PALLADIUM(II) COMPLEXES WITH CYTIDINE-GUANOSINE PAIR IN DMSO

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Solutions containing nucleosides: cytidine and guanosine, and Pd(II) ion in dimethylsulphoxide have been investigated using $^1$H NMR method. It has been found that the glycylglycinate-palladium(II) complex reacts with cytidine through its N$_{13}$ nitrogen atom as the fourth donor for Pd(II) ion, and with guanosine which binds with Pd(II) through N$_{17}$ of purine ring. The primary binding site of cytidine-guanosine pair for the glycylglycinate-Pd(II) complex was N$_{17}$ of guanosine. However, in the reaction of PdCl$_2$ with the cytidine-guanosine pair, equivalent binding sites were N$_{11}$ of guanosine and N$_{13}$ of cytidine giving CydN$_{13}$Pd-N$_{11}$Guo ternary complex.

For many years attempts have been undertaken to explain the mechanism of action of the anticancerogenic drug cis-dichlorodiammine-platinum(II) (CPD) on the molecular level (Rosenberg, 1978). It was found that an electrophilic attack of Pt(II) complex on DNA molecule releases the chloride ion from an inorganic complex (Macquet & Theophanides, 1975a). At the first stage of coordination of the Pt(II) complex with DNA no H$^+$ ions were released from DNA until guanine was completely bound. With an increase of the Pt(II) complex concentration above that of guanine, a release of the H$^+$ ions took place, which was ascribed to deprotonation of N$_{11}$ of guanine (Macquet & Theophanides, 1975b, 1976a). Evidence on chelation of the Pt(II) through N$_{17}$ and O$_{6}$ atoms of guanine has been presented (Macquet & Theophanides, 1975a,b, 1976a,b; Millard et al., 1975). These authors believe that on treatment of a cancer cell with the drug, the described type of coordination is responsible for inactivation of DNA. This conclusion has been confirmed with the use of other methods in vitro (Butour & Macquet, 1977; Macquet & Butour, 1978a,b).

So far investigations on coordination of Pt(II) complexes and their palladium(II) analogues to subunits of nucleic acids have been limited to solutions containing the inorganic complex and one nucleoside or nucleotide.
The coordination sites of the Pd(II) and Pt(II) ions in relation to nucleic bases have been determined by applying mainly the NMR method (Martin & Miriam, 1979).

The aim of the present paper was to investigate coordination of the Pd(II) complexed with glycylglycine$^1$ and PdCl$_2$ alone to each of the nucleosides: cytidine and guanosine separately, and to both of them in an equimolar solution. This last approach was considered particularly important in view of the fact that a complementary cytidine-guanosine pair constitutes a primary target of the electrophilic attack of the platinum drug on DNA molecule. Investigation of DMSO solutions by the NMR technique is doubly justified: (i) in DMSO solutions the interaction between guanine and cytosine resembles more closely the natural conditions existing in a DNA double helix (Katz & Penman, 1966) and (ii) under these conditions it is possible to observe resonance signals arising from exchangeable amine and imine protons of nucleic bases.

MATERIALS AND METHODS

The applied reagents were as follows: cytidine and guanosine from Fluka AG (Buchs, Switzerland), glycylglycine from Reanal (Budapest, Hungary), palladium(II) dichloride from POCh (Gliwice, Poland), deuteriodimethylsulphoxide from IBJ (Świerk, Poland).

The GlyGlyPd(II)(H$_2$O) complex was obtained by crystallization from concentrated aqueous solution of palladium(II) dichloride and glycylglycine (at a 1:1 molar ratio) at pH 8.8. The composition of the complex was determined on the basis of elementary analysis.

The $^1$H NMR spectra (100 MHz) were measured on a JEOL JNM PS 100 spectrometer. Concentrations of solutions were equal to 250 mM of the nucleoside. A chemical shift was referred to the DMSO methyl group signal and converted into a TMS scale using: $\delta_{\text{TMS}} = \delta_{\text{DMSO}} + 2.55$ ppm (Katz & Penman, 1966).

