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Stability Constants of the Copper(II) Complexes of Peptides and Peptide Amides Containing the α-Aminoisobutyric Acid Residue

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The proton and copper(II) association constants are measured for eight peptides and four peptide amides, which contain the α-aminoisobutyric acid (Aib) residue. The Aib peptides are slightly stronger bases than the corresponding glycyl peptides with a greater difference in the protonation constants for the carboxylate group (0.5–0.6 log unit) than for the amine functional group (0.0–0.25 log unit). The stepwise formation constants for the copper(II)–Aib_n peptide complexes indicate that both inductive and steric properties of the α-carbon methyl groups influence the relative stability of the copper(II) complexes of Aib peptides and glycyl peptides. The fully formed Aib complexes are 2.5–63 times more stable than the glycyl complexes. Steric interference to coordination is seen in the copper(II)–Aib_4 complex, where the third peptide nitrogen does not coordinate to copper(II) because of the bulk presented by the α-carbon methyl groups in the fourth residue.

Introduction

The occurrence of high proportions of α-aminoisobutyric acid (Aib), $NH_2C(CH_3)_2COOH$, in microbial polypeptide antibiotics[1-5] and the unusual ion conductance properties of membranes and artificial bilayers composed of these peptides[1,5,6] have stimulated studies concerning the effect of the Aib unit on the conformations available to small peptides in solution[7,8] and in the solid state.[7,9-12] Theoretical[13] and ^1H NMR[7,8] investigations have shown that dimethyl alkylation of the α-carbon sterically restricts the conformation of peptides containing the Aib unit.

In the course of our studies of the structure,[14] reactivity,[15] and photochemistry[15,16] of copper(III) complexes of the tripeptide, Aib_3, we have synthesized a number of other Aib-containing peptides and have characterized the copper(II) complexes of these peptides. The twelve ligands that we have prepared and studied are AAib, Aib_2, GAibG, $AAib_2$, Aib_3, G_2AibG, Aib_3G, Aib_4, Aiba, AAiba, Aib_2a, and Aib_3a, where G is glycyl, A is alanyl, and a is amide. Whereas previous studies have been concerned primarily with the steric restrictions introduced by dimethylation of the α-carbon in peptides, in the present work we are interested in understanding how the inductive and steric properties of α-carbon methyl groups affect the acid–base equilibria of these peptides and their ability to bind copper(II). In the microbial polypeptides adjacent Aib amino acid residues are present;[2-4] hence there is an interest in the Aib_n unit.

In this study the cumulative proton and copper(II) association constants for the Aib-containing peptides and peptide amides are compared to the corresponding values for the glycyl peptides (G_n) and peptide amides (G_na) and the alanyl peptides (A_n). The electron-releasing inductive property of α-carbon methyl groups is expected to increase the basicity, and therefore the donor strength, of the amine and peptide nitrogens and the carboxylate oxygen. The volume occupied by the methyl groups on the α-carbon in the Aib unit is much greater than that needed by the α-carbon hydrogens in the glycyl unit. Thus, some steric interference to copper(II)–peptide coordination is anticipated. The relative importance and magnitude of these two effects are examined with ligands varying in length from amino acid amides to tetrapeptides.

Experimental Section

Reagents. Copper(II) perchlorate, prepared from $CuCO_3$ and $HClO_4$, was standardized by EDTA titration with murexide indicator. Sodium perchlorate, prepared from Na_2CO_3 and $HClO_4$, was standardized gravimetrically. Carbonate-free sodium hydroxide was standardized with primary standard grade potassium hydrogen phthalate.

Ligand Synthesis. The peptides AAib, Aib_2, $AAib_2$, Aib_3, Aib_4, Aib_3G, Aib_2a, G_2AibG, and GAibG were prepared by the methods outlined by Kirksey et al.[15] The peptide amides AAiba and Aib_3a were prepared by the following procedure. The *tert*-butoxycarbonyl (BOC) and benzyl ester (OBz) blocked peptides BOCAAibOBz and $BOCAib_2OBz$ were synthesized from BOCAib or BOCA[17] and AibOBz by the dicyclohexylcarbodiimide (DCC) method of amino acid coupling.[15] Deblocking of $BOCAib_2OBz$ with trifluoroacetic acid[18] and DCC coupling with BOCAib gave $BOCAib_3OBz$. The benzyl ester group was removed from BOCAAibOBz and $BOCAib_3OBz$ by hydrogenolysis[15] using PdO catalyst to give $BOCAib_3$ or BOCAAib. Then the *o*-nitrophenol ester (ONP) of $BOCAib_3$ or BOCAAib was prepared according to the procedure of Bodansky et al.[18] Treatment of $BOCAib_3ONP$ or BOCAAibONP in tetrahydrofuran with ammonia gas gave $BOCAib_3a$ or BOCAAiba, which was deblocked with trifluoroacetic acid to yield the salt $Aib_3a \cdot CF_3COOH$ or $AAiba \cdot CF_3COOH$. The synthesis of Aiba was started with the ONP ester CBZAibONP (CBZ denotes carbobenzyloxy).[19] The ONP ester was treated with ammonia gas to give CBZAiba. Deblocking was done by hydrogenolysis over PdO. The amide was isolated as the hydrochloride salt. Satisfactory elemental analyses were obtained for all

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Table I. Cumulative Proton Association Constants for Peptide Ligands[a]

peptide	$\log \beta_{011}$[b]	$\log \beta_{021}$	pK_{COOH}[c]	ref
1. G_2	8.07	11.20	3.13	*d*
2. A_2	8.05	11.25	3.20	*d*
3. AAib	8.22 ± 0.008	11.71 ± 0.01	3.49	*e*
4. Aib_2	8.26 ± 0.007	11.93 ± 0.009	3.67	*e*
5. G_3	7.89	11.09	3.20	*d*
6. GAibG	8.20 ± 0.01	11.53 ± 0.02	3.33	*e*
7. $AAib_2$	8.29 ± 0.02	12.24 ± 0.03	3.95	*e*
8. Aib_3	8.11 ± 0.004	11.93 ± 0.006	3.82	*e*
9. G_4	7.87	11.05	3.18	*d*
10. G_2AibG	7.98 ± 0.006	11.53 ± 0.009	3.55	*e*
11. Aib_3G	8.33 ± 0.02	11.79 ± 0.02	3.46	*e*
12. Aib_4	7.78 ± 0.01	11.58 ± 0.01	3.80	*e*
13. Ga	7.95			*f*
14. Aiba	8.06 ± 0.003			*e*
15. G_2a	7.78			*f*
16. AAiba	7.93 ± 0.001			*e*
17. Aib_2a	7.93 ± 0.004			*e*
18. G_3a	7.75			*f*
19. Aib_3a	7.76 ± 0.006			*e*

