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Lack of Reciprocity of Proton Vicinal Coupling Constants and Chemical Shifts in Ternary Tridentate Dipeptide–Pd(II)-Nucleotide Complexes

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In tridentate dipeptide complexes of Pd(II) such as those with glycyltyrosine, the aromatic side chain is directed toward the metal ion. Binding of purine nucleosides or nucleotides at the fourth tetragonal position about Pd(II) results in a marked upfield chemical shift of the H(2) resonance when Pd(II) is at N(7). On the other hand in complexes with st at N(7). On the other hand in complexes with Pd(II) at N(7) neither the chemical shift of the aromatic tyrosyl protons nor the rotamer distribution about the α–β bond in the side chain is affected. Thus there is a lack or reciprocity evidenced by a marked upfield shift of the nucleic base component and the lack of a significant change in chemical shift or coupling constant of the aromatic amino acid side chain in ternary Pd(II) complexes. The results may be accounted for by edge-on binding of the nucleic base so that it is perpendicular to the face of the aromatic amino acid side chain.

Introduction

Dipeptides form a tridentate chelate about Pd(II) by pH 4 consisting of two 5-membered rings with amine, deprotonated amide, and a carboxylate oxygen donor atom [1]. The fourth position about the tetragonal Pd(II) may react with other ligands to form a ternary complex. In a recent study we investigated the effects of aromatic amino acid side chains in glycyl-amino acid dipeptides upon the chemical shifts of adenine nucleotides coordinated to the fourth Pd(II) position [2]. Compared to the Gly-Ala complex, the presence of an aromatic side chain in the Gly-Phe complex produced upfield shifts of up to 0.4 ppm for H(8) with Pd at N(7) and up to 0.7 ppm for H(2) with Pd at N(1). Unfortunately it was not possible to resolve the α and β hydrogens coupling pattern of the aromatic amino acid side chains in the Pd complexes. In binary dipeptide complexes aromatic and aliphatic side chains adopt a favored position over or at least toward the Pd(II)

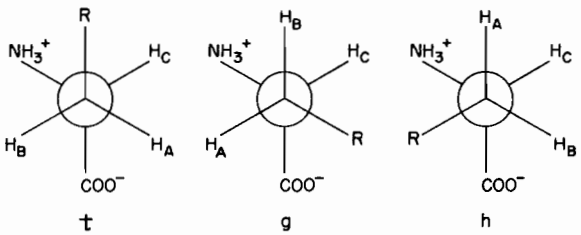

Fig. 1. Three staggered rotamers of α-amino acid with two β-hydrogens H_A and H_B.

[3]. During this study we have been able to resolve the α and β hydrogen couplings in some ternary complexes. Herein we report the effect of coordination of inosine and the 5′-nucleotides IMP and GMP on the disposition of phenylalanine, tyrosine, aspartate, valine, and isoleucine side chains in tridentate dipeptide Pd(II) complexes. The analysis closely follows one previously presented [2].

Experimental

Most experimental details are identical to those already described in a earlier article in this Journal [2]. In some cases excess nucleic base was added to supress binuclear complex formation. Experiments were performed at pH 4–5.

Results

The α and β hydrogens of amino acid side chains yield proton magnetic resonance spectra of 5–12 lines due to a three-spin ABX- or ABC-type system. These spectra are time averaged over three predominant staggered rotamers, designated for the purpose of labeling and expressing mole fractions as t, g, and h, as illustrated in Fig. 1. The bulky carboxylate and R groups are anti (*trans*) in the t rotamer, gauche in the g rotamer, and also gauche in the most hindered h rotamer, where all three substituent groups and all three carbon bound hydrogens are in adjacent posi-

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TABLE I. Vicinal Proton Coupling Constants and Rotamer Distribution in Tridentate Dipeptide Pd(II) Complexes.

