FTIR Study of the Secondary Structure of DNA in Complexes with Platinum Coordination Compounds

E Tymchenko¹, V Glovà¹, A Soldatova¹, E Chikhirzhina², A Polyanichko¹,²

¹ Faculty of Physics, Saint-Petersburg State University. 7-9 Universitetskaya nab. St. Petersburg, Russia 199034
² Institute of Cytology of the RAS. 4 Tikhoretsky ave., St. Petersburg, Russia 194064

E-mail: e.tymchenko@yandex.ru

Abstract. Mechanisms of interaction between antitumor compounds and biological molecules have been being studied for decades. One of the most widely applicable drug is dichlorodiammineplatinum(II) (DDP). In the present work we have analyzed effect of cis- and trans-DDP on secondary structure of DNA, bovine serum albums and HMGB proteins using FTIR spectroscopy. We have shown that both of the DDP isomers facilitate the formation of albumin dimers. We have also shown that DDP facilitates changes in DNA structure, attributed to DDP binding to DNA bases and DNA cross-linking. Thus, the application of hidden peak analysis of FTIR spectra shown to be informative to structural investigation of DDP complexes with nucleic acids and proteins.

1. Introduction
Cis-dichlorodiammineplatinum(II) (cis-DDP) also known as cisplatin – one of the best known antitumor drugs [1]. It is well established, that the main biological target of cis-DDP is DNA, and the structure of the complexes being formed determines its biological activity [2]–[4]. Nowadays, many successful antitumor compounds are derivatives of cisplatin [5]. It is also worth mentioning that trans-DDP is biologically inactive despite of its ability to interact with DNA [6]. We used FTIR-spectroscopy to examine the secondary structure of DDP-DNA complexes.

2. Materials and methods
Calf thymus DNA (Sigma) was used with further sonication as described elsewhere [7]. All aqueous solutions were prepared using purified water (Milli-Pore). For IR experiments heavy water (Astrachem, 99.9% D₂O) was used. The concentration of DNA in solutions and nativity of DNA were monitored spectrophotometrically [8]. cis-DDP and trans-DDP were purchased from Sigma-Aldrich.

DDP-DNA complexes were prepared by direct mixing of appropriate volumes of the DNA and DDP/NaCl solutions and incubated for 48 hours at 37°C. Then we dialyzed solutions against H₂O to get rid of unbound DDP. Complete deuteration was achieved by lyophilizing and redissolving all solutions two times in D₂O. The final concentration of DNA in the sample was 33 mg/ml ([P] = 0.107 M). DDP concentration was varied between 0 and 10.7 mM (0–0.1 [Pt]/[P]).

For FTIR measurements DNA samples were placed in a demountable cell composed of two BaF₂ windows separated by a 50 mkm Teflon spacer. The mid-IR absorption spectra were registered using Tensor 27 (Bruker) spectrometer equipped with MCT-detector. To obtain absorption the air spectrum was registered as background before each measurements series. The spectra obtained are the average of
264 accumulations at the 2 cm⁻¹ resolution. Absorption of solvent was registered as individual spectrum and was subtracted manually. Data preprocessing was performed using OPUS (Bruker) software provided with the spectrometer.

3. Results and discussion
We have used IR spectroscopy to examine the secondary structure of the DDP-DNA complexes. IR absorption spectra \( A = -\log \frac{I}{I_0} \) are presented in Figure 1.

![Figure 1. Spectra of cis-DDP-DNA (left) and trans-DDP-DNA (right) complexes in D₂O solutions at various molar ratios \( r = [\text{Pt}] / [\text{b.p.}] \).](image)

As the DDP concentration increases, the intensity of the absorption spectra generally significantly increased. All spectra of DNA and DDP-DNA complexes have 970, 896 and 837 cm⁻¹ bands corresponding to the B-form of DNA [9], [10].

