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Association of Terdentate Dipeptide–Pd(II) Complexes with Adenine Nucleotides

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Chemical shifts in proton nmr spectra of the nucleic base portion are assigned upon binding of AMP and ATP via N(7) and N(1) to three different chelated glycyl-amino acid dipeptide complexes of Pd(II) from pH 5 to 8. For AMP the H(8) and H(2) chemical shifts are dependent upon the state of ionization of the unbound phosphate. For both AMP and ATP the molar ratio of N(7)/N(1) bound species is about 1.3 for all (dipeptide)Pd complexes. The binuclear species occurs to only a minor extent in equimolar solutions. Compared to (glycylalanine)Pd upfield shifts occur upon binding of AMP or ATP to (glycylphenylalanine)Pd. These shifts are especially marked for H(8) with Pd at N(7) (up to 0.3 ppm) and for H(2) with Pd at N(1) (up to 0.70 ppm). The results are interpreted as suggesting a nearly perpendicular orientation of the aromatic rings on dipeptide and nucleotide in the Pd complex.

Introduction

Understanding interactions between nucleic acids and proteins and their constituents is essential for analysis of many biological processes. Bringing together elements of the two kinds of polymeric molecules under controlled conditions in aqueous solutions is difficult to achieve. Complexation of metal ions with peptides has been well understood for some time and the binding sites in nucleic bases have recently been elucidated [1]. Properly chosen metal ions may serve as a template for bringing peptides and nucleic bases into a position where they may interact. In particular palladium(II) complexes strongly with both peptides and nucleic bases in well understood ways and the diamagnetism of the metal ion permits direct observation of chemical shifts in ^{1}H nmr spectroscopy.

In this paper we compare the H(2) and H(8) chemical shifts of two adenine-5′-nucleotides, AMP and ATP, in the free ligand, when complexed to the diethylenetriamine complex of Pd^{2+}($dienPd^{2+}$), and when complexed to three dipeptide complexes of Pd^{2+}. The three dipeptide complexes investigated are those of glycyl-L-alanine, glycyl-L-phenylalanine, and glycyl-L-tyrosine. Over the pH range of this investigation the hydroxy group of tyrosine is always protonated and never coordinated.

$PdCl_4^{2-}$ in the presence of an equimolar amount of dipeptide titrates 2 equiv of base by pH 4 corresponding to deprotonation of ammonium and amide hydrogens [2]. The resulting complex consists of a terdentate dipeptide forming two five-membered rings chelated via amine nitrogen, deprotonated amide nitrogen, and carboxylate oxygen donor atoms. The fourth position about the strongly tetragonal Pd^{2+} is occupied by either H_2O or Cl^-. In these complexes side chains adopt a favored conformation in which an aromatic ring faces over the (dipeptide)Pd [3, 4]. Nucleic bases and their derivatives may substitute for H_2O or Cl^- in the fourth tetragonal position via a ring nitrogen donor.

Experimental

Dipeptides and nucleotides of the best quality available from Sigma Chemical Company were used without further purification. The dipeptide complex was prepared by addition of dipeptide to K_2PdCl_4 and two equivs of NaOH. Addition of nucleotide often aided solution of aromatic dipeptide complexes. Complexes were prepared in D_2O at 0.1 *M* for ^{1}H nmr spectra.

^{1}H nmr spectra were recorded on a 90 MHz Varian EM-390 spectrometer at a probe temperature of 34 °C with t-butyl alcohol as internal reference. Assignment of peaks due to H(8) in the purine bases was often aided by exchange with solvent deuterium upon Pd^{2+} binding at N(7) [1]. Recorded pH values are meter readings uncorrected for D_2O solvent. The resulting phosphate pK_a values in D_2O are nearly equal to those in H_2O due to the nearly offsetting effects of D_2O on pH meter readings and decreased acidity of acids [5].