[a] μ = 0.1 M $NaClO_4$, 25.0 °C. [b] $pK_{NH} = \log \beta_{011}$. [c] $pK_{COOH} = \log \beta_{021} - \log \beta_{011}$. [d] Reference 33a. [e] This work. [f] Reference 29.

peptides and peptide amides. Anal. Calcd for AAib, $C_7H_{14}N_2O_3$: C, 48.27; H, 8.10; N, 16.08. Found: C, 48.46; H, 8.05; N, 16.07. Calcd for AAiba·CF_3COOH, $C_9H_{16}N_3O_4F_3$: C, 37.63; H, 5.62; N, 14.63; F, 19.84. Found: C, 37.70; H, 5.37; N, 14.62; F, 19.66. Calcd for $AAib_2·H_2O$, $C_{11}H_{23}N_3O_5$: C, 47.64; H, 8.36; N, 15.15. Found: C, 47.52; H, 8.57; N, 15.26. Calcd for Aiba·HCl, $C_4H_{11}N_2OCl$: C, 34.67; H, 8.00; N, 20.21; Cl, 25.58. Found: C, 34.70; H, 7.96; N, 20.01; Cl, 25.38. Calcd for $Aib_2·H_2O$, $C_8H_{18}N_2O_4$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.65; H, 8.89; N, 13.32. Calcd for Aib_2a·HCl, $C_8H_{18}N_3O_2Cl$: C, 42.95; H, 8.11; N, 18.79; Cl, 15.85. Found: C, 43.12; H, 8.13; N, 18.60; Cl, 16.00. Calcd for $Aib_3·2H_2O$, $C_{12}H_{27}N_3O_6$: C, 46.58; H, 8.80; N, 13.58. Found: C, 46.73; H, 8.80; N, 13.30. Calcd for $Aib_3a·CF_3COOH·H_2O$, $C_{14}H_{27}N_4O_6F_3$: C, 41.58; H, 6.73; N, 13.85; F, 14.10. Found: C, 41.29; H, 7.00; N, 14.16; F, 14.35. Calcd for $Aib_4·H_2O$, $C_{16}H_{32}N_4O_6$: C, 51.04; H, 8.57; N, 14.88. Found: C, 51.33; H, 8.70; N, 15.14. Calcd for Aib_3G·CF_3COOH, $C_{16}H_{27}N_4O_7F_3$: C, 43.24; H, 6.12; N, 12.60; F, 12.82. Found: C, 43.11; H, 6.33; N, 12.45; F, 12.90. Calcd for G_2AibG, $C_{10}H_{18}N_4O_5$: C, 43.79; H, 6.61; N, 20.43. Found: C, 43.66; H, 6.33; N, 20.21. Calcd for GAibG, $C_8H_{15}N_3O_4$: C, 44.23; H, 6.96; N, 19.34. Found: C, 44.52; H, 7.00; N, 19.12.

Potentiometric Titrations. Potentiometric titrations were performed with an Orion Research Model 701A Digital Ionalyzer equipped with a Sargent-Welch S30050-15C glass electrode and a saturated sodium chloride calomel electrode. The reference electrode was connected to the titration cell via a salt bridge containing 0.1 M $NaClO_4$. The titrant, 0.1 M NaOH, was delivered from a calibrated Gilmont micrometer syringe, #S-4200, which has a capacity of 2.5 mL in 0.0001-mL divisions. All glassware was calibrated before use. During the titration the solution was blanketed with water-saturated argon. For a titration volume of 20 mL, the analytical concentration of copper(II) was varied from 1×10^{-3} to 5×10^{-3} M and at least three different L:Cu ratios (1.1:1 to 4.1:1) were titrated for each ligand. The ligands AAib,, $AAib_2$, GAibG, AAiba, and Aib_3G were titrated in a vessel specifically designed to use small amounts of peptides (4–10 mg) in a 4-mL solution volume.[20] The titration data were recorded in millivolts vs. milliliters of titrant added. Titrations of $HClO_4$ were performed before and after each set of data in order to convert potential readings to $[H^+]$ and to calculate K_w.[21] The ionic strength was maintained at 0.1 M $NaClO_4$, and the solutions were thermostated at 25.0 ± 0.1 °C. The potentiometric data were analyzed over the pH range 3–10.5 by a modified version[22] of the computer program SCOGS.[23] The standard deviation of titer varied from 3 to 10 μL. The fit of the calculated titration curve to the experimental data, as measured by the precisions quoted in Tables I–III, was poorer for the data taken in the small-volume titration vessel because a smaller titrant volume was required.

Results

The generalized cumulative formation reaction of peptide ligands with proton and copper(II) is given in eq 1, where L

$$mCu^{2+} + hH^+ + lL \overset{\beta_{mhl}}{\rightleftharpoons} Cu_m(H_hL_l) \quad (1)$$

is the negative species for the peptide ligands and the neutral amine for peptide amide ligands. Charges on the ligand and the copper(II) complexes are omitted for clarity in notation. The stability constant β_{mhl} is defined by eq 2. Analysis of

$$\beta_{mhl} = \frac{[Cu_m(H_hL_l)]}{[Cu^{2+}]^m[H^+]^h[L]^l} \quad (2)$$

the titration data for the peptide ligands in the absence of copper(II) gives the cumulative association constants for amine and carboxylate protonation (eq 3 and 4). For peptide amides

$$H^+ + L \overset{\beta_{011}}{\rightleftharpoons} HL \quad (3)$$

$$2H^+ + L \overset{\beta_{021}}{\rightleftharpoons} H_2L \quad (4)$$

no terminal carboxylic acid group is present, so only eq 3 is appropriate. The cumulative proton association constants determined for the peptides and peptide amides in this study are given in Table I together with the corresponding values for the G_n, G_na, and A_n series.

