ORGANIC LETTERS

2005
Vol. 7, No. 3
403−406

Binding of Amino Acids into a Novel Multiresponsive Ferrocene Receptor Having an Ene Backbone†

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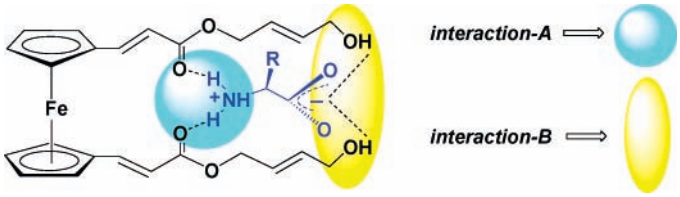
Received November 11, 2004

ABSTRACT



Receptor 1 featuring two open arms, multipoint binding sites, and unsaturated linkers on a ferrocene platform shows strong 1:1 binding to unprotected α-amino acids (UV–vis, fluorescence, CV, ITC, NMR, and ESI-MS). NMR and ESI-MS studies suggest an encapsulative binding mode involving the α,β-unsaturated carbonyl residue (site for $-NH_3^+$, interaction A) and the terminal –OH groups (site for $-COO^-$, interaction B).

Molecular recognition of unprotected α-amino acids is a challenging problem in supramolecular chemistry due to immense biological significance and practical importance of these molecules. The development of artificial receptors for amino acids at physiological pH is slow due to their hydrophilicity and bifunctional character. Effective recognition takes place only when the receptor is ditopic, with multiple binding sites to recognize the $-NH_3^+$ and the $-COO^-$ groups of the unprotected amino acids. In most receptors, crown ethers served as the ammonium ($-NH_3^+$) binding site, while quaternary ammonium, guanidinium, sapphyrin, or metals bind the carboxylate ($-COO^-$) anion.[1]

Though many ferrocene derivatives act as excellent ion-selective receptors,[2] to our knowledge, there is only one example of a ferrocene receptor for amino acids.[3] It involves a ferrocenecarboxylic acid−crown ether conjugate where the crown ether binds to the $-NH_3^+$ cation, while the carboxylic acid executes two-point hydrogen bonding with the $-COO^-$ moiety of the amino acid guest. Our interest in ferrocenes with an ene appendage[4] led us to propose that a receptor based on an open-arm framework, ene spacers, and hydrogen-bonding chromophores will be versatile depending on the number of ene groups and nature of the binding chromophore. Working on this hypothesis, here we report a ferrocene

† This work is dedicated in fond memory of Professor Bhaskar G. Maiya (1956−2004).

‡ Indian Institute of Technology.

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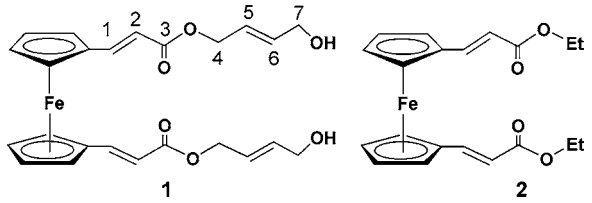
10.1021/ol0476772 CCC: $30.25
Published on Web 01/11/2005

Scheme 1. Receptor **1** and Probe **2** Used in This Study



receptor **1** (Scheme 1) that shows strong 1:1 binding to amino acids which is established through UV, fluorescence, CV, ITC, NMR, and ESI-MS studies. The presence of multiple binding sites in the design motif, fortuitous multiresponsive character of the receptor, and ability of the conjugated π-network to act as a favorable binding site in the recognition event are most noteworthy. Complex **2** with truncated arms, and without the terminal hydroxyl groups of receptor **1**, was used as a probe in binding-site determination studies.[5] The guests include five aliphatic amino acids, propionic acid, and ethylamine. Unless mentioned, all experiments were conducted with host and guests in same solvent mixture of acetonitrile and water in 55:45 v/v ratio at pH 7.2 (hereafter MeCN$-$H$_2$O) or in CD$_3$CN$-$D$_2$O at pD 7.6 (pD = pH +0.4). Under these conditions, Glu and Asp exist in anionic form, Gly and Gln in zwitterionic form, and Lys in cationic form. We could not study aromatic amino acids (e.g., tyrosine) due to precipitation at the experimental pH.

