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Capillary electrophoretic study of cisplatin interaction with nucleoside monophosphates, di- and trinucleotides

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Abstract

cis-Diamminedichloroplatinum(II) (cisplatin) is applied against different kinds of cancer although toxic side effects are known. Screening systems for alternative compounds with higher effectiveness but minimizing toxic side effects are required. We investigated the adduct formation of cisplatin with nucleoside monophosphates, di- and trinucleotides. Capillary electrophoretic separations were performed in a sodium phosphate buffer using an instrument equipped with a diode array detector. Adduct formation results in a significant shift of λ_{max} to lower energy compared to free nucleotides. Therefore, UV spectra are an important tool for peak identification. We could separate and identify all four common nucleotides and their major platinum adducts in a single run demonstrating the suitability of CE for these kinds of investigations. Furthermore, kinetic studies of these reactions are performed.

Keywords: Kinetic studies; Pharmaceutical analysis; Cisplatin; Nucleoside monophosphates; Nucleotides

1. Introduction

Cisplatin, *cis*-[$Pt(NH_3)_2Cl_2$], is a frequently used anticancer drug applied in cases of various malignancies [1], whereas best results are obtained in the treatment of testicular and ovarian cancer [2]. The proposed mechanism of action is based on coordinative interaction of cisplatin with nucleotides modifying the DNA structure and, therefore, its functionality. Although DNA has many components with lone pairs of electrons where metal ions might bind (phosphate groups, the sugar oxygen atoms and the heterocyclic nucleobases), studies have shown that cisplatin preferentially binds to nitrogen atoms of the nucleobases [2–4]. All four bases have such potential nitrogen binding sites, but cisplatin prefers the bases guanine and adenine [5,6]. Platinum binding occurs at guanine-N7 and adenine-N7, but also to a lower extent at cytidine-N3 and adenine-N1 [7]. Detailed studies have shown that guanine N7 is kinetically preferred. Nuclear magnetic resonance (NMR) techniques as well as various separation techniques like high-performance liquid chromatography (HPLC), fast protein liquid chromatography (FPLC) and flat bed electrophoresis contributed to a better understanding of involved coordinative sites [8–11]. The binding of cisplatin is based on a two-step mechanism with formation of monofunctional adducts predominantly at guanine as the first step, which is controlled by the rate of cisplatin hydrolysis [5,12–14]. The second step comprises the binding to another base resulting in bifunctional adducts. Main binding modes in vivo are (i) intrastrand cross-

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linking due to chelation to an adjacent or a next neighboring guanine-N7 as well as adenine-N7 or -N1 and cytosine-N3 in the same strand of double-helical DNA and (ii) interstrand cross-linking due to the chelation to another base in the opposite strand. The predominant lesion is the intrastrand chelation involving two adjacent guanines [5,15,16]. As a result of adduct formation the DNA bends and, subsequently its replication is inhibited [17,18]. Binding of cisplatin to RNA and to proteins has also been described [19–21].

Although toxic side effects are known [2,22] cisplatin became one of the leading anticancer drugs worldwide. Searching for alternative compounds with similar or even better effects but being less toxic resulted in competitive research. Selective, easy-to-handle and versatile methods are therefore required which allow a high throughput of samples but still providing reliable and meaningful data about the interaction with DNA.

Sharma et al. [23] published a CE method applying a laser-induced fluorescence detector for assaying DNA damage after exposition to cis- and carboplatin. The sensitivity of the method detecting one adduct within 10^4 nucleotides has not been reached before. Unfortunately, this method is an indirect one as samples have to be labeled with a fluorescence marker prior to analysis. Recently, HPLC and capillary electrophoresis (CE) have been compared for the separation and identification of DNA adducts with cisplatin and CE was considered as an efficient analytical tool [24].