The 1750 – 1550 cm⁻¹ region consists of strong bands arising from carbonyl stretching modes of DNA bases. The spectrum in this region is a superposition of these modes. Some bands may be at frequencies close to the frequencies of neighboring peaks. Such peaks can be covert or hidden. To analyze the corresponding vibrations in more details, we performed spectra decomposition using the second derivative approach (Figure 2), as it was described earlier [11]–[14]. The suggested assignment of the individual vibrations for the decomposed bands of DNA are summarized in Table 1 [9], [10], [15].

For cis-DDP-DNA complexes, the shift of the 1673 cm⁻¹ band (may be corresponding to C6=O Guanine, [10]) was observed. With increasing the [DDP]/[DNA] molar ratio \( r \) it shifts from 1673 cm⁻¹ (\( r=0 \)) to 1675 cm⁻¹ (\( r=0.02 \)) and then back to 1671 cm⁻¹ (\( r=0.1 \)). For trans-DDP-DNA complexes there was the stronger shift to 1675 cm⁻¹ with increasing the DDP/DNA molar ratio to \( r = 0.05 \) and then shift back to 1674 at \( r = 0.1 \). These shifts can testify to direct interaction of DDP with the corresponding groups in structure of DNA. The relative contribution of this band to the overall absorption also changed with increasing the DDP concentration (Table 2): it decreased with DDP/DNA molar ratio \( r = 0.05 \) and then grew a little for cis-DDP, and declined meanwhile the trans-DDP concentration grew. There were no other bands significant shifts in the 1750 – 1550 cm⁻¹ for these DDP/DNA ratios set observed. However, the intensity of 1697 cm⁻¹ band reduced by \( r = 0.02 \) and then grew back for cis-DDP-DNA. For trans isomer there was a growth by \( r = 0.05 \) and then reduction of this band intensity. For 1578 cm⁻¹ band the pattern of change was alike. The 1661 cm⁻¹ band absorption increased by \( r = 0.05 \) and then decreased (but still bigger than for naked DNA) for cis-DDP, and decreased by \( r = 0.05 \) and then grew (bigger than for DNA) for trans-DDP. The 1644 cm⁻¹ peak had been reducing with cis-DDP...
concentration growth and had a significant reduction for trans-DDP-DNA complexes in the $r = 0.02$, then grew back. There was a growth of 1622 cm$^{-1}$ peak for both DDP isomers with some setback at $r = 0.05$ for cis-DDP observed.

Table 1. The assignments of individual peaks of DNA spectra [9], [10], [15]

| Abs.$\text{max.}$ cm$^{-1}$ | Bond’s vibrations DNA bases | Abs.$\text{max.}$ cm$^{-1}$ | Bond’s vibrations Phosphates and sugars |
|-----------------------------|-----------------------------|-----------------------------|---------------------------------------|
| 1696                        | C2=C of Thymine             | 1089 Group PO$_2$           | (symmetrical)                         |
| 1675                        | C6=O of Guanine;            | 1055 C-O of deoxyribose     |                                       |
| 1661                        | C4=O of Thymine             | 1023 Deoxyribose ring       |                                       |
| 1643                        | Thymine ring                | 937 Deoxyribose ring        | B-form marker                         |
| 1621                        | Adenine ring                | 896 Deoxyribose ring        | B-form marker                         |
| 1575                        | C = N(H2) of Guanine        |                             |                                       |
| 1563                        | C = N7 of Guanine           |                             |                                       |
|                             |                             | 1023 for all concentrations of cis-DDP and to 1016 cm$^{-1}$ for all concentrations of trans-DDP observed. For normalized spectra of cis-DDP-DNA complexes there was a slight reduction of 1087 cm$^{-1}$ band (arises from the symmetric vibration of the O=PO=O group, [10]) while DDP concentration raised and a growth of 1023 cm$^{-1}$ band at the $r = 0.1$. For normalized spectra of trans-DDP-DNA complexes there was a slight reduction or 937 cm$^{-1}$ band by increasing DDP concentration. The 1087 cm$^{-1}$ band increased at $r = 0.02$ and then reduced at $r = 0.1$. Thus, it can be
assumed, that the DNA structure generally remains when interacting with the DDP. At the same time, we can assume some minor changes in the helix backbone.

