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Coordination of Adenosine and ATP to Pd(II)–Glycyl–L-aspartic Acid Complex

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The Pd(II) dipeptide complex forms the mono- and dimeric species with adenosine and ATP. At higher pH the Pd(II) favours the N1 over N7 coordination in nucleoside and nucleotide molecule. At pH above 10 adenosine is unbound and promotes a "double" hydrolysis of the Pd–dipeptide complex. The ATP forms much more stable complexes than adenosine and even at pH above 10 in 2:1 solution the Pd–N1 species is the dominant one (80% of ATP). The Pd–GLYASPA complex acts as a monodentate species and the coordination of two adjacent nucleotides has not been observed. Also the chelation by nucleoside or nucleotide is a minor form of interaction and it has not been found.

Introduction

There is a considerable interest in the interactions between metal ions and nucleotides or nucleosides because of their possible influence on the reactions of nucleic acids [1, 2]. It has been found that metal ions can stabilize or de-stabilize the structure of DNA or RNA [3], that they play a role in protein synthesis [4] and are thought to be involved in nucleotide transport processes [5]. The interactions of heavy metal ions with nucleic acids are usually harmful but they can be also beneficial as in the case of the platinum antitumor drugs [6, 7]. Palladium(II) ions interact with nucleosides at the same sites as platinum(II), but equilibria in the solutions can be different [8]. We have used the Pd(II) ions to investigate the interaction with adenosine and ATP because they react much more rapidly than the Pt(II) ones and they are more convenient in PMR studies. It was suggested also that due to the high affinity of chloride ion for Pt(II), the Pd(II) complexes may be better models for intracellular action of the corresponding Pt(II) complexes than the Pt(II) complexes themselves [15].

Experimental

K_2PdCl_4, glycyl–L-aspartic acid (GLYASPA) and ATP were used as received from Fluka. Adenosine was also commercially available (Merck) and used without further purification. PMR spectra were recorded on a JEOL 100 MHz JNM-PS-100 spectrometer using t-butanol as an internal standard. All chemical shifts were converted to DSS reference. The spectra were recorded at 25 ± 1 °C. The pH was measured on a Mera-Elmat N-5112 pH-meter. Analysis of the NMR spectra was made on a JEC-6 computer.

The concentration of Pd(II) ions in all measurements was 0.1 *M*. The solutions of 1:1, 2:1 and 1.5:1 Pd(II)–GLYASPA complex:ATP molar ratios were investigated. For adenosine only the 1:1 molar ratio solution was studied for comparison with the ATP one.

Results

The PMR spectra of the base portion of adenosine in D_2O consist of low field two singlets corresponding to H2 and H8 protons (Table I, Fig. 1).

TABLE I. GLYASPA–Pd–adenosine Solutions with 1:1 molar ratio.

Species	Chemical Shift [ppm]		Molar Fractions at pH			
	H2	H8	6.62	7.89	8.58	9.83
Adenosine	8.06	8.21	0.22	0.4	0.75	0.9
Pd–N1	8.56	8.27	0.50	0.4	0.25	0.1
Pd–N7	8.15	8.70	0.23	0.2	–	–
Dimer	8.66	8.79	0.05	–	–	–

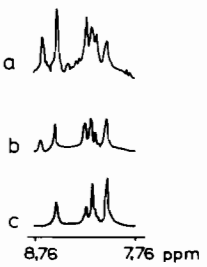

Fig. 1. PMR spectra of the base portion of adenosine in 1:1 molar ratio solutions at pH: 6.62 (a), 7.89 (b) and 8.58 (c).

After the complexation at pH = 6.62 the PMR of the purine base region contains as many as 8 lines. The spectrum depends on the pH; at pH = 9.83 4 lines are observed, and at pH above 11 only 2 lines remain. Peaks appear in pairs for each species present in solution. The metal-free adenosine signals are at 8.06 (H2) and 8.21 ppm (H8). The other pairs in the spectrum have been found by considering the intensity changes with pH and deuteration of H8 protons by heating of samples at 82 °C for 4.5 h.