	J_{AX}	J_{BX}	%t	%g	%h
Gly-Phe	5.5	3.4	9	28	63
Ino	5.5	3.0	6	28	66
IMP	3.9	3.7	12	14	74
GMP	4.6	3.4	9	20	71
Gly-Tyr	5.5	3.0	6	28	66
Ino	5.7	2.5	1	30	69
IMP	4.6	3.6	11	20	69
GMP	5.0	3.3	8	24	68
Gly-Asp	4.2		33		67
Ino	4.1		31		69
IMP	3.5		20		80
GMP	3.4		18		82
Gly-Val		3.4	9	91	
Ino		3.5	10	90	
IMP		3.3	8	92	
GMP		3.4	9	91	
Gly-Ileu		2.8	4	96	
Ino		3.0	6	94	
IMP		2.9	5	95	
GMP		2.7	3	97	

tions. Of the two β-hydrogens, in the labeling of Fig. 1 proton H_B is usually found at higher field than H_A [3–5]. When the ^1H NMR spectra exhibit a sufficient number of lines, observed vicinal, three-bond, proton spin coupling constants J_{AC} and J_{BC} may be individually determined and related to the mole fractions of each of the three rotamers and to the vicinal parameters J_G and J_T for gauche and anti positions of proton H_C with respect to H_A and H_B.

$$J_{AC} = tJ_G + gJ_T + hJ_G \quad (1)$$

$$J_{BC} = tJ_T + gJ_G + hJ_G \quad (2)$$

For some spectra, such as the aspartate side chains in this research, only the average of J_{AC} and J_{BC} may be determined, and from the previous two equations

$$2J_{av} = J_{AC} + J_{BC} = J_G(1 + h) + J_T(1 - h)$$

From eqn. 1 and 2 and with $t + g + h = 1$, it may be shown that the rotamer mole fractions are given by

$$t = (J_{BC} - J_G)/(J_T - J_G)$$

$$g = (J_{AC} - J_G)/(J_T - J_G)$$

$$h = (J_T + J_G - 2J_{av})/(J_T - J_G)$$

Thus the rotamer mole fractions may be estimated from the observed vicinal coupling constants J_{AC} and J_{BC} if sufficiently reliable values of the J_G and J_T parameters are known. Values of $J_G = 2.4$ Hz and $J_T = 13.3$ Hz have been suggested as especially applicable to amino acid side chains [5]. When only the average coupling constant can be determined, only the rotamer h mole fraction and sum of rotamer t and g mole fractions can be estimated. It should not be assumed that the rotamer t and g mole fractions are equal under these conditions.

As can be seen in Fig. 1, only in rotamer h will the side chain R group at 8 o'clock be directed over a metal ion chelated between the amino and carboxylate groups in the 10 and 6 o'clock positions, respectively.

Valine possesses two methyl substituents on the β-carbon. There is only one α- and one β-hydrogen. Without loss of generality it is convenient to consider hydrogen H_A in Fig. 1 as having undergone substitution by a methyl group. Then the coupling constant determined experimentally is J_{BC} and the population of rotamer t is given by

$$t = (J_{BC} - J_G)/(J_T - J_G)$$

Only the sum of rotamers g and h may be determined from $g + h = 1 - t$. For valine, rotamer t possesses anti α- and β-hydrogens while in rotamers g and h these two hydrogens are gauche to one another. Similarly for isoleucine with an ethyl R group in Fig. 1, a methyl group is viewed as replacing hydrogen H_A in Fig. 1. Analysis of the coupling constants and rotamer populations about the valine and isoleucine side chains is identical. Though the sum of rotamers g and h for valine and isoleucine cannot be resolved, it is of no consequence because they both dispose a hydrophobic side chain toward the metal ion in the tridentate dipeptide complex. Only in rotamer t is a hydrogen disposed toward the metal ion.

Table I tabulates the vicinal proton coupling constants and rotamer mole fractions of the amino acid side chains in the tridentate dipeptide Pd(II) complexes. For each listed dipeptide the first entry refers to the binary complex with water in the fourth tetragonal position. The three subsequent entries refer to the M_7BH_1 complexes of inosine, 5′-IMP, and 5′-GMP where the Pd(II) is coordinated at N(7) and N(1) is protonated [6].