The equilibria needed to fit the experimental titration curves for solutions of copper(II) and the peptide ligands under study here are given by eq 5–14. The accepted structures for the

$$Cu^{2+} + H^+ + L \overset{\beta_{111}}{\rightleftharpoons} Cu(HL) \quad (5)$$

$$Cu^{2+} + L \overset{\beta_{101}}{\rightleftharpoons} CuL \quad (6)$$

$$Cu^{2+} + L \overset{\beta_{1-11}}{\rightleftharpoons} Cu(H_{-1}L) + H^+ \quad (7)$$

$$Cu^{2+} + L \overset{\beta_{1-21}}{\rightleftharpoons} Cu(H_{-2}L) + 2H^+ \quad (8)$$

$$Cu^{2+} + L \overset{\beta_{1-31}}{\rightleftharpoons} Cu(H_{-3}L) + 3H^+ \quad (9)$$

$$Cu^{2+} + L \overset{\beta_{1-21}}{\rightleftharpoons} Cu(H_{-1}L)(OH) + 2H^+ \quad (10)$$

$$Cu^{2+} + L \overset{\beta_{1-31}}{\rightleftharpoons} Cu(H_{-2}L)(OH) + 3H^+ \quad (11)$$

$$Cu^{2+} + 2L \overset{\beta_{102}}{\rightleftharpoons} CuL_2 \quad (12)$$

$$Cu^{2+} + 2L \overset{\beta_{1-12}}{\rightleftharpoons} Cu(H_{-1}L)(L) + H^+ \quad (13)$$

$$Cu^{2+} + 2L \overset{\beta_{1-22}}{\rightleftharpoons} Cu(H_{-1}L)_2 + 2H^+ \quad (14)$$

species present during the stepwise chelation of copper(II) by peptide ligands are shown in Figure 1.[24-29] In general, the

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Table II. Cumulative Association Constants and Representative Equilibrium Constants for Copper(II) Peptides and Peptide Amide Complexes[a]

peptide	$\log \beta_{101}$	$\log \beta_{1-11}$	pK_{-1}[b]	$\log \beta_{1-21}$	pK_{-2}[b]	$\log \beta_{1-31}$	pK_{-3}[b]	ref
1. G_2	5.50	1.43	4.07	−7.85	9.28[c]			*d*
2. A_2	5.34	1.74	3.60	−7.74	9.48[c]			*d*
3. AAib	5.6 ± 0.1	2.18 ± 0.01	3.4	−6.68 ± 0.03	8.86[c]			*e*
4. Aib_2	6.03 ± 0.03	2.54 ± 0.005	3.49	−6.95 ± 0.01	9.49[c]			*e*
5. G_3	5.08	−0.03	5.11	−6.75	6.72			*d*
6. GAibG	5.55 ± 0.05	0.22 ± 0.03	5.33	−5.65 ± 0.03	5.87			*d*
7. $AAib_2$	5.83 ± 0.15	0.96 ± 0.02	4.87	−4.88 ± 0.02	5.84			*e*
8. Aib_3	5.41 ± 0.02	1.18 ± 0.007	4.23	−4.95 ± 0.007	6.13			*e*
9. G_4	5.10	−0.30	5.40	−7.10	6.80	−16.24	9.14	*d*
10. G_2AibG	5.18 ± 0.03	−0.03 ± 0.008	5.21	−7.74 ± 0.01	7.71	−15.85 ± 0.01	8.11	*e*
11. Aib_3G	5.27 ± 0.04	0.57 ± 0.04	4.70	−6.74 ± 0.02	7.31	−14.68 ± 0.02	7.94	*e*
12. Aib_4	4.59 ± 0.07	0.15 ± 0.007	4.44	−6.98 ± 0.01	7.13		>10.5	*e*
13. Ga	5.29	−1.63	6.92					*f*
	9.45[g]	2.54[h]	6.91[i]	−5.58[j]	8.12[k]			
14. Aiba	4.58 ± 0.02	−1.71 ± 0.03	6.29	−8.79 ± 0.005[l]				*e*
	9.28 ± 0.009[g]	2.29 ± 0.01[h]	6.99[i]	−5.25 ± 0.008[j]	7.54[k]			
15. G_2a	4.88	−0.19	5.07	−8.20	8.01	−18.02	9.82[m]	*f*
16. AAiba	4.42 ± 0.05	−0.03 ± 0.006	4.45	−7.34 ± 0.01	7.31	−16.77 ± 0.02	9.43[m]	*e*
17. Aib_2a	4.67 ± 0.04	0.28 ± 0.003	4.39	−7.63 ± 0.006	7.91	−17.60 ± 0.008	9.97[m]	*e*
18. G_3a	4.77	−0.51	5.28	−7.50	6.99	−16.19	8.69	*f*
19. Aib_3a	4.42 ± 0.07	−0.29 ± 0.006	4.71	−7.63 ± 0.01	7.34	−15.60 ± 0.01	7.97	*e*

[a] 0.1 M $NaClO_4$, 25.0 °C. [b] $pK_{-n} = \log \beta_{1-(n-1)1} - \log \beta_{1-n1}$. [c] pK_{-2} for

$$Cu(H_{-1}L)(OH_2) \overset{K_{-2}}{\rightleftharpoons} Cu(H_{-1}L)(OH) + H^+$$

[d] Reference 33a. [e] This work. [f] Reference 29. [g] $\log \beta_{102}$, eq 12. [h] $\log \beta_{1-12}$, eq 13. [i] $pK_{-1}^{bis} = \log \beta_{102} - \log \beta_{1-12}$. [j] $\log \beta_{1-22}$, eq 14. [k] pK_{-2}^{bis} for

$$Cu(H_{-1})(L) \overset{K_{-2}^{bis}}{\rightleftharpoons} Cu(H_{-1}L)_2 + H^+$$

[l] $\log \beta_{1-21}$, eq 10. [m] pK_{-3} for

$$Cu(H_{-2}L)(OH_2) \overset{K_{-3}}{\rightleftharpoons} Cu(H_{-2}L)(OH) + H^+$$

Table III. Copper(II)–Tripeptide and –Tetrapeptide Cumulative Association Constants for the Cu(HL) Complex[a]

peptide	$\log \beta_{111}$	pK'_{NH}[b]	ref
G_3	10.24	5.02	*c*
$AAib_2$	10.55 ± 0.06	4.72	*d*
Aib_3	9.99 ± 0.04	4.58	*d*
G_4	8.59	3.53	*c*
Aib_3G	10.20 ± 0.03	4.93	*d*
Aib_4	9.1 ± 0.3	4.5	*d*

[a] 0.1 M $NaClO_4$, 25.0 °C. [b] $pK'_{NH} = \log \beta_{111} - \log \beta_{101}$. [c] Reference 28. [d] This work.