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TABLE I. H(8), H(2) Chemical Shifts in AMP and ATP Complexes.[a]

	AMP		ATP	
	$ROPO_3H^-$	$ROPO_3^{2-}$	$ROPO_3H^-$	$ROPO_3^{2-}$
Free ligand				
B	7.22, 6.95	7.30, 6.95	7.26, 6.97	7.28, 6.97
$dienPd^{2+}$				
BM_1	7.24, 7.35	7.37, 7.35	7.21, 7.35	7.32, 7.36
M_7B	7.76, 7.06	8.02, 7.06	7.92, 7.02	8.00, 7.02
M_7BM_1	7.84, 7.50	8.12, 7.49	7.99, 7.50	8.05, 7.50
(glyala)Pd				
BM_1	7.22, 7.27	7.35, 7.30	7.26, 7.30	7.26, 7.30
M_7B	7.51, 7.00	7.76, 7.01	7.57, 7.02	7.58, 7.02
M_7BM_1	7.57, 7.40	7.81, 7.41	7.61, 7.41	7.63, 7.43
(glyphe)Pd				
BM_1	7.20, 6.77	7.32, 6.88	7.23, 6.88	7.25, 6.88
M_7B	7.28, 6.95	7.63, 6.95	7.43, 6.96	7.47, 6.97
M_7BM_1	7.22, 6.70	7.53, 6.85	7.33, 6.88	7.38, 6.88
(glytyr)Pd				
BM_1	7.17, 6.81	7.30, 6.87	7.21, 6.92	7.26, 6.92
M_7B	7.25, 6.93	7.53, 6.93	7.38, 6.97	7.42, 6.97
M_7BM_1	7.25, 6.77	7.53, 6.82	7.38, 6.88	7.42, 6.88

[a] In ppm downfield from t-butyl alcohol as internal reference.

Results

Chemical shifts of the adenine H(8) and H(2) protons for terdentate dipeptide Pd(II) complexes coordinated at the N(1) or N(7) nitrogens of adenine nucleotides are listed in Table I. Six species with Pd(II) at the nucleotides occur under the conditions of the experiments. Near pH 5 the free ligand nucleotide occurs with a neutral adenine ring system and a monoprotonated phosphate species, $ROPO_3H^-$. By pH 7.5 the phosphate group with pK_a = 6.2–6.5 in the free ligands becomes deprotonated to give the species, $ROPO_3^{2-}$. For both ionic forms of the phosphate residue, the dipeptide bound Pd(II) may bind to adenine nucleotides at N(1), to give a complex represented as BM_1, at N(7), to give M_7B, and at both N(1) and N(7) to give M_7BM_1. Assignments of chemical shifts to the several species are deduced from separate knowledge of the free ligand shifts and of M_7BM_1 shifts, from experiments with excess dipeptide complex, and by experience gained in this and previous studies from this laboratory which have proved consistent over several years [6, 7]. In all cases in this study some binuclear complexing occurred even in equimolar solutions of (dipeptide)Pd + AMP or ATP, but it was never the major species.

Table I lists assignments for complexes contained in solutions of (glycyl-L-alanine)Pd + AMP and provides a comparison with similar complexes with $dienPd^{2+}$ from our earlier study [7]. The results of Table I show that the chemical shifts are dependent upon the state of ionization of the unbound phosphate on the AMP ligand. By following the chemical shift of H(8) in the complex with (glyala)Pd bound at N(7) of AMP (M_7B) in D_2O, the pK_a is ~6.2, close to that found for the free ligand in the same solvent [7]. The ratio of N(7) to N(1) bound (glyala)Pd remains about 1.3 over the entire $5 < pH < 7.5$ range. These results contrast with those for $dienPd^{2+}$ and AMP where a depressed phosphate pK_a of 0.4 log units is coupled with an increased M_7B to BM_1 molar ratio from 0.5 in $ROPO_3H^-$ to 1.2 in $ROPO_3^{2-}$ [7].

In contrast to the complexes of $dienPd^{2+}$ + AMP the N(7)/N(1) mole ratio in complexes with ATP is about 1.3, independent of the state of protonation of the phosphate group. Many features and chemical shift assignments in the (glyala)Pd + ATP solutions are similar to those reported for (glyasp)Pd + ATP [8].