The structural characteristics of host **1** involve the α,β-unsaturated carbonyl residue near the Fc terminus, which was designed to favor a donor interaction from this residue to the $-NH_3^+$ end of the amino acid via $-NH\cdots O$ hydrogen bonding (interaction A). The conjugated ferrocene cap will further enhance such an interaction. In other words, the communication between binding site and the redox subunit will be enhanced. Such stabilization of the ammonium ion by the conjugated π-donor network might be the key to the success of amino acid recognition. Miranda et al. have postulated the importance of aromatic donor groups in promoting π-cation interaction with the ammonium group of L-glutamate, while Maynadie et al. proposed similar interactions between the conjugated carbonyl chromophore of a ferrocenyl receptor and calcium ion.[6] The second design feature invoked by us is to orient two allylic alcohol appendage at the termini of the receptor **1**, for simultaneous interaction with a $-COO^-$ group of amino acid via hydrogen bonding (interaction B). Ogoshi et al. reported such an interaction in metalloporphyrin receptors.[7]

The UV$-$vis spectrum of **1** in MeCN$-$H$_2$O shows two bands at λ_{max} 260 and 310 nm attributed to ene appendage/charge transfer and a broad band of lower intensity at 478 nm due to d$-$d transition. Addition of amino acid leads to a decrease of absorbance along with bathochromic shift of the d$-$d transition peak (Figure 1). A maximum red shift of

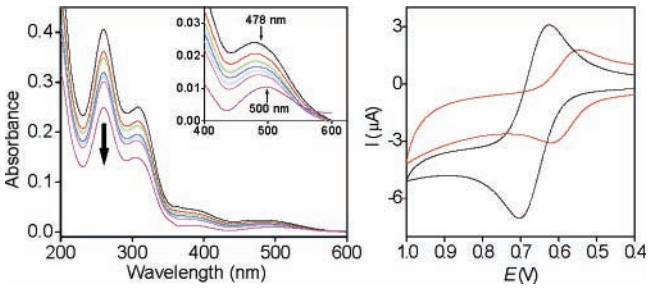


Figure 1. (Left) absorption titration profile of **1** (1×10^{-5} M) before and after addition of Glu (0.2$-$2.0 equiv) at 293 K; (inset) range 400$-$600 nm. (Right) (black line) CV of receptor **1** in MeCN$-$H$_2$O; (red line) after addition of Gly (6 equiv). Scan rate = 50mV/s. Solvent = MeCN$-$H$_2$O in all cases.

22 nm (from 478 to 500 nm) was observed in the case of glutamic acid. Absorption titration at 478 nm provided a linear Benesi$-$Hildebrand plot, indicating 1:1 stoichiometry for **1**$-$amino acid binding, from which the binding constants were determined (K_{red} in Table 1, plots in the Supporting Information).

Table 1. Binding Constants of **1** and $\mathbf{1}^+$ with Different Guests

guest	$K_{red} \times 10^{-3}$ (M^{-1})[a]	$K_{ox} \times 10^{-3}$ (M^{-1})[b]
Asp	93	2280
Glu	98	*c*
Gln	68	1540
Gly	39	890
Lys	21	310
$EtCO_2^-$	43	710
$EtNH_3^+$	7	*d*

[a] From absorption titration, error $<\pm 5\%$ from two runs. [b] From CV using eq 1, error $<\pm 5\%$ from two runs. [c] Not evaluated due to quasireversible nature of CV. [d] Not evaluated as $K_{red} < 10^4$ M^{-1}. Solvent = MeCN$-$H$_2$O in all cases.

