measurable abundance ratio [[A.A.$_1$]Cu^+]/[[A.A.$_2$]Cu^+] in, e.g., the metastable ion (MI) spectra of mass-selected [A.A.$_1$ + A.A.$_2$]Cu^+.

Because of the assumptions made, the kinetic method is best applied to ionic heterodimers of chemically related species that undergo simple dissociations. The method has successfully been used for the determination of proton affinities,[15,17] gas phase acidities,[18] metal and chloride ion affinities,[19,20] and electron affinities.[21] The kinetic method has provided relative proton affinities of amino acids[17a,b,d,g] and small peptides[22] that are in very close agreement with values measured by more accurate equilibrium or bracketing approaches.[22b,23] Discrepancies have however been documented, in particular with heterodimers composed of molecules from different chemical classes, i.e. when entropy effects do not cancel and/or reverse activation energies cannot be neglected.[23c,24,25] Recognizing such problems, the present investigation exclusively concerns amino acids and emphasizes the *qualitative* ordering of their Cu(I) affinities.

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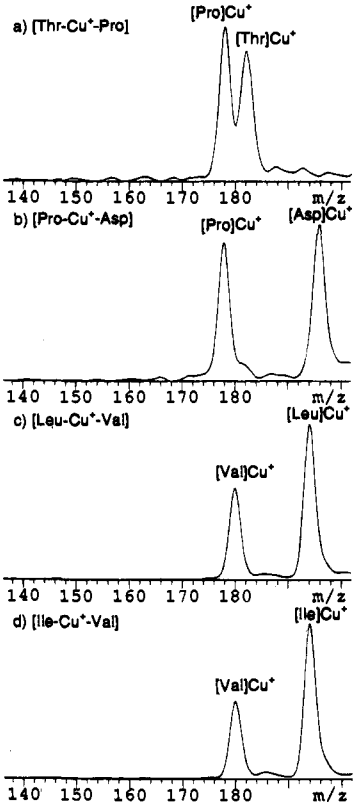

Figure 1. Typical MI mass spectra of Cu^+-bound heterodimers of amino acids: (a) [Pro + Thr]Cu^+, (b) [Asp + Pro]Cu^+, (c) [Leu + Val]Cu^+, and (d) [Ile + Val]Cu^+.

Experimental Section

A modified VG Autospec tandem mass spectrometer of E_1BE_2 geometry was used to perform the experiments described.[26] The Cu^+-bound heterodimers of amino acids were generated by FAB in a glycerol matrix using ~12 keV Cs^+ ions as bombarding particles. The precursor ion of interest was accelerated to 8 keV, mass-selected by MS-1 (E_1B), and allowed to dissociate spontaneously in the field-free region between the magnet and the second electric sector (FFR-3). The so formed fragments were dispersed by MS-2 (E_2) and recorded in the respective metastable ion (MI) mass spectrum. Collisionally activated dissociation (CAD) spectra of a few [A.A.$_1$ + A.A.$_2$]Cu^+ dimers and [A.A.]Cu^+ monomers were acquired similarly, by introducing He in one of the two collision cells situated in FFR-3. The MI and CAD spectra measured are multiscan summations and reproducible within $<\pm 5\%$.

The samples were prepared from saturated glycerol solutions of the amino acids and cupric chloride. To generate a heterodimer ion, aliquots of the stock solutions of the individual amino acids (ca. 0.5 mL) were mixed with an aliquot of the $CuCl_2$ solution (ca. 0.5 mL). A few microliters of the resulting mixture were then applied onto the FAB probe tip. This procedure maximized the intensity of [A.A.$_1$ + A.A.$_2$]Cu^+. Under the described conditions, the abundance of the heterodimer cations relative to base peak (usually protonated glycerol or a protonated amino acid) was approximately 1−3%. All substances were purchased from Sigma and were used without any modification.