Adenine ring H(8) and H(2) chemical shift assignments for binding of the aromatic ring side chain containing dipeptides glycyl-L-phenylalanine and glycyl-L-tyrosine complexes of Pd(II) to AMP and ATP also appear in Table I. The phenolic group of glytyr is not involved in metal ion binding; results for the glyphe complex are closely similar. The phosphate pK_a in the AMP complexes is little changed from the

free ligand. The concentration of the M_7B complex is consistently 30% greater than that for the BM_1 complex for both AMP and ATP in both phosphate ionic forms. The chemical shift assignments of Table I for (glytyr)Pd + ATP differs from those offered in another study where a N(7) bound mononuclear complex was not identified and two dimeric species were claimed [9]. We show that the N(7) bound mononuclear complex is 30% more common than the N(1) complex and need to identify only one kind of dimeric complex. We do agree that, in solutions at pH > 6 in equimolar solutions and becoming more evident at higher Pd to nucleotide ratios and at higher pH values than reported in this study, a new species with a marked upfield shift appears. This species remains unidentified and is not of major importance under the conditions of this investigation. An apparently similar species occurs in all (dipeptide)Pd complexes of adenine nucleotides. Though the ^1H nmr spectra is complicated by couplings, the aromatic protons of the amino acid side chains in the dipeptide complexes do not undergo major chemical shifts upon binding of adenine nucleotides.

Discussion

For both AMP and ATP and for all three kinds of complexes in both $ROPO_3H^-$ and $ROPO_3^{2-}$ forms, the H(2) chemical shifts for (glyala)Pd are 0.00 to 0.10 ppm less downfield from free ligand than are the shifts for $dienPd^{2+}$ complexes (Table I). For both forms of phosphate ionization the two kinds of complexes with Pd at N(7), M_7B and M_7BM_1, the H(8) chemical shifts of (glyala)Pd complexes are less downfield from free ligand than the same shifts in $dienPd^{2+}$ complexes by 0.25–0.31 ppm in AMP and 0.35–0.42 ppm in ATP. The lesser downfield shifts of AMP and ATP for (glyala)Pd are consistent with its net zero charge compared to a 2+ charge on $dienPd^{2+}$. The H(8) chemical shift with a metal ion at N(7) appears more susceptible to this charge difference than is the H(2) chemical shift with a metal ion at N(1).

For all the dipeptide complexes of Table I, mononuclear species with both N(1) and N(7) coordination of both AMP and ATP occur. In all cases with (dipeptide)Pd the N(7)/N(1) mol ratio is about 1.3. Binuclear complexes appear in equimolar solutions, but are always a minor species <15% of the total ligand. For the mononuclear complexes listed in Table I, only for the $dienPd^{2+}$ complex of AMP in the $ROPO_3H^-$ form is the N(7)/N((1) mol ratio other than about 1.3. In this single case it is 0.5 but becomes 1.24 upon phosphate deprotonation [7].

When the methyl group of the alanine side chain in glycyl-L-alanine is replaced by aromatic phenyl or tyrosyl side chains, pronounced upfield shifts occur in both H(2) and H(8) chemical shifts of AMP and ATP. In all cases the H(2) chemical shift occurs at even higher field in the aromatic peptide complexes than it does in the free ligand. Compared to the (glyala)Pd complexes the H(8) shift of BM_1 and the H(2) shift of M_7B move a relatively unaffected 0.00 to 0.08 ppm upfield in the presence of an aromatic side chain. In contrast other upfield shifts are: H(8) of M_7B and M_7BM_1, 0.11 to 0.35 ppm over both AMP and ATP; H(2) of BM_1, 0.38 to 0.50 ppm; and H(2) of M_7BM_1, 0.53 to 0.70 ppm. There is a tendency for the aromatic induced upfield shifts to be greater in AMP than in ATP.

The preceding comparisons of AMP and ATP chemical shifts of (glyphe)Pd and (glytyr)Pd with respect to (glyala)Pd complexes indicate aromatic amino acid side chain induced upfield shifts which are especially marked for H(2) when Pd binds at N(1). Unfortunately it was not possible to resolve the coupling pattern of the α and β hydrogens of the amino acid chain in the complexes with AMP and ATP. It is known, however, that in simple dipeptide and tripeptide complexes of Pd(II) the side chain conformation favors the rotamer in which the aromatic residue faces over the metal ion [3, 4]. Persistence of this conformation upon binding to dipeptide chelates of Pd(II), adenine nucleotides at either N(1) or N(7) in nearly edge-on positions would account for the observed H(2) and H(8) upfield shifts, respectively. Near edge-on binding by the adenine nucleotides accounts for the lack of a significant shift in protons of the aromatic side chains. Thus the nucleic base and aromatic amino acid side chain are evidently not stacked, but are approximately perpendicular in the mixed complexes.

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