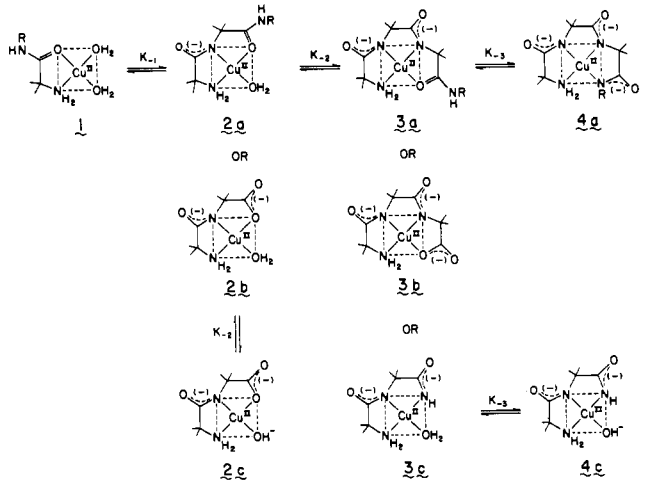

Figure 1. Stepwise coordination of peptide and peptide amide ligands by copper(II) with proposed structures **1–4c**.

structure for the fully formed copper(II) complex for the dipeptides is $Cu(H_{-1}L)(OH)$(**2c**), for the dipeptide amides $Cu(H_{-2}L)(OH)$ (**4c**), for the tripeptides $Cu(H_{-2}L)$ (**3b**), and for the tripeptide amide and the tetrapeptides, $Cu(H_{-3}L)$ (**4a**). However, titration of copper(II)–Aib_4 solutions required 1 equiv less of base than did copper(II)–G_2AibG or –Aib_3G solutions. The experimental titration curves for copper(II)–Aib_4 were best fit with use of eq 3–8, in which the third peptide nitrogen does not ionize so that the fully formed complex is $Cu(H_{-2}L)$ (**3a**), with R = $C(CH_3)_2CO_2^-$.

The lack of coordination of the third peptide nitrogen is supported by several other experimental facts. The wavelength maximum of the ligand field transition of copper(II)–Aib_4 in solution above pH 10 is 517 nm, whereas the same transition for $Cu^{II}(H_{-3}Aib_3G)^{2-}$ occurs at 482 nm, which indicates that there are stronger donors to copper(II) in the latter complex.[30] Also, the formal reduction potential, $E°'$, for the $Cu^{III,II}(H_{-3}Aib_3G)^{-,2-}$ couple is 0.38 V[31] vs. NHE whereas $E°'$ for the copper(II)–Aib_4 complex is 0.73 V vs. NHE.[32] The $E°'$ value for copper(III, II)–Aib_4 is even higher than the value found for the $Cu^{III,II}(H_{-2}Aib_3)^{0,-}$ couple, which indicates that there are weaker donor atoms to copper(II) in the copper(II)–Aib_4 complex than in the $Cu^{II}(H_{-2}Aib_3)^-$ complex. This is consistent with the proposed $Cu^{II}(H_{-2}Aib_4)^-$ structure, **3a**.

For an Aiba:Cu(II) ratio of 1.7:1 the $Cu(H_{-1}Aiba)(OH)$ species was required to fit the titration data (eq 10). The analogous $Cu(H_{-1}Ga)(OH)$ species was not seen.[29] The values of the cumulative copper(II) association constants, $\log \beta_{mhl}$, for the Aib-containing peptides and peptide amides are presented in Tables II and III along with the values for the

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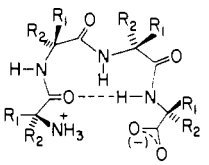
G_n, G_na, A_2, and A_3 ligands for comparison.

Discussion

Peptide Protonation Constants. Increased basicity of the amine and carboxylate functional groups for the Aib-containing ligands relative to the glycyl ligands can be attributed to the inductive properties of the α-carbon methyl groups. Specifically, carboxylate basicity is increased 0.5–0.6 log unit, whereas amine basicity is increased less than 0.25 log unit (with the exception of Aib_3G). The same trend is seen with monofunctional amine[33a,b] and carboxylic acid[33c] derivatives. For example, the pK_a values for protonated amine (μ = 0, 25 °C) are 10.64 ($CH_3CH_2NH_2$), 10.67 ($(CH_3)_2CHNH_2$), and 10.69 ($(CH_3)_3CNH_2$), a range of only 0.05 log unit, while the pK_a values for the corresponding carboxylic acids (μ = 0, 25 °C) are 4.87 (CH_3CH_2COOH), 4.85 ($(CH_3)_2CHCOOH$), and 5.03 ($(CH_3)_3CCOOH$), a range of 0.16 log unit. Solvation of the ionic forms, RCO_2^- and RNH_3^+, may be hindered by the hydrophobic nature of the α-carbon methyl groups. This effect would lessen the increase in basicity of the amine nitrogen and enhance the increase in basicity of the carboxylate group due to methyl group inductive effects. For those ligands with only one α-carbon methyl group in the amine terminal residue, AAib, $AAib_2$, and AAiba, the amine nitrogen is about equal to or is slightly more basic than Aib_2, Aib_3, and Aib_2a, respectively. This can be attributed to opposing methyl group effects of increased basicity due to electron release and decreased basicity due to steric interference with solvation of the protonated amine.