We studied the adduct formation of nucleoside monophosphates, dinucleotides d(GpG) and trinucleotides d(GpTpT) and d(GpTpG) with cisplatin by CE to evaluate the possibilities of this technique. CE in combination with diode array detection (DAD) has proven to be the technique of choice. Analytes are separated due to different electrophoretic mobilities. Both, main hydrolysis products of cisplatin *cis*-$[PtCl(NH_3)_2(H_2O)]^+$ and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ [8,25], which are supposed to be the active forms under physiological conditions [26–29], as well as the major adducts formed with the nucleotides differ in their electrophoretic mobilities. Furthermore, DAD has shown to be of great help for the identification of the adducts. This detector allows to find the λ_{max} of each analyte. Binding of cisplatin to nucleotides results in a shift of the absorption maximum to lower energy [30,31]. Due to this characteristic shift we were able to identify adducts without having corresponding reference compounds. As an extension of these investigations, we have also entered the area of kinetic studies demonstrating a huge potential of this screening technique.

2. Experimental

2.1. Capillary electrophoresis

Analysis were performed on a Hewlett-Packard ^{3D}capillary electrophoresis system equipped with a DAD system. Fused-silica capillaries (effective length 41.5 cm×75 μm I.D.) were purchased from ICT (Vienna, Austria) and fused-silica capillaries with extended lightpath (effective length 56 cm×50 μm I.D.) from Hewlett-Packard (Palo Alto, CA, USA). Once a day the capillary was flushed with 0.1 *M* sodium hydroxide solution (HPCE grade; Fluka, Buchs, Switzerland) for 2 min. Prior to each analysis the capillary was flushed with bidistilled water for 2 min and afterwards with separation buffer [20 m*M* sodium phosphate buffer, pH 7 or 89 m*M* Tris–borate buffer, pH 7.4 with 1.5% hydroxyethylcellulose (HEC)] for 4 min. Temperature of the capillary as well as of the sample tray was kept at 30°C. All samples were dissolved in the separation buffer and injected by pressure (p=10 mbar, t=5 s). During the separation mode the voltage was kept constant at 20 kV. Alterations of the standard separation mode are described in the corresponding figure legends. Figures show detection at λ_{max} of the major analytes.

2.2. Standards

cis-$[PtCl_2(NH_3)_2]$ was prepared according to a standard procedure [32]. $[PtCl(NH_3)_2(H_2O)]^+$ (monoaqua) and $[Pt(NH_3)_2(H_2O)_2]^{2+}$ (diaqua) were synthesized by adding one or two equivalents of $AgNO_3$, respectively, to a solution of cisplatin and filtrating them afterwards. Both standards were stored as 10 m*M* aqueous solutions at pH of 2. The purity was verified by CE (20 m*M* citrate buffer, pH

4) showing that both standards contained about 10% of the respective other substance (monoaqua or diaqua), which corresponds to the equilibrium state at pH of the separation buffer (pH 4; pK_a of diaqua= 3.6).

2.3. Interaction of nucleotides with cisplatin

Stock solutions of each nucleoside monophosphate (10 m*M* of AMP, CMP, GMP and TMP) and cisplatin (10 m*M*) were prepared in the separation buffer. Monophosphates were incubated at 37°C for 24 h with cisplatin corresponding a ratio of 5:1. Non-reacting hydrolysis products of cisplatin were prevented by adding nucleoside monophosphates in excess. Samples were diluted with separation buffer (1:10) prior to injection. The studies were recorded at 200 nm to detect hydrolysis products of cisplatin and at the absorption maxima of each nucleotide (Table 1).

2.4. Competitive studies of nucleotides with cisplatin

Nucleotides (2 m*M* each) were incubated with cisplatin (0.4 m*M*) at 37°C for 20 h within the separation buffer in a ratio of 5:1. We diluted the samples with 10 m*M* instead of 20 m*M* sodium phosphate buffer (pH 7) making use of the stacking effect. The separation conditions were the same as described above with exception of the voltage which was raised to 30 kV. Data were collected at 200 nm and 254 nm.