Receptor **1** expectedly shows good electrochemical response. CV of a 1.0 mM solution of **1** in MeCN$-$H$_2$O shows a single reversible redox wave (Fe^{2+}/Fe^{3+} $E_{1/2}$ = 664 mV Vs Ag/Ag$^+$, $E_{pa} - E_{pc}$ = 80 mV, Figure 1). Addition of excess amino acid replaces the redox wave of **1** by a new wave due to host$-$guest complexation ($E_{1/2}$ = 566$-$595 mV; $E_{pa} - E_{pc}$ = 73$-$103 mV; i_{pa}/i_{pc} = 1.1$-$1.5; Supporting Information).[8]

According to the EC scheme (Scheme 2), host$-$guest interaction can be rationalized using eq 1 where K_{ox} and K_{red} are the apparent association constants of the guest with the ferricenium and ferrocene form of the receptor, respectively.[9,10] For low K_{red} (<1 M^{-1}), redox titration alone would

(5) Probe **2** was synthesized from 1,1′-bisformylferrocene and ethoxycarbonylmethylenetriphenylphosphorane. Receptor **1** was prepared from **2** and but-2-ene-1,4-diol, using 1-hydroxy-3-(isothiocyanato)tetrabenzyldistannoxane as catalyst (Supporting Information). **1** is soluble in polar organic solvents and in organic$-$aqueous medium.

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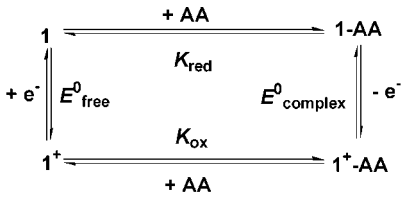
Scheme 2. Square Scheme Relating the Apparent Association Constants between **1** and Amino Acid (**AA**)



$$\Delta E_{1/2} = E_{1/2}^{\text{free}} - E_{1/2}^{\text{complex}} = (RT/nF)\ln(K_{ox}/K_{red}) \quad \text{. . . eqn. 1}$$

provide the binding constant for the oxidized receptor (K_{ox}). When K_{red} is significant ($>10^4$ M^{-1}) and the measurement of $\Delta E_{1/2}$ is reliable, eq 1 may be applied to calculate K_{ox} by incorporating the K_{red} data from other experiment. In cases where $1 < K_{red} < 10^4$, there is no direct method to determine K_{ox} values.[9d] We incorporated the K_{red} data from absorbance studies in eq 1 to calculate the binding constant of $\mathbf{1}^+$ (K_{ox}) for cases where $K_{red} > 10^4$ M^{-1}. While similar methods for determination of K_{ox} values are known in the literature,[10] we suggest that the data should be treated for comparative analyses only. Even though the preliminary data set for K_{ox} and K_{red} values in Table 1 is limited, an inspection shows that the binding constant values for amino acids may be grouped into three classes.[11] They are (a) low K for the cationic amino acid guest (Lys), (b) intermediate K for zwitterionic guests (Gly, Gln), and (c) comparatively higher K for anionic amino acids (Glu, Asp). Lower binding affinities of propionic acid and ethylamine compared to the amino acids indicate multipoint binding of amino acids by receptor **1**.

To further corroborate the recognition of amino acids by **1**, fluorescence studies were done with **1** and glutamic acid as a representative amino acid. In the absence of Glu, when excited at 473 nm, the emission spectrum of **1** in MeCN−H_2O displayed only one band with a maximum at 554 nm. Independently, glutamic acid showed no fluorescence. In the presence of Glu (0.2−2 equiv), the emission intensity of **1** increased along with shift in the maximum from 554 to 550 nm (Figure 2). Thus, compound **1** enlists to the rare examples of ferrocenes[12] having both fluorescence and redox signaling units. Emission titration provided an association constant value $K = 9800$ M^{-1} (error <10%) and a 1:1 stoichiometry. The difference in binding constants obtained from absorbance and emission titration is not surprising. While the former shows binding of the receptor in the ground electronic state, the latter reflects binding in the excited electronic state (**1***).

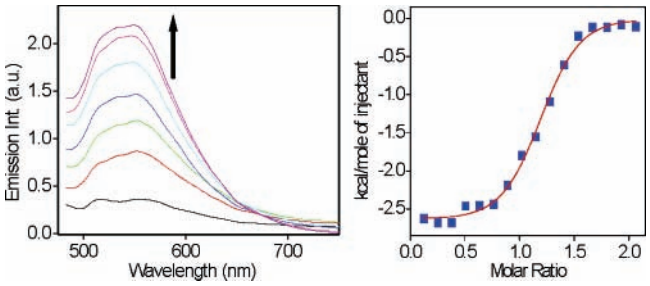


Figure 2. (Left) emission titration profile of **1** (1×10^{-4}) M before and after addition of Glu (0.2−2.0 equiv) at 293 K. Excitation wavelength: 473 nm. (Right) isothermal titration calorimetry for binding of **1** (1 mM) with Glu (41.6 mM) at 303 K. Solvent = MeCN−H_2O in all cases.