The difference of 0.55 log unit between log β_{011} for Aib_3G and Aib_4 is surprising for such similar peptides. The amine protonation determination was repeated for both tetrapeptides, and the same results were obtained. Possibly there is some "head-to-tail" interaction between the protonated amine and the carboxylate anion in the Aib_3G system that is not possible in the Aib_4 system. This zwitterion stabilization, only possible in the longer peptides, would increase amine basicity (as noted above) and carboxylate acidity (as seen by comparing pK_{COOH} for G_2AibG and Aib_3G). The crystal structures of several peptides containing Aib residues show that a 3_{10}-helical conformation is favored.[7,9-11] For a tetrapeptide, a 3_{10} conformation (**5**) would bring the protonated amine and carboxylate



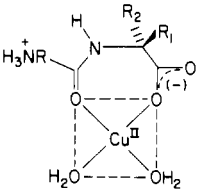
5

ends in close proximity. In aqueous solution this conformation might be disrupted.[12] Circular dichroism studies of Aib-containing peptides in 95% EtOH show helical structure,[34] but it is not possible to distinguish between the α-helical and 3_{10}-helical conformations. It is difficult to see why Aib_3G can be involved in a "head-to-tail" electrostatic interaction and Aib_4 cannot unless there is steric hindrance between the first and last residues.

Copper(II)–Peptide Association Constants. Cu(HL). On the basis of electronic absorption data and charge neutralization effects on the magnitude of pK'_{NH} for eq 15, Kaneda

$$Cu(HL) \overset{K'_{NH}}{\rightleftharpoons} CuL + H^+ \qquad (15)$$

and Martell[28] assign the Cu(HL) structure as the amine protonated species, **6**. The pK'_{NH} value (log β_{111} − log β_{101})



6

measures the relative stability of the copper(II) complex in **6** vs. the complex shown in **7** (Figure 5). The trends in pK'_{NH} (Table III) parallel the trends in pK_{NH} (log β_{011}). Depending on the relative values of log β_{111}, log β_{101}, and log β_{1-11}, the Cu(HL) species at pH 3–5 may be a minor component (Aib_4 in Figure 2A) or a significant fraction of the total copper complexes (Aib_3 in Figure 2B, $AAib_2$ in Figure 2C, and Aib_3G in Figure 2D).

CuL. For both the glycyl and the Aib series of ligands, the cumulative association constant, β_{101} (for formation of the CuL species, **1**), increases as the basicity of the amine nitrogen increases as illustrated in Figure 3. However, the sensitivity of log β_{101} to changes in the basicity of the amine nitrogen (log β_{011} = pK_{NH}) is dependent upon the class of ligands considered. The CuG_na series and the CuL'_n series (where L′ refers to an amino acid residue) for the G, A, and Aib members of the peptide ligands (L'_n) have essentially the same large, positive slope for the log β_{101} vs. log β_{011} plot (points 1–10 and 12 in Figure 3). Because of a possible "head-to-tail" interaction noted above, the Aib_3G ligand is thought to have an anomalously high value of log β_{011} and is an exception to the correlation.

In contrast to the L'_n series the slopes of the log β_{101} vs. log β_{011} plots for the individual peptide amide (L'_na) series are large and negative as shown in Figure 3 for L'_3a, L'_2a, and L'_1a. On the other hand, the log β_{101} values for the glycyl amides (points 13, 15, and 18) fall along the same correlation line as the peptides in Figure 3. However, the amide ligands that have α-carbon methyl groups in the first residue (points 19, 17, 14) are relatively independent of the value of log β_{011}. Since the proposed chelate structure, **1**, for all the CuL'_n and CuL'_na species is the same, the structure of the R group in **1** must cause the difference. This suggests that the carboxylate group in the L'_n (L′ = A or Aib) series, which is significantly more basic for the Aib-containing peptides, is associated with copper(II) either by axial coordination (possible for L'_3) or by hydrogen bonding to coordinated water. Such an interaction would stabilize $CuAib_n$ relative to $CuAib_n$a. The invariance of log β_{101} with changes in log β_{011} for the $CuAib_n$a series will be discussed further in the $Cu(H_{-1}L)$ section.

Cu(H_{-1}L). With the exception of Aiba the cumulative association constant, β_{1-11}, for formation of $Cu(H_{-1}L)$ (**2a** or **2b**) is larger (log β_{1-11} less negative) for the Aib series of ligands than for the glycyl series. Log β_{1-11} will reflect variations in log β_{101} as well as the **1** to **2** structural change. A more convenient value to describe the stability of **2** relative to **1** is the difference log β_{101} − log β_{1-11}, which is defined as pK_{-1}, the pK_a for deprotonation of a peptide nitrogen (eq 16).

$$CuL \overset{K_{-1}}{\rightleftharpoons} Cu(H_{-1}L) + H^+ \qquad (16)$$

A less positive value of pK_{-n} indicates stronger metal complexation. The difference $pK_{-1}^{Gly} - pK_{-1}^{Aib}$ ranges from 0.57 to 0.96, indicating the Aib-containing ligands, including the amides, consistently form a more stable $Cu(H_{-1}L)$ complex (**2a** or **2b**) than the G_n or G_na ligands as indicated by the general negative slopes in Figure 4. Note also that pK_{-1} for $Cu(H_{-1}Aib_2)$ is 3.5, which means at pH values as low as 3.5 the peptide nitrogen deprotonates by coordination to copper-

(33) Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum Press: New York: (a) 1974; Vol. 1. (b) *Ibid.*, 1975; Vol. 2. (c) *Ibid.*, 1977; Vol. 3.

(34) Oekonomopulos, R.; Jung, G. *Biopolymers* **1980**, *19*, 203.