Based on the above data we can conclude that the DDP binding to DNA, affect the vibrations of the sugar-phosphate backbone, that may indicate a change in their mutual arrangement, but there is no damage of DNA double helical structure. This data correlates well with the existing model of formation of DNA cross-linking due to cis-DDP interaction [4], [6].

Table 2. the relative contribution of bands to the total absorption in 1750 – 1550 cm⁻¹ region in %

| peak  | DNA | Cis-DDP-DNA | Trans-DDP-DNA |
|-------|-----|-------------|---------------|
|       |     | r = 0.02    | r = 0.05      | r = 0.01      | r = 0.02    | r = 0.05    | r = 0.01    |
| 1718  | 3.7 | 3.5         | 3.4           | 3.6           | 3.8         | 3.6         | 3.5         |
| 1697  | 14.8| 12.0        | 14.1          | 14.6          | 14.8        | 15.5        | 12.2        |
| 1673  | 28.0| 27.2        | 24.2          | 25.4          | 27.0        | 26.8        | 25.5        |
| 1661  | 4.1 | 5.7         | 7.8           | 6.8           | 4.3         | 3.5         | 7.8         |
| 1644  | 19.4| 18.7        | 18.6          | 16.6          | 17.8        | 19.2        | 19.1        |
| 1622  | 14.0| 16.1        | 15.5          | 16.9          | 14.9        | 15.1        | 15.8        |
| 1601  | 2.1 | 2.7         | 2.5           | 2.7           | 2.8         | 2.5         | 2.3         |
| 1578  | 9.2 | 10.4        | 9.9           | 9.6           | 9.8         | 9.3         | 9.3         |
| 1563  | 4.8 | 3.8         | 4.1           | 3.7           | 4.8         | 4.6         | 4.3         |

4. Conclusions
We have shown that both of the DDP isomers facilitate the formation of albumin dimers. We have also shown that DDP facilitates changes in DNA structure, attributed to DDP binding to DNA bases and DNA cross-linking. Thus, the application of hidden peak analysis of FTIR spectra shown to be informative to structural investigation of DDP complexes with nucleic acids and proteins.

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References
[1] Wong E and Giandomenico C 1999 Chem. Rev. 99 pp 2451–2466
[2] Mansy S, Rosenberg B and Thomson A 1973 J. Am. Chem. Soc. 95 pp 1633–40
[3] Roos I, Thomson A and Mansy S 1974 J. Am. Chem. Soc. 96 pp 6484–6491
[4] Jamieson E and Lippard S 1999 Chem. Rev. 99 pp 2467–2498
[5] Kelland L 2007 Nat. Rev. Cancer 7 pp 573–584
[6] Sherman S and Lippard S 1987 Chem. Rev. 87 pp 1153–1181
[7] Polyanchiko A 2004 Nucleic Acids Res. 32 pp 989–996
[8] Spirin A 1958 Биохимия 23 pp 656–662
[9] Taboury J, Liquier J and Taillandier E 2003 Can. J. Chem. 63 pp 1904–1909
[10] Banyay M, Sarkar M and Gräslund A 2003 Biophys. Chem. 104 pp 477–488
[11] Al-Aidarosis A, Satheesh S and Devassy R 2016 Thalass. An Int. J. Mar. Sci. 32 pp 37–42
[12] Belaya I, Chikhirzhina E and Polyanchiko A 2017 J. Mol. Struct. 1140 pp 148–153
[13] Polyanchiko A et al 2014 Cell tissue biol. 8 pp 352–358
[14] Plotnikova L et al 2019 Cell tissue biol. 13 pp 130–135
[15] Taniguchi H and Saito M 2009 J. Phys. Condens. Matter 21 064242