RESULTS

Glycylglycinate-palladium(II) complex

Glycylglycine in the dianionic form is a tridentate ligand in the GlyGlyPd(II) complex. Signals of methylene protons in the $^1$H NMR spectrum for the GlyGlyPd(II) complex are shifted by about 0.14 (CH$_2$ of Gly1) and 0.18 (CH$_2$ of Gly2) ppm downfield in relation to dipeptide in the monoanionic form (spectra recorded for $^2$H$_2$O solutions). The

$^1$ Abbreviations: GlyGly, glycylglycine; GlyGlyPd(II), glycylglycinate-palladium(II) complex; Cyd, cytidine; Guo, guanosine; DMSO, deuteriodimethylsulphoxide.
position of the d-d absorption band (335 nm) indicates that two nitrogen atoms of the dipeptide: amine and amide are coordinated with one Pd(II) ion (Jeżowska-Trzebiatowska et al., 1978, 1980; Kozłowski et al., 1979). From the results shown above one can conclude that donor atoms of dipeptide in the square-planar Pd(II) complex are amine and amide nitrogen atoms, oxygen atom of dipeptide carboxyl group and also oxygen atom of water molecule. The $^1$H NMR spectrum for this complex does not change on addition of cytidine or guanosine.

*Coordination of the glycylglycinate-palladium(II) complex to cytidine and guanosine*

In DMSO solutions containing GlyGlyPd(II)(H$_2$O) and guanosine, the ternary GlyGlyPd-Guo complex is formed, for which the H$_{\text{B}}$ proton signal of guanosine is shifted by about 0.64 ppm downfield (Fig. 1, Table 1). This signal arises from the well-known Pd-N$_{(7)}$Guo type complex (Pneumatikakis et al., 1978; Jeżowska-Trzebiatowska et al., 1980). As far as complexation equilibrium is inert on the NMR scale, hydrogen donor-acceptor equilibrium is labile on this scale. Therefore, one can observe only one set of resonances arising from N$_{(11)}$H and NH$_2$ protons of guanosine, common for free and bound nucleoside. These signals shift gradually downfield with increasing concentration of the GlyGlyPd(II) complex, the complex containing a few proton acceptor groups, e.g. carboxyl and carbonyl groups of dipeptide and H$_2$O molecule (Table 1, Fig. 1a - d).

In DMSO solution containing GlyGlyPd(II) and cytidine one can observe a spectrum of free nucleoside and a typical spectrum of cytidine coordinated through N$_{(3)}$ to the Pd(II) ion (Coletta et al., 1976; Ettore,

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*Table 1*

$^1$H NMR chemical shift values of guanosine for solutions containing the nucleoside and GlyGlyPd(II)

Resonances have been assigned in accordance with Dehand & Jordanov (1976), Kong et al. (1976), Chatterji et al. (1977), and Cini et al. (1977).

<table>
<thead>
<tr>
<th>Solution</th>
<th>Chemical shift (in ppm) of</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N$_{(13)}$H</td>
<td>H$_{\text{B}}$</td>
<td>NH$_2$</td>
<td></td>
</tr>
<tr>
<td>Guo</td>
<td>10.84</td>
<td>8.06</td>
<td>6.55</td>
<td></td>
</tr>
</tbody>
</table>
| GlyGlyPd(II):Guo  | 11.16$^a$   | 8.06$^b$| 6.79$^a$| $^b$
| 1:1               | 8.70$^b$    |        |        |
|                   | 11.26$^a$   | 8.06$^a$| 6.87$^a$| $^b$   |
| 1.5:1             | 8.70$^a$    |        |        |
|                   | 11.33$^a$   | 8.71$^b$| 7.09$^a$| $^b$   |
| 2:1               |             |        |        |