For binary complexes of Pd(II) and the first three dipeptides of Table I, the mole percentage of rotamer h is 63–67%. Thus in the tridentate dipeptide complex about 2/3 of the side chains are disposed toward the tetragonal Pd(II). In the free ligand the percentage of rotamer h is about 20% [3]. Reasons for the increase in the mole percentage

of rotamer h on going from free to tridentate ligand have been discussed [3]. Table I shows that the rotamer mole percentages of the amino acid side chains in the tridentate ligands are almost unaffected by the binding of inosine, IMP, or GMP in the fourth coordination position about Pd(II). The largest effect appears to occur with glycylaspartate where binding of IMP and GMP lowers the coupling constant about 0.7 cps and raises the mole percentage of rotamer h to about 81%. A similar increase has been reported upon binding of (Gly-Asp)Pd(II) to ATP in a binuclear complex [7]. Though different from the free ligands [3], Table I shows that the side chain rotamer distributions in the tridentate complexes of Gly-Val and Gly-Ileu also remain unaffected by the binding of nucleic base in a M_7BH_1 complex.

Chemical shifts of inosine and GMP and IMP move markedly upfield in the presence of an aromatic side chain in the dipeptide. We use as a reference state the chemical shifts of the nucleic base bound to (Gly–Ala)Pd(II). Nucleic base chemical shifts fall into two distinct groups: those that are shifted <0.13 ppm and >0.4 ppm. The relatively unshifted protons include shifts due to all H(8) and H(2) protons in the Gly-Asp and Gly-Val complexes, to H(2) in all M_7BH_1 complexes, and to H(8) in all BM_1 complexes. Pronounced upfield shifts of 0.4 to 0.75 ppm occur in H(8) of M_7BH_1 and M_7BM_1 and in H(2) of M_7BM_1 and BM_1 complexes of Gly-Phe and Gly-Tyr. Similar induced upfield shifts occur in the last two dipeptide complexes with AMP and ATP [2]. Thus the pronounced upfield shifts in the nucleic base proton adjacent to the Pd(II) binding site induced by the aromatic amino acid side chain becomes a general effect in purine nucleosides and nucleotides.

In contrast to the upfield shifts induced in the nucleotide by the aromatic side chain in the ternary complexes, chemical shifts of the aromatic side chain are relatively unaffected by binding of inosine or the two 5′-nucleotides. A detailed study was conducted in the (Gly-Tyr)Pd(II) complex where four peaks due to protons on the aromatic ring are discernible. Upon binding of inosine, IMP, or GMP by N(7) to the fourth coordination position about Pd(II), the shifts in the tyrosyl peaks are all small, the largest being only 0.06 ppm. The same lack of aromatic amino acid shift was observed in similar ternary complexes with AMP and ATP [2].

There is a lack of reciprocity in the mutual effects of aromatic amino acid side chain and nucleoside or nucleotide in the ternary Pd(II) complexes. On the one hand, the aromatic amino acid side chain induces pronounced upfield shifts in H(8) of M_7BH_1 and M_7BM_1 and in H(2) of M_7BM_1 and BM_1 complexes. On the other hand, there is an insignificant chemical shift difference in the aromatic amino acid side chain upon binding of purine nucleosides or nucleotides. Nor is there any significant change in α–β coupling constants of the aromatic side chain upon binding of purine nucleosides or nucleotides. Binding of a purine nucleoside or nucleotide to a terdentate dipeptide Pd(II) complex does not affect the chemical shifts or rotamer distribution of an aromatic amino acid side chain, but chemical shifts of nucleoside or nucleotide protons adjacent to the metal ion binding site undergo a marked upfield shift.

Examination of molecular models reveals that an aromatic amino acid side chain in a tridentate dipeptide complex, even if disposed toward the Pd(II) in rotamer h, is unable to reach far enough to interact with a nucleic base bound *via* N(1) or N(7) in the fourth tetragonal position about Pd(II). Thus the upfield chemical shifts of the nucleic base protons adjacent to the binding site result from through space interactions. All the evidence may be accounted for by edge-on binding of the nucleic base so that it is perpendicular to the face of the aromatic amino acid side chain. The aromatic side chain then affects the nucleic base without being itself affected in turn. A corollary of this conclusion is that the aromatic amino acid side chain is more nearly parallel than perpendicular to the chelate plane.

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