The greatest intensity signal pair at pH = 6.62 is at 8.56 (H2) and 8.27 ppm (H8). They can be assigned as the species with Pd–N1 bond because of the small changes of the H8 proton chemical shift (–0.06 ppm) and of the considerable variation of H2 proton shift (–0.5 ppm). The signals at 8.15 (H2) and 8.70 ppm (H8) correspond to the Pd–N7 coordination. There are two very weak signals at 8.66 (H2) and 8.79 ppm (H8) of the dimer Pd–N1 + Pd–N7 species. These assignments are in agreement with those of Lim and Martin for the [enPd]–adenosine system [8]. The spectrum of dipeptide ligand at pH = 6.62 consists of two glycine residue singlets at 2.33 and 2.61 ppm and of two aspartic acid residue doublets at 1.63 and 1.75 ppm (β-CH_2 protons). The α-CH proton multiplets are overlapped by the ribose proton signals. This spectrum corresponds to the 1:1 Pd(II) to dipeptide tridentate complex described earlier [9].

The A_2X type of spectrum [10] for the aspartic acid residue of both signals proved that the dipeptide acts as the tridentate ligand and only one coordination site occupied by Cl^- ion can be used to coordinate the adenosine molecule. The signal intensity of metal-free adenosine also increased with the pH increase. At pH = 9.83 only the Pd–N1 complex lines are observed besides the unbound adenosine ones. The Pd–N7 species is less stable than Pd–N1 and its spectrum is not observed already at pH above 8.5.

The dipeptide part of spectrum is changing considerably with the pH increment. The glycine residue protons become of the AB type and the aspartic acid ones of the ABX type. The PMR parameters are very similar to those for 1:2 Pd(II)–dipeptide complex [9]: $\Delta\nu_{AB}$ = 24.5 Hz and J_{AB} = –16.0 Hz with multiplet center at ν_c = 2.16 ppm for glycine residue. For aspartic acid protons: ν_A = 1.32 ppm, ν_B = 1.55 ppm, ν_X = 3.10 ppm and J_{AX} = 10.7 Hz, J_{BX} = 4.5 Hz, J_{AB} = –14.9 Hz. At pH above 11 only such a spectrum is observed. In 2:1 solution containing the Pd(II)–GLYASPA complex and ATP at pH = 5.01 only one pair of base proton signals is observed at 8.72 (H2) and 8.88 ppm (H8). The assignment of signal for H2 and H8 proton has been made by deuteration of H8, as described above. The considerable variation of chemical shifts of both protons compared to metal-free ATP (0.64 ppm for H2 and 0.47 ppm for H8) allows to assign this spectrum to the Pd–N1 + Pd–N7 dimer species with two Pd–dipeptide complexes bound to one ATP molecule. The dipeptide region consists of two glycine singlets at 2.20 and 2.35 ppm with intensity ratio of 2:3. The β-CH_2 aspartic acid proton doublets are at 1.46 (J_{AX} = 4 Hz) and 1.63 ppm (J_{AX} = 3.5 Hz) and the intensity ratio of these two signals is also 2:3.

As was mentioned above in this case the Pd dipeptide complex is tridentate as well and the ATP is bound by substituting the Cl^- ion. Two sets of chemical shifts for dipeptide protons could result from the unequivalent surrounding of this ligand in the ternary complex. The unequal intensity, however, suggests that the difference is not due to the coordination of Pd(II) complex to two different sites in the nucleotide. The same spectrum is observed at pH = 6.17 for the same molar ratio solution. At pH equal 7.82 the nucleic base spectrum consists of six lines and three pairs may be distinguished at 8.25 (H2) and 8.86 ppm (H8), 8.55 (H2) and 8.68 ppm (H8), 8.74 (H2) and 8.90 ppm (H8) (Fig. 2, Table II). The latter

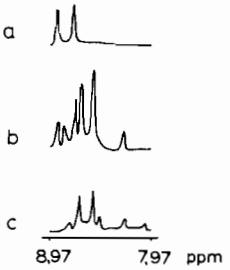

Fig. 2. PMR spectra of the purine base protons of ATP in 2:1 molar ratio solutions at pH: 6.17 (a), 7.82 (b) and 11.1 (c).

TABLE II. GLYASPA–Pd–ATP 2:1 Solutions.

Species	Chemical Shift [ppm]		Molar Fractions at pH			
	H2	H8	5.01	6.17	7.82	11.1
ATP	8.08	8.41	–	–	–	0.1
Pd–N1	8.55	8.68	–	–	0.55	0.8
Pd–N7	8.25	8.86	–	–	0.10	0.1
Dimer	8.72	8.89	1.0	1.0	0.35	–

one belongs to the dimeric species mentioned above. The lines at 8.25 and 8.86 ppm correspond to the Pd–N7 complex and those at 8.55 and 8.68 ppm to the Pd–N1 one. The assignment is made by considering the chemical shift variations for each pair of signals.