2.5. Incubation of the di- and trinucleotides with cisplatin

Oligonucleotides (10 m*M*) and cisplatin (0.5 m*M*) were incubated at 37°C for 24 h within the separation buffer corresponding to a ratio of 20:1. Aliquots of the incubated sample taken after 20 h, 40 h, 70 h, 160 h and additionally after 190 h in case of trinucleotides, were analyzed by directly injecting into the CE system and recording online at 200 and 260 nm.

2.6. Kinetic studies

Cisplatin (5 m*M*) and GMP (10 m*M*) were dissolved in the separation buffer. Equimolar amounts of both analytes (300 μ*M*) were mixed and filtered prior to injection. During the analysis the sample tray was kept at 30°C. Aliquots for analysis were taken from the same sample vial. Calibration was done externally by a calibration curve (R^2= 0.995). The kinetic study was recorded at 200 nm to detect hydrolysis products of cisplatin and at 258 nm to identify GMP adducts.

2.7. Chemicals

Disodium salts of guanosine 5′-monophosphate (GMP), adenosine 5′-monophosphate (AMP), cytidine 5′-monophosphate (CMP) and thymidine-5′-monophosphate (TMP) and the sodium phosphate buffer (20 m*M*, pH 7, HPCE grade) as well as the citrate buffer (20 m*M*, pH 4, HPCE grade) were purchased from Fluka. Dinucleotides d(GpG) and trinucleotides d(GpTpT) and d(GpTpG) were syn-

Table 1
UV absorption maxima of nucleotides and major cisplatin adducts

Nucleotide	λ_{max} (nm)	Major cisplatin adduct	λ_{max} (nm)
AMP	259	*cis*-[Pt(NH_3)$_2$(AMP-N7)$_2$]$^{2-}$	264
CMP	271		n.m.[a]
GMP	252	*cis*-[Pt(NH_3)$_2$(GMP-N7)$_2$]$^{2-}$	258
TMP	267		n.m.
d(GpG)	252	*cis*-[Pt(NH_3)$_2$(G-N7-G-N7)]$^{+}$	258
d(GpTpT)	260	*cis*-[Pt(NH_3)$_2$(G-N7-TT)$_2$]$^{2-}$	264
d(GpTpG)	254	*cis*-[Pt(NH_3)$_2$(G-N7-TG-N7)]	262

[a] n.m.=Not measured.

thesized by VBC-Genomics (Vienna, Austria). The oligonucleotides are abbreviated as GG, GTT, GTG. Tris buffer solution (89 m*M*) containing boric acid (332 m*M*) and EDTA (2 m*M*) with pH of 7.4 (HPCE buffer grade) and HEC were purchased from Hewlett-Packard. Bidistilled water was further purified by an Elgastat water purification system (conductivity of 18 mΩ cm^{-1}, Bucks, UK). All solutions were filtered prior to analysis by a 0.2-μm filter (Millipore, Bedford, MA, USA) and degassed ultrasonically.

3. Results and discussion

In general the peak identities of the nucleoside monophosphates were established on the basis of their migration behavior and spectral properties. Major adducts were identified by their typical λ_{max}-shift of their UV spectra, their migration time and in case of GMP-adducts on NMR experiments. Monoadducts were obtained by incubation of nucleotides with cisplatin in the separation buffer (20 m*M* sodium phosphate buffer, pH 7) in presence of Cl^- ions (100 m*M*). Consequently, adduct formation slowed down significantly because chloride is a competing ligand favoring the monoadduct formation (data not shown). The hydrolysis products of cisplatin were identified by dotting sample solutions with separately synthesized standards. These indications together with the knowledge about the general mechanism of cisplatin binding resulted in the peak assignment presented.