Results and Discussion

Unimolecular Chemistry of [A.A.$_1$ + A.A.$_2$]Cu^+ Ions. Irrespective of the amino acids involved, the metastable ion (MI) spectra of the [A.A.$_1$ + A.A.$_2$]Cu^+ precursors contain [A.A.$_1$]-Cu^+ and [A.A.$_2$]Cu^+ fragments, generated according to eq 2. This is illustrated for four different A.A.$_1$ + A.A.$_2$ combinations in Figure 1. The competitive eliminations of one A.A. unit (eq 2) remain as the principal decomposition channel also upon collisionally activated dissociation (CAD), as documented in Figure 2 by the CAD spectra of the homodimer [Val]$_2Cu^+$ and the heterodimers [Leu + Val]Cu^+ and [His + Lys]Cu^+. These data verify that the two amino acids in [A.A.$_1$ + A.A.$_2$]Cu^+ are loosely bonded by the Cu^+ ion, viz. A.A.$_1$−Cu^+−A.A.$_2$, a prerequisite for applying the kinetic method to determine relative A.A.−Cu^+ bond strengths (*vide supra*).

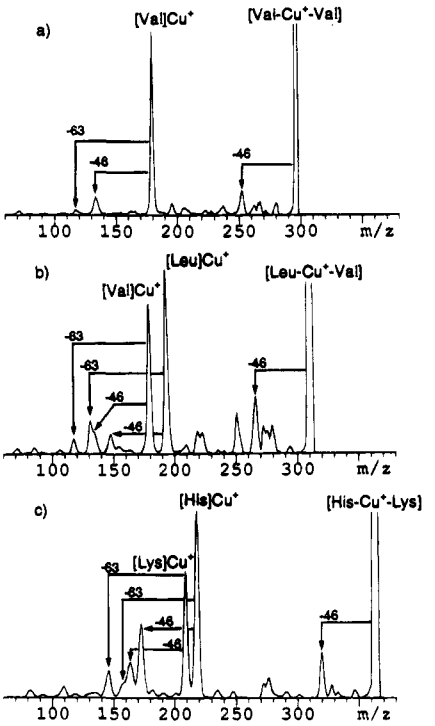

Figure 2. CAD mass spectra of [A.A.$_1$ + A.A.$_2$]Cu^+ ions: (a) homodimer [Val]$_2Cu^+$, (b) heterodimer [Leu + Val]Cu^+, and (c) heterodimer [His + Lys]Cu^+. The numbers on the arrows give the masses of a few important neutral losses (see text).

Collisional activation enables several new dissociation pathways, most notably loss of CH_2O_2 (46 u) from the [A.A.$_1$ + A.A.$_2$]Cu^+ precursor ion and consecutive fragmentations from the major [A.A.$_1$]Cu^+ and [A.A.$_2$]Cu^+ fragments by rupture of CH_2O_2 (46 u) and Cu (63 u), cf. Figure 2. The consecutive nature of the two latter processes is substantiated by the observation that such reactions also take place upon CAD of authentic [A.A.]Cu^+ produced in the FAB ion source.[27] Of importance for the present study is the fact that all activated dimer ions undergo decompositions of similar type, independent of the amino acids contained. This finding suggests that the dimers are structurally comparable, as needed to minimize entropy effects upon their dissociation (*vide supra*).

Copper(I) Affinity Order of Amino Acids. The MI spectra of >60 different [A.A.$_1$ + A.A.$_2$]Cu^+ heterodimers were evaluated in order to derive the Cu^+A order of the 20 common α-amino acids. Most amino acids were compared to at least three others. Figure 1 shows representative MI spectra, including those of [Pro + Thr]Cu^+, [Asp + Pro]Cu^+, [Leu + Val]-Cu^+, and [Ile + Val]Cu^+. The larger abundance of [Pro]Cu^+ in Figure 1a indicates that Pro has a higher Cu^+ affinity than Thr. Similarly, the [Asp]Cu^+ and [Pro]Cu^+ intensities in Figure 1b reveal that the Cu^+ affinity of Asp is larger than that of Pro, thereby establishing the Cu^+A order Thr < Pro < Asp. Because Leu and Ile are isobaric, they could not be compared directly,