$^a$ Free guanosine.
$^b$ Guanosine coordinated through N$_{(3)}$.
Fig. 1. The 5.5 - 12.0 ppm region of $^1$H NMR spectra for solutions of guanosine and solutions containing guanosine and GlyGlyPd(II) complex.
Fig. 2. The 5.5 - 8.5 ppm region of $^1$H NMR spectra for cytidine and 1:1 GlyGlyPd(II):Cyd solutions.
1978; Jeżowska-Trzebiatowska & Wołowiec, 1982). In the $^1$H NMR spectrum of the GlyGlyPd-N$_{(3)}$ complex the H$_{(6)}$ and H$_{(5)}$ doublets are shifted by about 0.16 and 0.23 ppm downfield (Fig. 2, Table 2), respectively. Coordination of Pd(II) to N$_{(3)}$ and contribution of the NH$_2$ group of Cyd to intermolecular hydrogen bondings with carbonyl and carboxyl groups of the dipeptide affect the chemical shift of cytidine amine group.

Table 2

$^1$H NMR chemical shift values of cytidine for solutions containing cytidine and cytidine and GlyGlyPd(II) complex at a molar ratio of 1:1

Reonances have been assigned in accordance with Dehand & Jordanov (1976), and Cini et al. (1977).

<table>
<thead>
<tr>
<th>Solution</th>
<th>Chemical shift (in ppm) of</th>
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<tbody>
<tr>
<td></td>
<td>H$_{(6)}$</td>
</tr>
<tr>
<td>Cyd</td>
<td>7.85</td>
</tr>
<tr>
<td>GlyGlyPd(II):Cyd, 1:1</td>
<td>7.85$^d$</td>
</tr>
<tr>
<td></td>
<td>8.01$^e$</td>
</tr>
</tbody>
</table>

$^c$ Position of center of doublet, J$_{5,6}$ = 7Hz.
$^d$ Free cytidine.
$^e$ Cytidine coordinated through N$_{(3)}$.

Coordination of the glycylglycinate-palladium(II) complex to equimolar cytidine-guanosine mixture

The $^1$H NMR spectrum of an equimolar cytidine and guanosine mixture in DMSO shows proton signals of amine group and N$_{(1)}$H of guanosine and amine group of cytidine distinctly shifted downfield in relation to the spectra of these nucleosides in separate solutions (Fig. 3a,b). This shift is connected with the formation of hydrogen bonds between the nucleosides in dimethylsulphoxide, which is a weak proton acceptor (Katz & Penman, 1966). The values of shifts in given conditions (temp. 25°C, C(Cyd) = C(Guo) = = 250 mM) connected with formation of hydrogen bonds are: N$_{(1)}$H 0.99 ppm, NH$_2$(Guo) 0.57 ppm and NH$_2$(Cyd) 0.48 ppm downfield.

On addition of the GlyGlyPd(II) complex to the 1:1 Cyd:Guo solution, a new signal of guanosine appears at the first stage (at 8.68 ppm, Fig. 3c, Table 3), which is connected with the coordination of Pd(II) to N$_{(7)}$ of guanosine, whereas the interaction between cytidine and guanosine is only slightly perturbed. With an increase in the concentration of Pd(II) complex a new spectrum of cytidine appears, corresponding to cytidine coordinated via N$_{(3)}$ to the Pd(II) ion (H$_{(6)}$ doublet centered at 8.04 ppm, Table 3). Additionally the signals of amine groups and N$_{(1)}$H of guanosine shift downfield due to increased concentration of proton-acceptor groups.
Fig. 3. The 6.5 - 12.0 ppm region of $^1$H NMR spectra for solutions containing: b, 1:1 Cyd:Guo; c-e GlyGlyPd(II), Cyd and Guo. Spectrum a is superposition of spectra for guanosine and cytidine in DMSO.
Table 3

$^1H$ NMR chemical shift data for cytidine and guanosine for solutions of GlyGlyPd(II):Cyd:Guo

$H_{15}$, Cyd doublets are not separated from ribose signals.