It should be noted here that the downfield chemical shift of H8 proton compared to the metal-free ATP is quite large (0.27 ppm) for a Pd–N1 species.

The Pd–N1 complex is the most stable one even at pH above 11. The intensity ratio of this species signal to the metal-free ATP is 6.5:1 at pH = 11.1. The

spectra of adenine protons for some pH are presented in Fig. 2. and Table II.

The GLYASPA part of the spectrum is almost the same in the whole pH range. At pH = 11.1 three singlets are observed for glycine protons and three doublets for β-CH_2 aspartic acid protons, at 2.16, 2.26 and 2.33 ppm, and at 1.47, 1.63 and 1.73 ppm, respectively. The intensity ratios are almost 1:1:1 and there are only traces of the ABX spectrum for aspartic acid protons.

The PMR spectrum of a 1:1 solution (complex to ATP) at pH = 4.35 consists of 6 lines, in the purine region (Fig. 3, Table III). Three pairs can be assigned

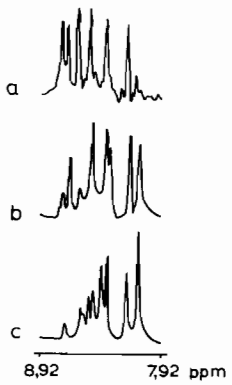

Fig. 3. PMR spectra of the base protons of ATP in 1:1 molar ratio solutions at pH: 5.77 (a), 8.43 (b) and 10.09 (c).

TABLE III. GLYASPA–Pd–ATP 1:1 Solutions.

Species	Chemical Shift [ppm]		Molar Fractions at pH			
	H2	H8	4.34	5.77	8.43	10.09
ATP	8.16	8.46	–	0.06	0.26	0.42
Pd–N1*	8.62	8.50	0.24	0.33	0.36	0.30
Pd–N7	8.24	8.86	0.33	0.28	0.30	0.24
Dimer	8.74	8.92	0.43	0.33	0.08	0.04

as previously. The lines at 8.26 (H2) and 8.85 (H8) correspond to Pd–N7 bonding, those at 8.72 (H2) and 8.90 ppm (H8) to dimeric species and those at 8.62 (H2) and 8.50 ppm (H8) to the Pd–N1 one.

It should be noted here that the chemical shifts of signal pair attributed to Pd–N1 bond are different for both molar ratio solutions 1:1 and 2:1.

At pH above 6 two additional signals of the unbound ATP appear at 8.17 (H2) and 8.48 ppm (H8). They are shifted downfield compared to metal-free ATP solutions, because of the stacking of ATP bases in the presence of its complexes [8]. The concentration of the unbound ATP increases but even at pH above 10 there are considerable amounts of Pd–N1 and Pd–N7 species in solution (Table III).

The dipeptide part of the spectrum is similar as in 2:1 solutions but the intensity of ABX spectrum of aspartic acid residue is higher (about 30% of the whole β-CH_2 proton spectrum at pH = 10.09). The PMR spectra of 1.5:1 solutions at pH = 6.01 and 7.77 (Fig. 4) are analogous to those already presented. In these spectra we can observe both above mentioned signal pairs which could correspond to the Pd–N1 bonding at 8.62 and 8.46 ppm (1:1 solution) and at 8.56 and 8.66 ppm (2:1 solution) simultaneously.

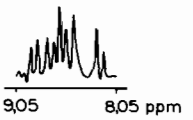

Fig. 4. PMR spectrum of the base protons of ATP in 1.5:1 molar ratio solutions at pH 7.77.