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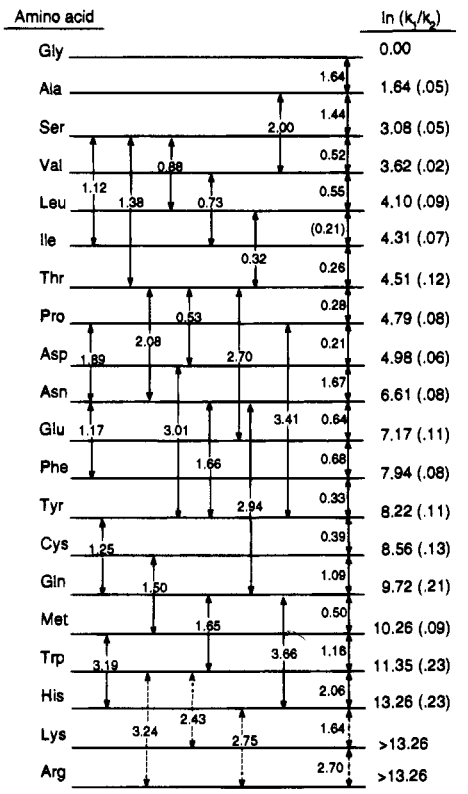

Figure 3. Measured $\ln(k_1/k_2)$ values for Cu^+-bound dimers of amino acids. The data presented under the heading $\ln(k_1/k_2)$ are average cumulative values expressed relative to glycine. The numbers given in parentheses are the estimated errors resulting from the measurement of abundance ratios. The dimers compared are connected by arrows; dimers containing Lys or/and Arg (for which eq 6 is not valid because of significant entropy effects) are indicated by dotted arrows.

and their ranking was determined from their heterodimers with Val (Figures 1c,d) and Ser.

With 41 of the >60 $[A.A._1 + A.A._2]Cu^+$ pairs studied, the difference in Cu^+ affinities between $A.A._1$ and $A.A._2$ was sufficiently small to allow for the observation of both possible fragments, $[A.A._1]Cu^+$ and $[A.A._2]Cu^+$, in the corresponding MI spectrum (as in the examples of Figure 1). The $\ln(k_1/k_2)$ values resulting from these 41 measurements are presented in ladder form in Figure 3. Such a stair-step display makes it easy to cross-check the behavior of different dimers and examine the validity of the assumptions made. It can be seen that the results are internally consistent for all Cu^+-bound pairs, except those containing Lys and Arg. For example, $\ln(k_1/k_2)$ for [Pro + Tyr]Cu^+ is 3.41; a very similar value is obtained by summing the $\ln(k_1/k_2)$ values of the five intermediate steps, viz. 3.53 = 0.21 + 1.67 + 0.64 + 0.68 + 0.33. This accord confirms that entropic effects, which tend to be non-additive, are indeed negligible with the majority of amino acids.

On the other hand, the substantial disagreement between cumulative and added sequential $\ln(k_1/k_2)$ values for heterodimers with Lys and Arg points out that the dissociation of such species must be associated with non-negligible entropy changes. The CAD spectra of Lys or Arg containing $[A.A._1 + A.A._2]Cu^+$ parallel those of the other pairs (e.g., compare Figure 2b vs Figure 2c). The loss of one amino acid unit dominates in all cases, in keeping with the $A.A._1-Cu^+-A.A._2$ connectivity for all precursor ions, as stated above. Nonetheless, entropy effects can still arise with Lys and Arg, if their conformation changes drastically during the dimer ion fragmentation (eq 2).[24,25,28] The strongly basic side chains of these amino acids may fold to provide multidentate binding in the $[Lys]Cu^+$ and $[Arg]Cu^+$ products emerging from the dissociating heterodimer, thus bringing upon a significant entropy change. Entropy effects of this type have been documented in the decomposition of H^+-bound amino acid dimers that contain Lys or Arg.[24,25b]

Relative Cu^+−A.A. Bond Strengths. In order to convert the $\ln(k_1/k_2)$ order to a Cu^+A scale in kcal/mol, it is necessary to determine the effective temperature of the metastable $[A.A._1 + A.A._2]Cu^+$ heterodimers (eq 6). T_{eff} could be calibrated by using dimers [A.A. + B]Cu^+, in which B is a reference base of known copper(I) affinity and chemically similar to amino acids. In such a case, eq 6 can be rewritten as

$$\ln(k_{A.A.}/k_B) = -Cu^+A(A.A.)/RT_{eff} + Cu^+A(B)/RT_{eff} \quad (7)$$

and a plot of $\ln(k_B/k_{A.A.})$ vs $Cu^+A(B)$ would give both T_{eff} and $Cu^+A(A.A.)$. Unfortunately, the Cu^+As of amino acids, amines, or other suitable reference compounds are not available.