3.1. Interaction of nucleoside monophosphates with cisplatin

Cisplatin has two available binding sites for nucleotides. In order to obtain the major bisadduct *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)_2]^{2-}$ we incubated cisplatin with GMP added in excess. Temperature and pH were kept close to physiological conditions (37°C, pH 7) under which the predominant hydrolysis products of cisplatin are the aquated complexes *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. These are supposed to be the active structures in vivo [8]. Minor hydrolysis products are *cis*-$[Pt(NH_3)_2(OH)(H_2O)]^+$and *cis*-$[Pt(NH_3)_2Cl(OH)]$ [25]. Incubation of GMP with the metal complex for 24 h resulted in two major signals (Fig. 1a), the expected adduct *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)_2]^{2-}$ as well as the excess of GMP. The identification of the adduct is based on its migration time and on the spectral information. Binding of cisplatin to nucleotides results in a characteristic shift of UV-absorption maxima to lower energy (λ_{max}-shift; Table 1). In addition to this indirect evidence our corresponding NMR experiments confirmed the proposed structure of the major adduct *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)_2]^{2-}$ (data not shown). However, cisplatin and its hydrolysis products could not be detected anymore. The non reactive hydrolysis products of cisplatin are in equilibrium with the active aqua complex. Probably due to the excess of GMP all reactive hydrolysis products are bound thus shifting the equilibrium. Furthermore, the monoadduct *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)Cl]^-$ was not detected.

Comparative results are obtained for interaction of cisplatin with AMP (Fig. 1b). After incubation with cisplatin the fast migrating component is the major adduct *cis*-$[Pt(NH_3)_2(AMP\text{-}N7)_2]^{2-}$. The second one corresponds to AMP which was added in excess.

Incubation of cisplatin with CMP and TMP did not result in any comparable adduct formation. In both cases only minor adduct peaks were detected (Fig. 1c, peak 1 and d, peak 2) but could not be identified due to the small amounts formed.

3.2. Interaction of nucleoside monophosphates with cisplatin under competitive conditions

We investigated the kinetic preference of the four nucleotides GMP, AMP, CMP and TMP for cisplatin [33,34]. All nucleotides were used in equimolar amounts to obtain a competitive situation for cisplatin. Fig. 2 shows the control sample (a) in comparison to the sample with cisplatin (b). As a result we found two additional, not completely resolved signals (R=0.85) which we identified as *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)_2]^{2-}$ (peak 1) and *cis*-$[Pt(NH_3)_2(AMP\text{-}N7)_2]^{2-}$ (peak 2). The group of four later eluting signals are due to the excess of GMP, AMP, TMP and CMP, respectively. According to our initial experience there is no evidence for any adduct formation in case of TMP and CMP. Peak