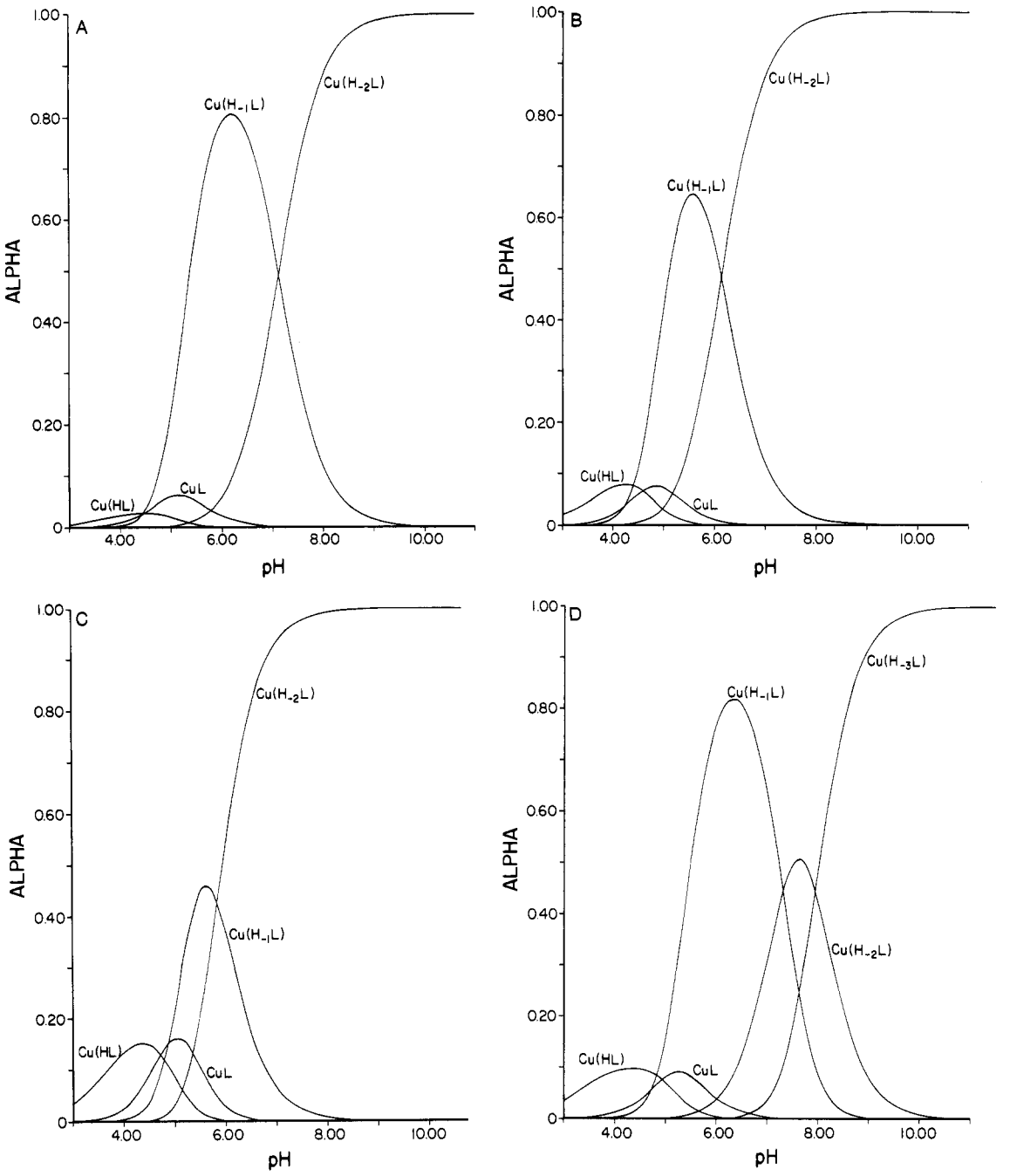


Figure 2. Species distribution diagram for complexation of copper(II) by peptide ligands. Alpha is the fraction of Cu_T present as each species ($[Cu(II)]_T = [L]_T = 2.00 \times 10^{-3}$ M, μ = 0.1 M $NaClO_4$, 25.0 °C): (A) L = Aib_4; (B) L = Aib_3; (C) L = $AAib_2$; (D) L = Aib_3G.

(II)! Since the pK_a for the amide nitrogen in RCONHR′ is ~16,[35] this low pK_{-1} value emphasizes the affinity of copper(II) for the deprotonated peptide nitrogen donor. The dipeptide complexes are approximately 1 log unit more stable than the tri- and tetrapeptide complexes as shown in Figure 4B because carboxylate oxygen (**2b**) is a stronger donor than carbonyl oxygen (**2a**).

Recall that the Aib-containing amides and Aib_4 form less stable CuL complexes, (**1**) than G_na and G_4, respectively. In the **1** to **2** conversion, a stronger donor to copper(II) is being formed by deprotonation of the first peptide nitrogen. Figure 5 emphasizes the bond angles and distances that change in the **1** to **2** equilibrium. The values[26] for structure **7** are from the crystal structure of $Cu(G_3)Cl \cdot {}^1/_2H_2O$, which has the geometry of **1**, with two equatorial waters replaced by Cl^- and the carboxylate tail of a second G_3 molecule. For structure **8** the average values determined by Freeman[26] for a structure of this type are given. Since angle *a* closes, angle *b* opens, and bond length *d* decreases, formation of **8** results in reduction of the crowding of the α-carbon R groups, the amine hydrogens, and the amide proton around the periphery of the five-membered ring in **7**. Steric repulsions felt in **7** should be relieved in **8**. Thus, the bulky methyl groups at the α-carbon in **7** decrease the stability of CuL for Aib-containing peptides relative to the glycyl ligands regardless of the basicity of the amine nitrogen. These same steric problems are present in the di- and tripeptide complexes, but the charge neutralization effect of the terminal carboxylate discussed above more than compensates for any steric hindrance to coordination in the CuL complexes. Also, the reduced dependence of log β_{101} on log β_{011} for the Aib_na series points to the uniformity of structure **7** for all Aib_na with R varying in length but not interacting with copper(II).

$Cu(H_{-2}L)$ or $Cu(H_{-1}L)(OH)$. For the dipeptides the second

(35) Hendrickson, J. B.; Cram, D. J.; Hammond, G. S. "Organic Chemistry"; McGraw-Hill: New York, 1970; p 304.