<table>
<thead>
<tr>
<th>Solution GlyGlyPd(II):Cyd:Guo</th>
<th>Chemical shift (in ppm) of</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Cyd</td>
</tr>
<tr>
<td></td>
<td>$H_{(6)}$</td>
</tr>
<tr>
<td>0:1:1</td>
<td>7.90</td>
</tr>
<tr>
<td>1:1:1</td>
<td>7.90$^{f}$</td>
</tr>
<tr>
<td>1.5:1:1</td>
<td>7.90$^{f}$</td>
</tr>
<tr>
<td>2:1:1</td>
<td>8.04$^{e}$</td>
</tr>
<tr>
<td>3:1:1</td>
<td>8.04$^{e}$</td>
</tr>
</tbody>
</table>

$^{a}$ See Table 1.
$^{e}$ See Table 2.
$^{f}$ Signals arising from not coordinated nucleosides.

(Fig. 3c-e, Table 3). A doublet from $H_{(5)}$ proton of cytosine ring is overlapped with ribose $H_{(1)}$ doublets of the nucleoside moiety, and because of this respective regions of the spectra are not presented in Fig. 3.

**Coordination of PdCl$_2$ to cytidine-guanosine pair**

In the spectrum recorded for the 1:2:2 Pd(II):Cyd:Guo solution two sets of cytidine and guanosine resonances appear (Fig. 4b-e, Table 4). The first of them is characteristic for the free cytidine-guanosine pair (designated $f$ in Table 3). The second consists of the cytidine $H_{(6)}$ doublet shifted downfield (centered at 8.01 ppm) and the resonance of cytidine NH$_2$ group shifted highfield and split into a doublet, while the intensity of guanosine $N_{(1)}$H signal is distinctly diminished. The NH$_2$ group signal of guanosine is shifted highfield and split into a doublet similarly as for cytidine. From the observations of chemical shifts of amine protons one can conclude that in the Cyd:Guo pair a weakening of hydrogen bonds takes place. The ratio of spectra intensity of cytidine and guanosine bound with Pd(II) and of free nucleosides is constant which suggests that the nucleosides are bound with Pd(II) to form a ternary complex, in which the Pd(II) ion replaces $N_{(1)}$H proton of guanosine in the complementary Cyd:Guo pair. An increase of the Pd(II) ion concentration causes disappearance of $N_{(1)}$H proton signal with simultaneous formation of the new guanosine $H_{(8)}$ signal at 8.65 ppm. The position of the latter indicates that additional coordination of Pd(II) to $N_{(7)}$ of guanosine occurs (Fig. 4c-e, Table 4). As a result of coordination of three nitrogen donors of cytidine
Fig. 4. The 6.5 - 12.0 ppm region of $^1$H NMR spectra for solutions containing: a, 1:1 Cyd-Guo; b-e, PdCl$_2$, Cyd and Guo.
Table 4

$^1H$ NMR chemical shift data of cytidine and guanosine for solutions containing PdCl$_2$, cytidine and guanosine

H$_{(6)}$, Cyd doublets are not separated from ribose signals.

<table>
<thead>
<tr>
<th>Solution PdCl$_2$:Cyd:Guo</th>
<th>Chemical shift (in ppm) of Cyd</th>
<th>Guo</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>H$_{(6)}$</td>
<td>NH$_2$</td>
</tr>
<tr>
<td>0:1:1</td>
<td>7.90</td>
<td>7.63</td>
</tr>
<tr>
<td>1:2:2</td>
<td>7.90$^f$</td>
<td>7.63$^f$</td>
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<tr>
<td></td>
<td>8.01$^g$</td>
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<td></td>
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<td>7.46$^g$</td>
</tr>
</tbody>
</table>

$^b$ See Table 1.
$^c$ See Table 2.
$^f$ See Table 3.
$^g$ Signals arising from nucleosides bound in the CydN$_{(3)}$-Pd-N$_{(1)}$Guo ternary complex.