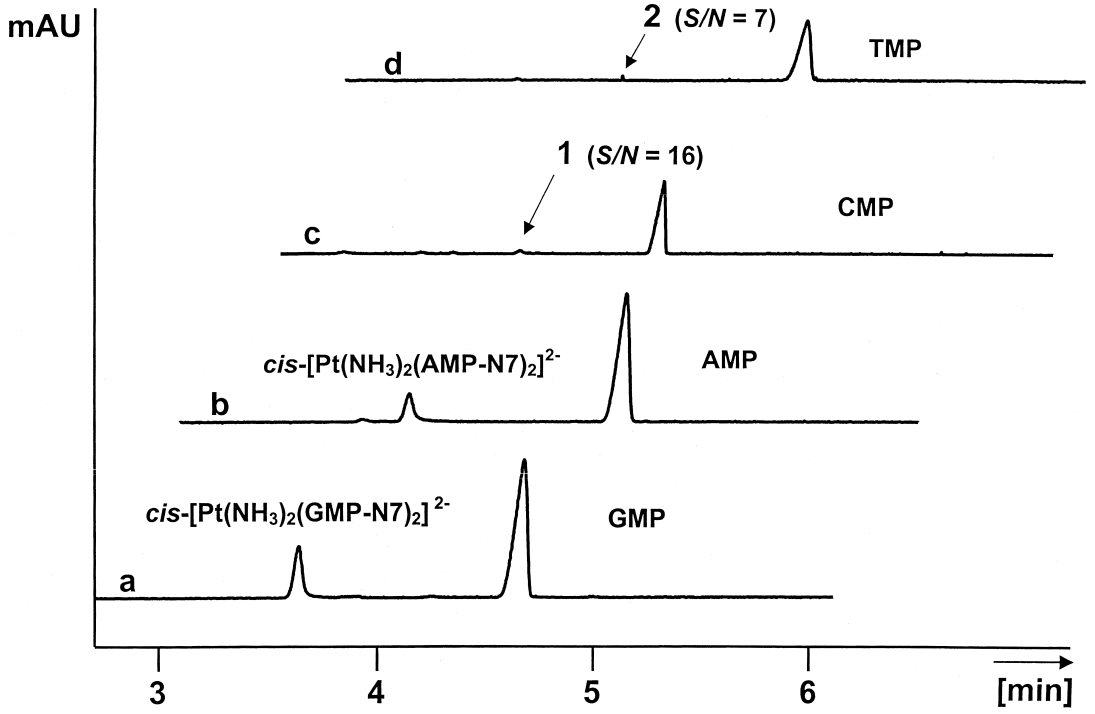

Fig. 1. Electropherogram of nucleoside monophosphates after incubation of cisplatin with (a) 5′-GMP, (b) 5′-AMP, (c) 5′-CMP and (d) 5′-TMP, in a ratio of 1:5; λ=260 nm.

area of TMP and CMP remained constant before as well as after addition of cisplatin, whereas signals corresponding to GMP and AMP decreased proportionally to the increase of the adducts. Again, excess of nucleoside monophosphates prevented any formation of monoadducts and detection of cisplatin or its hydrolysis products.

3.3. Kinetics of GMP interaction with cisplatin

The time course of the reaction was studied over a period of 42 h (Fig. 3). After 1.3 h cisplatin as well as the monoaqua complex *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ were separated (data not shown) whereas none of the adduct could be detected at this time. As reported earlier, binding of cisplatin to GMP is controlled by the rate of hydrolysis of the metal complex ($t_{1/2}$=1.9 h at 37°C, pH 6.5, [13]). The predominant bisadduct *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)_2]^{2-}$ as well as the minor monoadduct *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)Cl]^-$ (Fig. 3, peak 1) were found for the first time after 5.2 h. After this incubation period about 30% of GMP was bound to cisplatin. About 50% of GMP was bound within 15.3 h. At the termination of this study, after 42 h, the two major components were the bisadduct and the free GMP, with only minor amounts of the monoadduct $[Pt(NH_3)_2(GMP\text{-}N7)Cl]^-$ and an unidentified adduct (about 5% of the total area of all four signals, S/N=6.1; peak 2). Although GMP and cisplatin were added in equimolar amounts about 22% of the initial GMP were still free.

3.4. Interaction of the dinucleotide 5′-GG-3′ with cisplatin

After 20 h of incubation a first adduct (Fig. 4a, peak 2) of the dinucleotide 5′-GG-3′ (Fig. 4a, peak 1) was formed with cisplatin. Due to the small amounts unambiguous identification was not possible. This small adduct peak disappeared after 160 h and, therefore, it might be a monoadduct. A third component (Fig. 4b, peak 3) was detected after 40 h. The same elution pattern was obtained after 70 h (Fig. 4c) with the fast migrating peak increasing