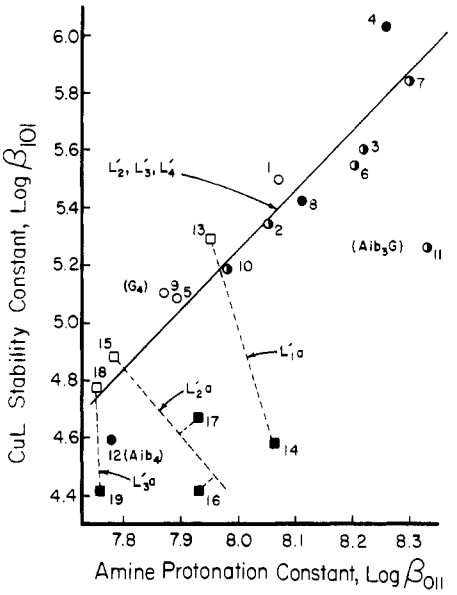


Figure 3. Comparison of the stability constant for CuL (log β_{101}) with the amine protonation constant (log β_{011}) for L'$_2$, L'$_3$, L'$_4$ (circles) and for L'a, L'$_2$a, L'$_3$a (squares). Open symbols are for peptides and amides with only G, closed symbols with only Aib, and half-closed symbols with G or A and Aib. Numbers refer to peptides in Tables I and II. The solid line is the least-squares fit for all points except 11, 14, 16, 17, and 19: slope 2.1 ± 0.2.

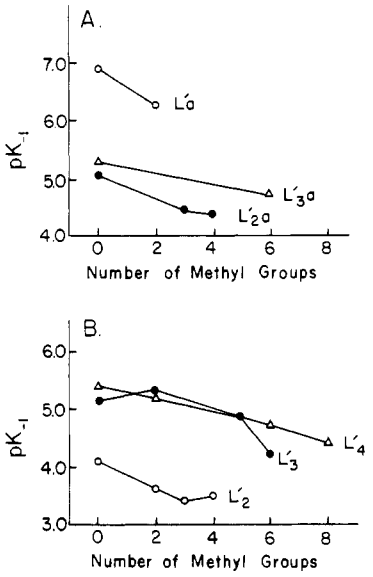


Figure 4. Plot of pK_{-1} vs. the number of α-carbon methyl groups in L for the Cu^{II}(H$_{-1}$L) complex: (A) peptide amides; (B) peptides.

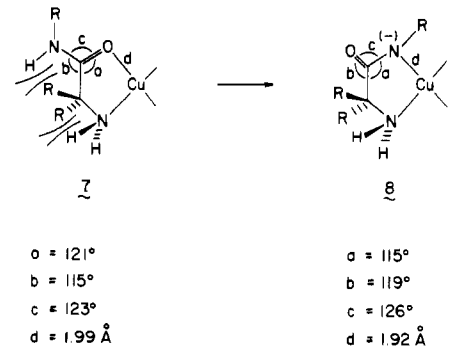


Figure 5. Comparison of the bond angles and distances for the Cu^{II}L and the Cu^{II}(H$_{-1}$) species. Data are taken from ref 26.

proton is lost from an equatorially coordinated water molecule, **2c**. It is not clear why pK_{-1} (**2b** to **2c**) for AAib is 0.5 log unit

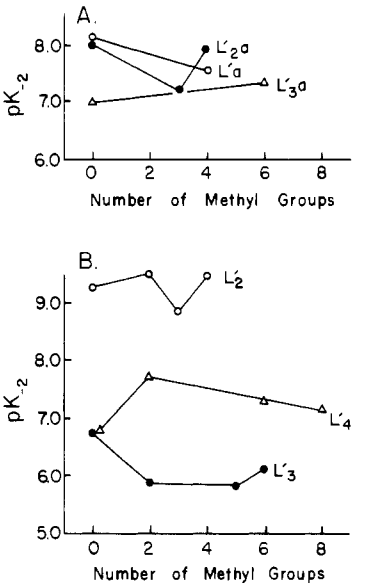


Figure 6. Plot of pK_{-2} vs. the number of α-carbon methyl groups in L for the Cu^{II}(H$_2$L) or Cu^{II}(H$_{-1}$L)(OH) complexes: (A) peptide amides; (B) peptides.

lower than pK_{-1} for the remaining dipeptides. For the tripeptides a second peptide deprotonation occurs that is 2–3 pK_a units lower (i.e. a more favorable reaction) than loss of a proton from coordinated water, as seen in Figure 6B, where K_{-2} corresponds to eq 17. Again, an increase in stability (less

$$\mathrm{Cu(H_{-1}L)} \overset{K_{-2}}{\rightleftharpoons} \mathrm{Cu(H_{-2}L)} + \mathrm{H^+} \qquad (17)$$

negative log β_{1-21} or smaller pK_{-2}) is seen for the Aib-containing tripeptides relative to G$_3$ with pK_{-2} for GAibG, AAib$_2$, and Aib$_3$ 0.72, 0.88, and 0.59 log unit lower, respectively, than pK_{-2} for G$_3$. For the tripeptides Cu(H$_{-2}$L) has a carboxylate oxygen instead of a carbonyl oxygen coordinated in the fourth position (**3b**). The lower pK_{-2} for Aib$_3$, GAibG, and AAib$_2$ compared to that for G$_3$ indicates the basicity of both the carboxlate oxygen and the peptide nitrogen are increased by the electronic effects of the α-carbon methyl groups.

The tetrapeptides and the tripeptide amides show a reversal in the trend to lower pK_{-2} values for the Aib-containing ligands compared to that for G$_4$ and G$_3$a. The Aib peptide nitrogens are 0.33–0.91 log unit more difficult to deprotonate than G$_4$ and G$_3$a as shown in Figure 6. Deprotonation of the second peptide nitrogen gives the 5–5–5 chelate system (**4a**), which is known to be strained.[29,36] Possibly, Cu–N bond length changes and peptide ligand angle adjustments to properly orientate the ligand for maximum overlap with the copper(II) bonding orbitals are significant in the **2a** to **3a** structural change. With the ligands derived from the Aib amino acid, the increased basicity of the donor atoms and increased steric hindrance to structural change[13] makes the 5–5–5 ring system less stable relative to the glycyl ligands. Therefore, the Aib systems require a larger driving force, i.e., higher pH, to form **3a**.

Cu(H$_{-3}$L) and Cu(H$_{-2}$L)(OH). For the dipeptide amides, the last proton is lost from an equatorially bound water molecule (**4c**). The pK_{-3} value for G$_2$a and Aib$_2$a are about the same; however, pK_{-3} for AAiba is ~0.5 log unit lower. A similar discrepancy was seen for the ionization of an equatorial water in the dipeptide series.

