An Unnatural Amino Acid Bearing Bipyridyl Backbone: Selective Formation of *mer*-Isomers for Iron(II) Tris-chelate Complexes

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The *fac*/*mer*-ratios of the iron(II) complexes with the derivatives of an unnatural amino acid, 5′-*N*-acetylamino-2,2′-bipyridine-5-carboxylic acid, were investigated. It was found that the amide derivatives with secondary amines selectively yielded the *mer*-isomers independently of the ligand structures. On the other hand, for the amides with primary amines, the ratios of the *fac*- and *mer*-complexes increased and decreased, respectively, with the increasing sizes of the substituents, suggesting stabilization of the *fac*-isomers by electrostatic interaction (or hydrogen bondings) between the ligands.

The molecular design of functional peptides by utilizing unnatural amino acids and/or incorporating synthetic molecules into the peptides has attracted much attention.[1,2] As a strategy for the *de novo* design of peptides, metal (*e.g.*, iron(II))-assisted stabilization of triple α-helical[3] and collagenous peptides bundles[4] to which bipyridyl groups are attached at the *N*-terminus is known. There are two possible isomers, *fac* and *mer* of the iron(II) tris(bipyridine)-type complexes (Figure 1), and during reactions, the *fac*-isomers are stabilized by interaction between the peptides.

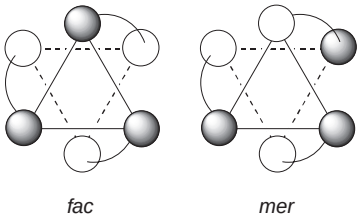
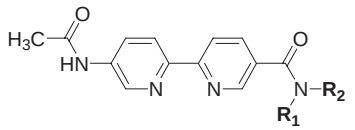


Figure 1. *fac*- and *mer*-Isomers for metal tris-chelate complexes.

We have proposed the molecular design of a peptide into which three residues of an unnatural amino acid, 5′-amino-2,2′-bipyridine-5-carboxylic acid (H-**5Bpy**-OH), are incorporated, leading to a definite folding structure by coordinating with the metal ion.[5] In the molecular design, the central metal complexes, which stabilize the folding structures of the peptides, are *mer*-isomers, and therefore, it is important to control the structure of the octahedral metal complexes. Moreover, the isomer ratios of the *fac*/*mer*-complexes have attracted considerable interest from the viewpoint of supramolecular architectures functionalized with the polypyridine complexes.[6] Recently, Fletcher *et al.* have reported that the *fac*-isomers of the ruthenium(II) tris(2,2′-bipyridine-5-carboxylic acid ester) are favorably formed.[7] The isomer ratios could occur from the thermodynamic difference between the *fac*- and *mer*-complexes based on the differences in their interligand interactions. The complexes of iron(II), which are "substitution-labile," would be good probes for determining the differences in the interligand interactions and thermodynamics between the isomers. In this study, the derivatives of the unnatural amino acid **5Bpy** especially with various amide groups at the *C*-terminus, whereas the acetyl group is unified for protection at the *N*-terminus, were synthesized (Figure 2), and the structural effects of the ligands on the isomer ratios of the iron(II) complexes were investigated.



Ligands	R_1	R_2
Ac-**5Bpy**-NHMe	-H	$-CH_3$
Ac-**5Bpy**-NH^{t}Bu	-H	$-C(CH_3)_3$
Ac-**5Bpy**-NH(^{c}Hex)	-H	H
Ac-**5Bpy**-NMe_2	$-CH_3$	$-CH_3$
Ac-**5Bpy**-N^iPr_2	$-CH(CH_3)_2$	$-CH(CH_3)_2$
Ac-**5Bpy**-N(^{c}Hex)$_2$	H	H

Figure 2. Unnatural amino acid derivatives.

The unnatural amino acid, H-**5Bpy**-OH, was prepared according to a modified procedure[5] reported in the literature.[8] The derivatives were synthesized as follows: the unnatural amino acid was acetylated with acetic anhydride to give acetyl-**5Bpy**-OH. It was treated with $SOCl_2$ to yield acetyl-**5Bpy**-Cl, which was amidated with primary or secondary amines in benzene to produce the derivatives.

The iron(II) tris-chelate complexes were prepared by mixing ammonium iron(II) sulfate hexahydrate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) with the ligands in CH_3OH/H_2O. The solution of iron(II) complexes showed absorption bands at 520–540 nm based on a metal-to-ligand charge transfer (MLCT) in CH_3OH/H_2O (1:1 v/v), and coordination of iron(II) with 3 equiv. of the ligands was confirmed from the Job plots. The maximum wavelengths of the absorption bands for the primary amine derivatives (Ac-**5Bpy**-NHR) were 538–539 nm, which were longer than the ones for the secondary amine derivatives (Ac-**5Bpy**-NR_2): 529 nm for R = Me, 524 nm for R = ^{i}Pr, ^{c}Hex. $[Fe(bpy)_3]^{2+}$ shows the maximum wavelength at 523 nm in the same solution.