Isothermal calorimetric titration (ITC) provided evidence of thermodynamic dominance for the binding of **1** with amino acids.[13] ITC analyses for the complexation of **1** with Glu in MeCN−H_2O revealed 1:1 binding (n = 1.14) with an association constant $K = 43600 \pm 1200$ M^{-1} at 303 K (Figure 2). The complexation is synergistically driven by negative enthalpy change ($\Delta H = -2.7 \pm 0.02$ kcal/mol) and positive entropy change ($\Delta S = 12.3$ cal/(mol K)).

Electrospray mass spectrometry is an excellent probe to investigate molecular association in solution.[14] The ability to generate either a cationic or an anionic species under ES+ or ES− mode attracted us as it offers an opportunity to probe the binding of amino acids through ammonium ($-NH_3^+$) or carboxyl ($-COO^-$) terminus. The study was conducted in MeCN−H_2O (1:1 v/v) with receptor **1** and compound **2** lacking the terminal −OH groups. Guests studied included amino acids (Gly, Glu), propionic acid (a model for $-COO^-$), and ethylamine (a model for $-NH_3^+$). In all cases, the scan range was extended to locate the highest mass ion peak. Major observations from the above studies are highlighted below (Figure 3 and Supporting Information).

1. In ES+ and ES− mode, receptor **1** shows mass ion peaks at 489 $[M + Na]^+$ and 465 $[M - H]^-$ respectively (Figure 3 and Figure S3a,b in the Supporting Information).

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(11) The K_{ox}/K_{red} ratio linearly correlates with $\Delta E_{1/2}$ values (Supporting Information). Note that $\Delta E_{1/2}$ reflects the balance of interactions with the guest between the oxidized and neutral receptor rather than the strength of the interaction between the receptor and the guest. For an analogous argument, see: Ion, I.; Moutet, J.-C.; Popescu, A.; Saint-Aman, E.; Tomazeswski, L.; Gautier-Luneau, I. *J. Electroanal. Chem.* **1997**, *440*, 145.

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(14) Cole, R. B. *Electrospray Ionization Mass Spectrometry-Fundamentals, Instrumentation, And Applications*; VCH: Weinheim, 1997.

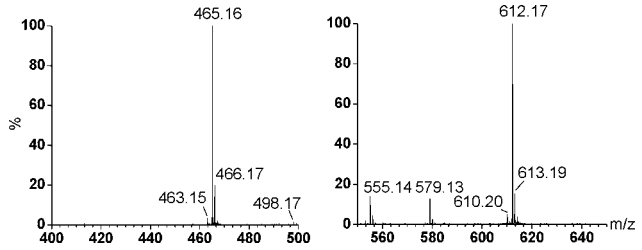

Figure 3. ESI-MS spectra (ES− mode) of (left) free **1** in MeCN−H_2O; (right) a 1:1 mixture of **1** and Glu in MeCN−H_2O.

2. Binding of **1** and amino acid in 1:1 stoichiometry is clearly indicated in both ES+ and ES− modes. In case of Glu, the highest peak in ES− at 612 corresponds to (**1**−Glu−COO^-) (Figure 3 and Figure S3d in the Supporting Information), while in ES+ the peak at 614 represents the complex (**1**−Glu−NH_3^+) (Figure S3e in the Supporting Information). Gly shows similar behavior (Figure S3f,g in the Supporting Information).

3. With propionic acid, receptor **1** shows molecular association only in ES− mode—the highest mass ion peak at 539 corresponds to (**1**−$EtCOO^-$) (Figure S3h in the Supporting Information).

4. In contrast to amino acids and carboxylic acids, complexation with ethylamine is observed in ES+ mode only − the highest mass ion peak at 512 corresponds to (**1**−$EtNH_3^+$) (Figure S3i in the Supporting Information**I**).