(36) Yamauchi, O.; Nakao, Y.; Nakahara, A. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 2119.

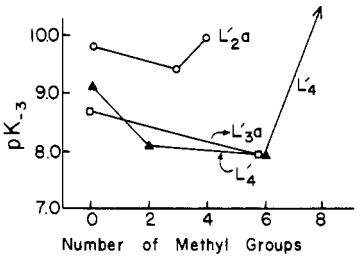


Figure 7. Plot of pK_{-3} vs. the number of α-carbon methyl groups in L for the $Cu^{II}(H_{-3}L)$ or $Cu^{II}(H_{-2}L)(OH)$ complexes. The arrow for the Aib_4 point indicates the lower limit for pK_{-3}.

For the tetrapeptides and the tripeptide amides, deprotonation of the third peptide nitrogen (K_{-3}, eq 18) occurs at 0.7–2

$$Cu(H_{-2}L) \overset{K_{-3}}{\rightleftharpoons} Cu(H_{-3}L) + H^+ \qquad (18)$$

pK_a units lower than loss of the third proton from equatorial coordinated water in the dipeptide amide series as shown in Figure 7. The pK_{-3} value is lowest for the Aib series relative to the glycyl series in accord with the inductive effect of the α-carbon methyl group. For the tetrapeptides, coordination of the third peptide nitrogen is possible only as long as the fourth amino acid residue is not an Aib. The tetrapeptide Aib_4 does not form the $Cu(H_{-3}L)$ species because of steric hindrance to coordination between the rest of the complex and the fourth residue. Structure **3a** is proposed for $Cu^{II}(H_{-2}Aib_4)^-$ rather than a structure similar to **3c** because an equatorially coordinated water molecule would be expected to ionize at pH values below 10.5.

Conclusions

The volume occupied by the α-carbon methyl groups in the Aib residue is an important steric effect that restricts the conformation of peptides containing this residue. This study shows that the Aib units can hinder copper(II) coordination in a few cases. Thus, coordination of the third peptide nitrogen in the Aib_4–copper(II) complex does not occur. A destabilizing effect due to the steric requirements of the α-carbon methyl groups is seen in the formation of the first chelate ring (**1**) and in the formation of the first 5–5–5 fused chelate system (**3a**). However, in general, the copper(II)–peptide complexes containing Aib residues are more stable than the corresponding alanyl or glycyl complexes. The increased stability of the Aib-containing peptide complexes of copper(II) is due largely to the inductive effect of the α-carbon methyl groups. The increase in overall stability varies with the length of the peptide and is largest for the tetrapeptides (with the exception of Aib_4). Thus, $Cu^{II}(H_{-3}Aib_3G)^{2-}$ is ~40 times more stable than $Cu^{II}(H_{-3}G_4)^{2-}$. Overall, the relative stability of the Aib complexes is determined by a balance between enhanced stability due to the inductive effects and destabilization due to the space requirements of the α-carbon methyl groups.

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Registry No. AAib, 84799-80-4; Aib_2, 39692-70-1; GAibG, 87453-23-4; $AAib_2$, 83917-78-6; Aib_3, 50348-89-5; G_2AibG, 82628-39-5; Aib_3G, 87453-24-5; Aib_4, 50348-91-9; Aiba, 16252-90-7; AAiba, 87453-25-6; Aib_2a, 87453-26-7; Aib_3a, 82628-40-8.

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Use of a Metal-Containing Plasma Polymer Coating To Prepare a Prussian Blue Surface Modified Electrode

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Graphite electrodes are surface modified by coating them in a glow-discharge plasma chamber with iron pentacarbonyl (the resulting deposit is called an iron-containing plasma deposit) or iron pentacarbonyl and ethane in a 1:1 molar ratio (the resulting deposit is called an iron-containing plasma polymer). Both modified electrodes have surface bound redox-active iron centers that are characterized by cyclic voltammetry. Either of these surface modified electrodes may be further modified by electrochemical reaction with hexacyanoferrate to form surface adherent Berlin green, Prussian blue, and Everitt's salt, which may be interchanged reversibly by changing the potential of the electrode. Well-defined cyclic voltammograms

$$\underset{\text{Berlin green}}{[MFe^{3+}Fe^{II}(CN)_6]_{1/2}[Fe^{3+}Fe^{III}(CN)_6]_{1/2}} \xleftarrow{-1/2e^-,\ -1/2M^+} \underset{\text{Prussian blue}}{MFe^{3+}Fe^{II}(CN)_6} \xrightarrow{e^-,\ M^+} \underset{\text{Everitt's salt}}{M_2Fe^{2+}Fe^{II}(CN)_6}$$

$$M^+ = K^+, Na^+$$

are obtained in neutral aqueous solution by using the Prussian blue surface modified working electrode. Surface adherence of the Prussian blue persists over several thousand cycles. Concentrations of surface adherent redox-active Prussian blue increase to as high as 2×10^{-7} mol/cm^2 with increased nominal coatings of iron-containing plasma polymer or iron-containing plasma deposit on the graphite electrode surface. The Prussian blue modified electrode is permeable to both K^+ and Na^+ ions, and the kinetics of the oxidation–reduction processes at the electrode surface are controlled by diffusion of the electrolyte cation in and out of the lattice. Experiments with mixed electrolytes in aqueous solution demonstrate a cation preference in the order $K^+ > Na^+ >> Li^+$.

Introduction

Research into modified electrode surfaces, which began only a decade ago, has developed into an active area of current interest.[2] Various techniques have been utilized to modify the surface of an electrode; these include dipping, electrodeposition, covalent attachment, and plasma polymerization of

(1) (a) Duke University. (b) Research Triangle Institute.

(2) See, for example, the following review articles: (a) Ryan, M. D.; Wilson, G. S. *Anal. Chem.* **1982**, *54*, 20R. (b) Murray, R. W. *Acc. Chem. Res.* **1980**, *13*, 135. (c) Snell, K. D.; Keenan, A. G. *Chem. Soc. Rev.* **1979**, *8*, 259.

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