Discussion

In the 1:1 Pd(II)–GLYASPA complex, as was found in our previous work [9], the dipeptide acts as a tridentate ligand at pH 4–13. In the very high pH region (above 11) the Cl^- ion is substituted by OH^- group but the ligand is still tridentate. Breaking of the metal–carboxyl bond leads to the ABX spectrum of aspartic acid protons, which is observed in the 1:2 complex [9]. The reaction of this complex with adenosine and ATP is pH-dependent. In the lower pH region (4–7) in the adenosine containing solutions three modes of coordination were found (Table I). The most stable is the Pd–N1 bonding, existing up to pH =11, and above this pH only unbound adenosine is present in solution. The intensity of the ABX spectrum of aspartic acid protons increases with pH and at pH above 11 the whole spectrum of this residue is of the ABX type. It suggests that adenosine promotes hydrolysis of the Pd–GLYASPA complex with breaking of the metal–carboxyl bond. At pH above 11 in solution there is mainly the unbound adenosine and Pd–GLYASPA$(OH)_2$ hydrolysed complex with bidentate coordination of dipeptide [9]. In the 2:1 solutions containing the ATP and the palladium complex at pH 5 to 7 there exists only one species with one complex molecule bound to N-1 and another one bound to N-7. This dimeric ternary complex is present in solution up to pH about 8.5. Of the monomeric species with Pd–N1 and Pd–N7 coordination observed at pH above 7 the former one is more stable. It is striking to see that such form is the dominant one also at pH above 11 (Table II). There is only a small amount of the unbound ATP at pH = 11.1 (about 11% of ATP present in solution). The dipeptide part of the spectrum shows that almost the whole of this ligand is coordinated as tridentate also in the Pd–dipeptide complex unbound to the ATP, contrary to adenosine solutions.

The Pd–N1 species is also more stable than the dimeric and the Pd–N7 one in the higher pH region in 1:1 solutions. There are, however, two main differences between both kinds of ATP solutions. One of them is a various chemical shift for Pd–N1 species. The differences in chemical shifts are 0.07 ppm (H2) and –0.18 ppm (H8). There is a considerable change on H8 proton. There is rather no bidentate coordination of two adjacent nucleotide molecules by Pd(II) complex because of no change in the dipeptide spectrum (see above). It is, however, possible that after the dimeric species is decomposed (breaking of Pd–N7 bond) in 2:1 solution the remaining Pd dipeptide complex molecule can interact around the N7 atom by a weaker bond, *e.g.* a hydrogen one. In 1:1 solution this possibility is less likely.

In 1:1 solution in the high pH region the amount of unbound ATP is greater than in the 2:1 one (Table II and III) but the concentrations of Pd–N1 and Pd–N7 species are much higher than in the adenosine case.

In 1.5:1 solutions, among the others, both Pd–N1 species are observed as can be seen in Fig. 4 and Table IV. From the shape of aspartic acid residue spectra

TABLE IV. GLYASPA–Pd–ATP 1.5:1 Solutions.

Species	Chemical Shift [ppm]		Molar Fractions at pH	
	H2	H8	6.01	7.77
ATP	8.18	8.46	–	0.1
Pd–N1*	8.62	8.48	0.26	0.32
Pd–N1	8.54	8.68	0.11	0.21
Pd–N7	8.26	8.86	0.18	0.21
Dimer	8.74	8.91	0.45	0.16

(30% of ABX spectrum) it can be concluded that a greater amount of ATP in 1:1 solutions also promotes some double hydrolysis of Pd–dipeptide complex but with much less effect than the adenosine does.

The J_{AX} value for A_2X type of spectrum of aspartic acid residue of a complex bound to ATP (dimeric species) is about 3.5 Hz, indicating that the *gauche* rotamer is even more favourable here than in pure palladium dipeptide complex [9]. Its population exceeds 80%.

Conclusions

From the results presented above it is clear that nucleosides and nucleotides can interact in the same coordination sites but the species formed at various pH are quite different. The ATP forms more stable ternary complexes with Pd–GLYASPA than adenosine does and the equilibria existing in solutions at various pH are different for nucleotide and nucleoside. Thus it seems very important to remember that the simple transfering of the conclusions from metal–nucleoside studies to metal–nucleic acid systems could be unjustified.

The X-ray studies of purine nucleoside and nucleotide complexes with d-electron metal ions [11–14] have suggested that the most common binding site in the solid sate is the N7 one. It might mean that such complexes are easier to crystallize than the metal–N1 ones.

The results obtained here are also important for the Pd–GLYASPA complex. In our previous paper [9] the explanation for the AB spectrum of glycine residue in the 1:2 complex was suggested as a mutual influence of two five-membered rings. In this paper it was shown that also in the 1:1 complex the AB type of spectrum was observed, after the breaking of metal–carboxyl bond and hydrolysis of the complex. It is more likely that the aspartic acid residue which can be treated as a side-chain in the bidentate ligand is the cause of the glycine CH_2 proton unequivalence.

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