and guanosine, i.e. N$_{(3)}$Cyd, and N$_{(1)}$ and N$_{(7)}$ of Guo, in solution at the high Pd(II) concentration multinuclear complexes may form, e.g. CydN$_{(3)}$-Pd-N$_{(1)}$GuoN$_{(7)}$Pd-N$_{(7)}$GuoN$_{(1)}$Pd-N$_{(3)}$Cyd (4:3 nucleoside:Pd(II) molar ratio) and CydN$_{(3)}$-Pd-N$_{(1)}$GuoN$_{(7)}$Pd (1:1 nucleoside:Pd(II) molar ratio). Splitting of amine group proton signals of cytidine and guanosine can result from magnetic inequivalence caused by: (i) restricted rotation in the C-NH$_2$ bonds resulting from NH$_2$--Pd interaction (this type of interaction has been found in the solid dichlorobis(1-methylcytosine)palladium(II) complex — Sinn et al., 1977) or (ii) magnetic field gradient deriving from neighbouring heteroaromatic ligand ring current. In this case, however, specific plane arrangement of coordinated bases, different from coplanar and perpendicular, is necessary.

In the light of the presented data it is obvious that coordination of the Pd(II) ion in the form of PdCl$_2$ salt to cytidine and guanosine in their 1:1 solution takes a quite different path than it was observed in the case of the Pd(II) ion with its three metal coordination sites occupied by the GlyGly dipeptide. Addition of PdCl$_2$ to the 1:1 Cyd:Guo solution led to simultaneous coordination of Pd(II) to N$_{(3)}$ of cytidine and N$_{(1)}$ of guanosine, accompanied by a release of proton from the purine ring.
DISCUSSION

It is thought that the Hg(II) ions intercrosslink DNA, binding mainly to AT rich regions, which causes a red shift of the absorption band and a decrease and a red shift of Cotton effect in the ORD spectrum of DNA (Yamane & Davidson, 1961; Cheng, 1965). Similar changes in UV, ORD and CD spectra are caused by coordination of Pd(II) ion to DNA (Pillai & Nandi, 1977). It is known that Pt(II) forms more stable complexes with guanosine, cytidine and adenosine than with thymidine (Scovell & O'Connor, 1977; Mansy et al., 1978) and binds mainly within GC rich regions of DNA, and this suggests that Pt(II) and probably Pd(II) may intercrosslink DNA. The dienPt(II) and cis-(NH₃)₂Pt(II) cannot coordinate in such a manner. Nevertheless, Macquet & Butot (1978a,b) observed a distinct shortening of polynucleotide chain of nicked circular PM2 DNA on binding of both trans- and cis-(NH₃)₂Pt(II). Our results suggest that, in the case of the Pd(II) ion, intercrosslinking is possible if an inorganic complex possesses two trans-labile groups (PdCl₂ in DMSO solution). Otherwise, this type of coordination is obviously excluded, and the N(7) of guanine is mainly involved as a binding site. Recently Lippert (1981) stated that binding of plasma component X to the cis-Pt(NH₃)₂Cl₂ giving the Pt(NH₃)₂ClX can promote a release of NH₃ from platinum complex. Therefore, it is not possible to exclude that Pt(NH₃)(H₂O)Clₓ complexes are the reactive forms of a platinum drug in vitro. From the results presented here one can conclude that intercrosslink involving N(1) of guanosine and N(3) of cytidine sites could be, in addition to N(7) of guanine, the probable Pt(II) binding site of DNA.

REFERENCES


KOMPLEKSY PALLADU(II) Z PARĄ CYTYDYNA-GUANOZYNA W DMSO

Streszczenie

Metodą protonowego rezonansu magnetycznego zbadano roztwory zawierające cytydynę i guanozynę oraz jon palladu(II) w sulfotlenku dwumetylu. Stwierdzono, że kompleks glicylo-glicynianu z palladem(II) koordynuje do N(3) cytydyny oraz N(7) guanozyny. Jednak w roztworze zawierającym chlorek palladu(II) oraz równomolową mieszaninę cytydyny i guanozy ny donorami nukleozydów są N(1) guanozyny i N(3) cytydyny.

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