5. The above results corroborate the presence of ditopic binding sites in **1** for favorable interaction with $-COO^-$, and $-NH_3^+$ group. To identify the binding sites in receptor **1**, ESI-MS study was extended to all the four guests with compound **2** as probe. Note that **2** possess the α,*β*-unsaturated carboxylate functionality near the Fc-terminus, which is present in receptor **1**, but lacks the side chain extension containing the terminal −OH groups. In the ES+ mode, **2** shows 1:1 complexation with only Gly, Glu, and ethylamine (Figure S3j−l in the Supporting Information), but not with propionic acid. In contrast, in the ES− mode **2** fails to show any complexation with all four guests Gly, Glu, propionic acid, ethylamine. These results clearly identify the α,*β*-unsaturated carbonyl residue in **1** as the binding site for $-NH_3^+$ (interaction A), and the terminal −OH groups as the binding site for $-COO^-$ group (interaction B).

Attempted ^1H NMR titration of a mixture of **1** and Glu presented problems due to the presence of multiple peaks within a narrow spectral range. However, meaningful insight was obtained from ^{13}C NMR titration with **1** and Glu (0−1.25 equiv) (Figure 4 and Figure S2a,b in the Supporting Information). Most pronounced is the downfield chemical shift of carbonyl C3 and olefinic C1 carbons from 167.36 and 145.16 ppm to 168.17 and 145.77 ppm, respectively, which indicates a donor interaction from α,*β*-unsaturated carbonyl residue of **1** to $-NH_3^+$ end of amino acid, and supports our proposal (on interaction A). An additional interaction between the allylic alcohol appendage of **1** and terminal

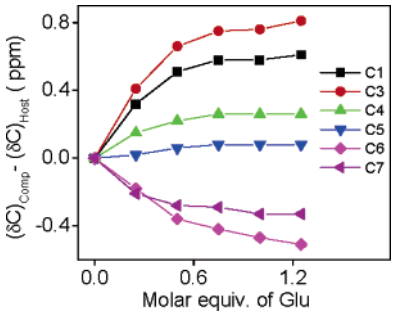

Figure 4. Chemical shift variation of C_1, C_3, C_4, C_5, C_6, and C_7 carbons of **1** (1.6×10^{-1} M) versus the molar equiv of Glu. Solvent CD_3CN-D_2O (pD 7.6). The shifts in other carbons are not significant and, hence, are omitted for clarity.

$-COO^-$ group of amino acid is indicated by the upfield chemical shift of C6 and C7 carbons from 134.69 and 58.57 ppm to 134.18 and 58.24 ppm, respectively, and supports our proposal (on interaction B). The titration curves reach saturation after adding nearly one molar equivalent of Glu, which also augments **1**−amino acid binding in 1:1 stoichiometry (also see Figure S1i,j in the Supporting Information).

Two models can be invoked for **1**−amino acid interaction: end-cap binding or encapsulative binding. In an end-cap binding mode only the terminal hydroxyl groups will interact with the guest. While an encapsulative binding mode will involve both α,*β*-unsaturated carbonyl residue (site for $-NH_3^+$, interaction A), and the terminal −OH groups (site for $-COO^-$ group, interaction B). Results from ESI-MS and NMR studies are consistent with the encapsulative binding mode, however it is too early to comment on the relative strengths of the two interactions.[15]

In conclusion, we note that while the design feature of the open-arm receptor **1** accounts for multipoint recognition of amino acid, further validation of the encapsulative binding mode is warranted. Synthetic studies are also underway to improve the conjugated linker design for better communication and to substitute the −OH groups in the open-arm termini of the receptor with stronger hydrogen-bonding residues, e.g., $-NH_2$, $-CONH_2$, −COOH, and N-heterocycles. We believe the above features will further improve amino acid selectivity. Not surprisingly, our final aim is to cap the arm with chiral spacers to achieve enantioselective recognition of amino acids.

Acknowledgment. P.D. and M.B. thank the Institute and CSIR, respectively, for senior research fellowship.

Supporting Information Available: Experimental procedures including a table, figures, and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Positive value of K_{ox}/K_{red} (see CV, Table S1, Supporting Information), offers an initial suggestion that interaction B is strengthened on going from ferrocene to ferricinium receptor. Note that since the binding sites are far away from the redox site contribution from through-space electrostatic interactions (if any) will be much smaller.