The iron(II) complexes exist in the mixtures of the *fac*- and *mer*-isomers in solution. The *fac*/*mer*-ratio is considered to be 25:75 from a simple statistical analysis.[9] The NMR spectra for

the *fac*-complexes should show equivalent signals for the ligands due to C_3-symmetry, while all ligands in the *mer*-isomers should exhibit non-equivalent signals due to the C_1-symmetry. The NMR spectra of the iron(II) complexes in D_2O actually showed that the contents of the *mer*-isomers were greater than the *fac*-ones for all the tested ligands. To more precisely determine the *fac*/*mer*-ratios, HPLC analyses using TOSOH ODS-80Ts as the column and 0.1%-trifluoroacetic acid (TFA)/H_2O/CH_3CN as the eluent were carried out. The peak assignments were also confirmed by comparison with the corresponding ruthenium(II) complexes, which were synthesized and isolated.[2,10] The *fac*/*mer*-ratios for all the ligands are summarized in Table 1. For the derivatives with secondary amines (Ac-**5Bpy**-NR_2), the *mer*-complexes selectively formed (>91%) regardless of the types of amide groups. On the other hand, for the derivatives with primary amines (Ac-**5Bpy**-NHR), the contents of the *mer*- and *fac*-isomers decreased and increased, respectively, with the increasing bulkiness of the amide groups at the *C*-termini.

Table 1. Ratios of *fac*/*mer*-iron(II) complexes with the derivatives of 5′-amino-2,2′-bipyridyl-5-carboxylic acid in CH_3OH/H_2O (1:1 v/v) at 298 K

Ligands	Ratio of isomers	
	mer	*fac*
Ac-**5Bpy**-NHMe	80.7	19.3
Ac-**5Bpy**-NH^{t}Bu	71.5	28.5
Ac-**5Bpy**-NH(^{c}Hex)	68.7	31.3
Ac-**5Bpy**-NMe_2	91.8	8.2
Ac-**5Bpy**-N^iPr_2	93.4	6.6
Ac-**5Bpy**-N(^{c}Hex)$_2$	93.4	6.6

When a bulky group is introduced into an unsymmetrical bidentate ligand at one side, the *fac*-iron(II) tris-chelate complex, in which the bulky substituents gather along the C_3-axis, would be destabilized due to steric hindrance. As such steric hindrance is probably not influenced by solvent effects, we examined the *fac*/*mer*-ratios for $[Fe(Ac\text{-}\mathbf{5Bpy}\text{-}N(^cHex)_2)_3]^{2+}$ in CH_3OH/H_2O with various CH_3OH contents (Figure 3). The *fac*/*mer*-ratios showed a highly selective *mer*-preference, which is not influenced by the CH_3OH contents in solution. Similar phenomena were observed for the other derivatives with secondary amines (Ac-**5Bpy**-NR_2). These results indicate that the ligands Ac-**5Bpy**-NR_2 destabilize the *fac*-isomers of the iron(II) tris-chelate complexes by steric hindrance. On the contrary, for the derivatives with primary amines (Ac-**5Bpy**-NHR), the contents of the *fac*-isomers increased with the increasing bulkiness of the substituents. In the iron(II) tris-chelate complexes, there is either a hydrophobic or electrostatic interaction (including hydrogen bonding) between the inter-ligands in the *fac*- or *mer*-isomers rather than steric hindrance. For a series of iron(II) complexes with Ac-**5Bpy**-NHR, the *fac*-contents increased with the increasing bulkiness of the R groups, and the rates of the *fac*- and *mer*-isomers increased and decreased, respectively, with the increasing CH_3OH contents in solution (see Ac-**5Bpy**-NH(^{c}Hex) in Figure 3). These results suggest that an electrostatic interaction (or hydrogen bonding) exists between the amide groups in the ligands in the *fac*-complexes with Ac-**5Bpy**-NHR, and that the

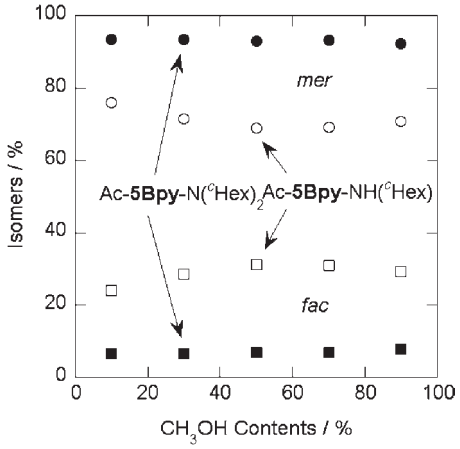


Figure 3. Effects of CH_3OH contents in CH_3OH/H_2O solution on *fac* (square)/*mer* (circle)-isomers for iron(II) complexes with Ac-**5Bpy**-N(^{c}Hex)$_2$ (closed) and Ac-**5Bpy**-NH(^{c}Hex) (open).

fac-isomers are stabilized when the interligand interaction is enhanced by increasing the hydrophobicity of the ligands and/or of the solution.

In conclusion, for the unnatural amino acid derivatives with secondary amines, Ac-**5Bpy**-NR_2, the *mer*-isomers of the iron(II) tris-chelate complexes are selectively formed mainly due to steric hindrance which destabilizes the *fac*-isomers. On the other hand, for the derivatives with the primary amines, Ac-**5Bpy**-NHR, the existence of an electrostatic interaction (or hydrogen bonding) between the amide groups in the *fac*-iron(II) complexes is suggested. This information is valuable for controlling the structures of the octahedral metal complexes with unsymmetrical bidentate ligands, and it would further lead us to design functional peptides with the unnatural amino acid and the supramolecular architectures functionalized with polypyridine complexes.

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