The effective temperature of the dissociating cluster ion is a measure of its internal energy and primarily depends on the structure and lifetime of this ion as well as the ionization mode.[15,18b,19b,d,25a,29,30] Several investigations have shown that different dimer ions (of chemically similar molecules), generated under identical experimental conditions and having the same lifetime, also have fairly similar T_{eff}s, independent of the central ion holding them.[19b,d,31] Hence, T_{eff} of $[A.A._1 + A.A._2]Cu^+$ can be approximated by the effective temperature of the corresponding H^+-bound heterodimers, coproduced upon FAB from the same sample. Application of eq 7 to such H^+-bridged A.A. dimers yields $T_{eff} = 508 \pm 10$ K.[32] With this T_{eff} and the average cumulative $\ln(k_1/k_2)$ values listed in Figure 3, eq 6 leads to the copper(I) affinity scale of amino acids given in Table 1. Note that only relative values, ΔCu^+A, are assessed due to the aforementioned lack of appropriate reference reagents of known copper(I) affinity. Glycine, which exhibits the lowest Cu^+A (i.e. the weakest Cu^+−A.A. bond), was arbitrarily chosen as the zero point of the relative scale.

From all metastable [A.A. + Arg]Cu^+ pairs studied, [Arg]-Cu^+ is formed with higher abundance than the alternative [A.A.]-Cu^+ fragment. A parallel result is obtained for the [A.A. + Lys]Cu^+ dimers investigated, while [Lys + Arg]Cu^+ yields more [Arg]Cu^+ than [Lys]Cu^+ (Figure 3). These data suggest that copper(I) affinities increase in the order His < Lys < Arg. However, no exact ΔCu^+A values are provided for Lys and Arg in Table 1, because the above described entropy effects associated with the dissociation of heterodimers containing one or both of these amino acids preclude the use of the simplified eq 6 (which ignores entropy effects).

Copper(I) has a filled d shell (d^{10}) and behaves like a soft Lewis acid in aqueous solution chemistry,[34] preferring coordination with soft amino acid ligands.[1,37,38] This reactivity must be intrinsic, since it is also encountered in the solvent free environment of the gas phase. As attested by Table 1, amino acids with soft donor groups (e.g., cysteine, methionine, or

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(30) Note that T_{eff} is not a true thermodynamic temperature because the dimer ions undergoing dissociation are not in thermal equilibrium. It should rather be viewed as a proportionality constant in eqs 6 and 7.[17g]

(31) Chen, L.-Z.; Miller, J. M. *J. Organomet. Chem.* **1993**, *448*, 225−232.

(32) Average from three sets of [A.A. + B]H^+ dimers, namely [Thr + B]H^+ (set 1), [Phe + B]H^+ (set 2), and [Met + B]H^+ (set 3). B includes Ser, Asp, Leu, Ile, Met, and Asn in set 1, Thr, Met, Asn, and Glu in set 2, and Tyr, Asn, Trp, and Glu in set 3. The PA values used for these amino acids are listed in Table 1.[17b,d,g,25b,33]

(33) Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. 1.

Table 1. Copper(I) Affinity Order and Copper(I) Affinity Values Relative to That of Gly (ΔCu^+A in kcal/mol). Relative Proton Affinities (ΔPA in kcal/mol) and the Orders of Proton and Sodium Ion Affinities Are Included for Comparison

amino acid	Cu^+ order	ΔCu^+A^a	H^+ order	ΔPA^b	Na^+ order[c]
Gly	1	0.0	1	0.0	1
Ala	2	1.7	2	3.2	2
Ser	3	3.1	4	5.6	7
Val	4	3.7	5	6.5	4
Leu	5	4.1	7	7.1	5
Ile	6	4.3	8	7.6	6
Thr	7	4.6	9	7.6	9
Pro	8	4.8	15	10.8	11
Asp	9	5.0	6	6.5	12
Asn	10	6.7	13	10.5	14
Glu	11	7.2	14	10.7	17
Phe	12	8.0	10	8.3	10
Tyr	13	8.3	11	9.1	13
Cys	14	8.6	3	3.4	3
Gln	15	9.8	17	15.3	18
Met	16	10.4	12	9.4	8
Trp	17	11.5	16	11.9	16
His	18	13.3	18	22.4	19
Lys	19	>13.3	19	23.7	15
Arg	20	>13.3	20	33.6	20

[a] Estimated error, ±0.3 kcal/mol. [b] Relative to PA(Gly) = 211.6 kcal/mol[33] and based on proton affinities reported in refs. 33 (Ala), 17b (Cys), 25b (His and Lys), 17d (Arg), and 17g (all other amino acids). [c] Reference 19d.

histidine) clearly exhibit large Cu^+ affinities; the largest *measurable* Cu^+A value is found for histidine (13.3 kcal/mol higher than glycine) which is a favored binding site for Cu^+ in several enzymes (e.g., the dioxygen transport proteins).[1] Despite their high copper(I) affinity in the gas phase, Lys and Arg are not common Cu^+ ligands in aqueous biochemistry.[1,37,38] This can be understood by keeping in mind that the side chains of Lys and Arg, which bring upon the superior binding to Cu^+ in the gaseous state, are protonated (and thus disabled) at physiological pH.[39]

Comparison of the ΔCu^+A Order to Proton and Sodium Ion Affinities. Inspection of Table 1 reveals that the orders of copper(I) and proton affinity are substantially different from each other, giving rise to a poor correlation between ΔCu^+A and ΔPA values (viz. Figure 4). For example, Cys has a high Cu^+A but a low PA; the reverse is true for Pro. These discrepancies can be accounted for by considering that Cu^+ and H^+ are Lewis acids of markedly distinct hardness. As a much softer acid, Cu^+ should form particularly stable bonds with amino acids containing soft donor groups (such as RS− or aromatic rings).[40] Indeed this class of A.A.s exhibits larger copper(I) affinities, appearing above the regression line in Figure 4.

The order of Cu^+−A.A bond strengths also is distinct from the recently reported order of Na^+−A.A. bond strengths.[19d] Again, the hard/soft acid/base principle helps explain the deviating trends. Na^+ is harder than Cu^+ and should preferentially bind to hard amino acids. Accordingly, such amino acids (e.g., O-rich Ser, Asp, and Glu) have higher rankings in the Na^+ than in the Cu^+ affinity scale (Table 1).

It is also noticed from Table 1 that, with few exceptions, multifunctional amino acids form particularly stable bonds. Thus, Arg ligands produce the strongest cation−A.A. bond with

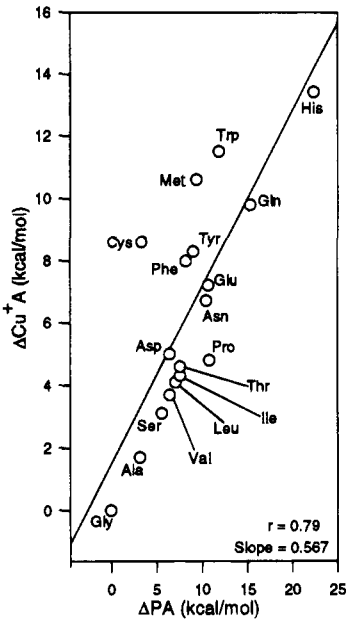

Figure 4. Comparison of the relative affinities of amino acids to Cu^+ and H^+ (ΔCu^+ vs ΔPA). The solid line is a least-squares fit to the data.

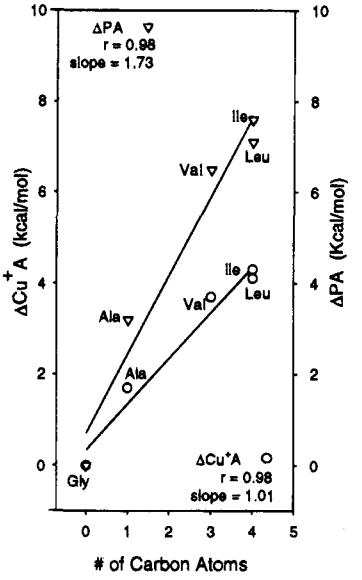

Figure 5. Relative copper(I) and proton affinities of alkyl-substituted amino acids (ΔCu^+A and ΔPA, respectively) vs the number of C-atoms in the alkyl side chain. The solid lines are least-squares fits to the data.

(34) Lewis acids (electrophiles) and Lewis bases (nucleophiles) are classified as soft or hard based on the energy level of their lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO), respectively.[35,36] Soft acids (e.g., Cu^+, Hg^+, BH_3) have a low-energy LUMO and hard acids (e.g., H^+, Na^+, Al^{3+}) have a high-energy LUMO. Soft bases (e.g., RSH, R_3P, aromatic rings) have a high-energy HOMO and hard bases (e.g., H_2O, ROH, RNH_2) a low-energy HOMO. Hard−hard and soft−soft interactions are preferred. Generally, soft acids have lower positive charge, larger size, and readily oxidizable outer electrons; the reverse is true for hard acids.[36] On the other hand, hard bases are highly electronegative, of low polarizability, and difficult to oxidize and they bear no low-lying empty orbitals; the opposite characteristics are found in soft bases.[36]

(35) Flemming, I. *Frontier Orbitals and Organic Chemical Reactions*; John Wiley & Sons: Chichester, 1976; pp 34−40.

(36) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper & Row Publishers: New York, 1987; pp 318−322.

(37) Hay, R. W. *Bio-inorganic Chemistry*; Ellis Horwood Limited: Chichester, 1987.

(38) Kaim, W.; Schwederski, B. *Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life*; John Wiley & Sons: Chichester, 1994.

(39) In aqueous solution, the pK values of the side chains of His, Lys, and Arg are 6.00, 10.53, and 12.48, respectively: Lide, D. R., Ed. *Handbook of Chemistry and Physics*, 71st ed.; CRC Press: Boca Raton, 1990; p 7−1.

(40) For a listing of hard, borderline, and soft acids/bases see Tables 3−1 and 3−2 in ref 35.

all three cations listed, and His and Trp possess high affinities across the board. The high Lewis acid affinities of the multifunctional A.A.s are most probably due to their ability to provide multiple coordination sites.

The copper ion affinities of the 20 common α-amino acids fall within a <20 kcal/mol range, compared to a range of 34 kcal/mol for their proton affinities. The smaller spread of Cu^+A values indicates that the Cu^+-A.A. bond is weaker (and hence longer) than the H^+-A.A. bond. This is substantiated by the magnitude of inductive effects on copper(I) vs proton affinities. A plot of ΔCu^+A and ΔPA vs the number of side chain C-atoms of alkyl substituted A.A.s (Figure 5) shows a much more pronounced increase in ΔPA than in ΔCu^+A as the alkyl chain and, thus, the electron-donating inductive effect on the A.A.−cation bond becomes larger. Such effects provide the highest stabilization for short bonds. Hence, the A.A.$-Cu^+$ bond must be longer than the A.A.$-H^+$ bond. The A.A.$-Cu^+$ bond could have some covalent character by delocalization of electrons from the attached A.A. ligand into the empty 4s or 4p orbitals of Cu^+.[41] Repulsion of the donated electrons by copper(I)'s filled $3d^{10}$ shell would reduce the extent of such an interaction, leading to a A.A.$-Cu^+$ bond of less covalent and more electrostatic nature than the A.A.$-H^+$ bond.[41] The smaller degree of covalency in A.A.$-Cu^+$ agrees well with the above mentioned lowered strength and raised length of this bond vis à vis A.A.$-H^+$.

(41) Operti, L.; Tews, E. C.; Freiser, B. S. *J. Am. Chem. Soc.* **1988**, *110*, 3847−3853.

Conclusions

With careful consideration of the assumptions underlying the kinetic method, this method was used to semiquantitatively evaluate the copper(I) affinities of amino acids. The order of Cu^+ affinities of these polar, labile biomolecules differs from the orders of their H^+ or Na^+ affinities, and this is thought to originate from the distinct intrinsic properties of the cations (H^+, Na^+, Cu^+) and of the amino acids. One important factor in determining an amino acid's affinity toward a specific metal ion is the hard/soft Lewis acid/base relationship between the two partners. Acid/base pairs of comparable softness (or hardness) yield relatively strong bonds. Similar trends are expected to also operate with other biomolecules. Finally, it is worth mentioning that the preferred binding sites of Cu^+ in biological systems include amino acids that also exhibit high Cu^+ affinity in the gas phase (Table 1). For example, His is a favored location of Cu^+ attachment in several proteins, while 35% of the amino acids in copper-storing metallothioneins are cysteine residues.[1,37,38]

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