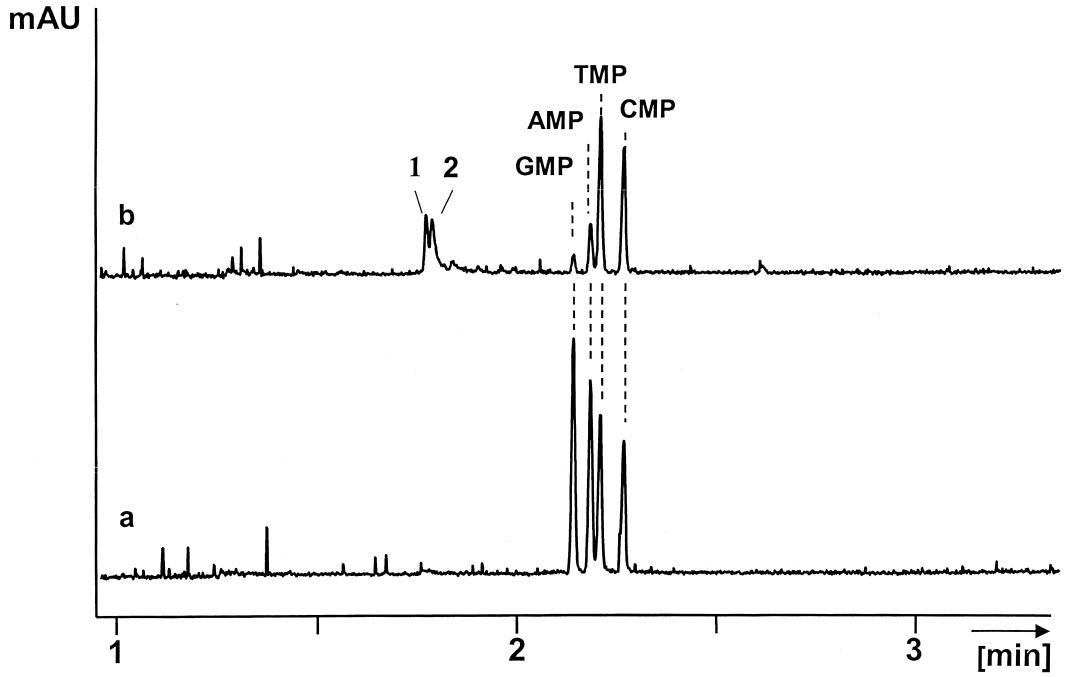

Fig. 2. Electropherogram of competitive study of nucleoside monophosphates with cisplatin: (a) control sample with equimolar amounts of AMP, GMP, CMP and TMP; (b) nucleoside monophosphates after incubation with cisplatin; λ=260 nm.

during the time course of the reaction. Due to the retention time and the absorption maximum we assume the following structure *cis*-$[Pt(NH_3)_2$(G-N7-G-N7)$]^+$ as the final product. As expected, only two compounds were detected after 160 h (Fig. 4d), the latter peak corresponding to free GG. It is known from literature that two adjacent guanine bases are preferred in the coordinative binding with cisplatin forming intrastrand cross-linking [5,30,35–41]. Although GG was added in excess, no *cis*-$[Pt(NH_3)_2(GG)_2]$ was formed.

3.5. Interaction of the trinucleotide 5′-GTT-3′ with cisplatin

N3 of thymine is protonated at the conditions chosen (pH 7) and therefore not available as a binding site for cisplatin. Consequently, coordinative binding of cisplatin can only be formed with 5′-guanine of 5′-GTT-3′. After 20 h of incubation five components could be separated with the first and the second peak being not completely resolved (Fig. 5a). Due to the absorption maxima the first four peaks could be identified as adducts with the last eluting peak corresponding to free GTT added in excess (peak 1). After 40 and 70 h (Fig. 5b and c) similar electropherograms were obtained but with significantly increased area of all adduct peaks. Incubation for 190 h resulted in a notably changed electropherogram (Fig. 5d). A certain adduct (peak 2) was increased at the expense of free GTT. Due to the λ_{max} shift, the preference of cisplatin binding and the mobility we propose the structure *cis*-$[Pt(NH_3)_2$(G-N7-TT$)_2]^{2-}$ with guanine N7 as the major binding atom. The minor adducts (peak 3 and 4) could not be baseline separated. By changing the separation buffer to a Tris solution with hydroxyethylcellulose as a sieving additive resolution could be increased from 1.1 to 2.2. Because of the elution order, the absorption maximum and the supposed mechanism of hydrolysis as well as the possibilities of interaction, we propose the two structures *cis*-$[Pt(NH_3)_2Cl$(G-N7-TT)$]^-$ and *cis*-$[Pt(NH_3)_2$(OH)(G-N7-TT)$]^-$. The component appearing between the major adduct

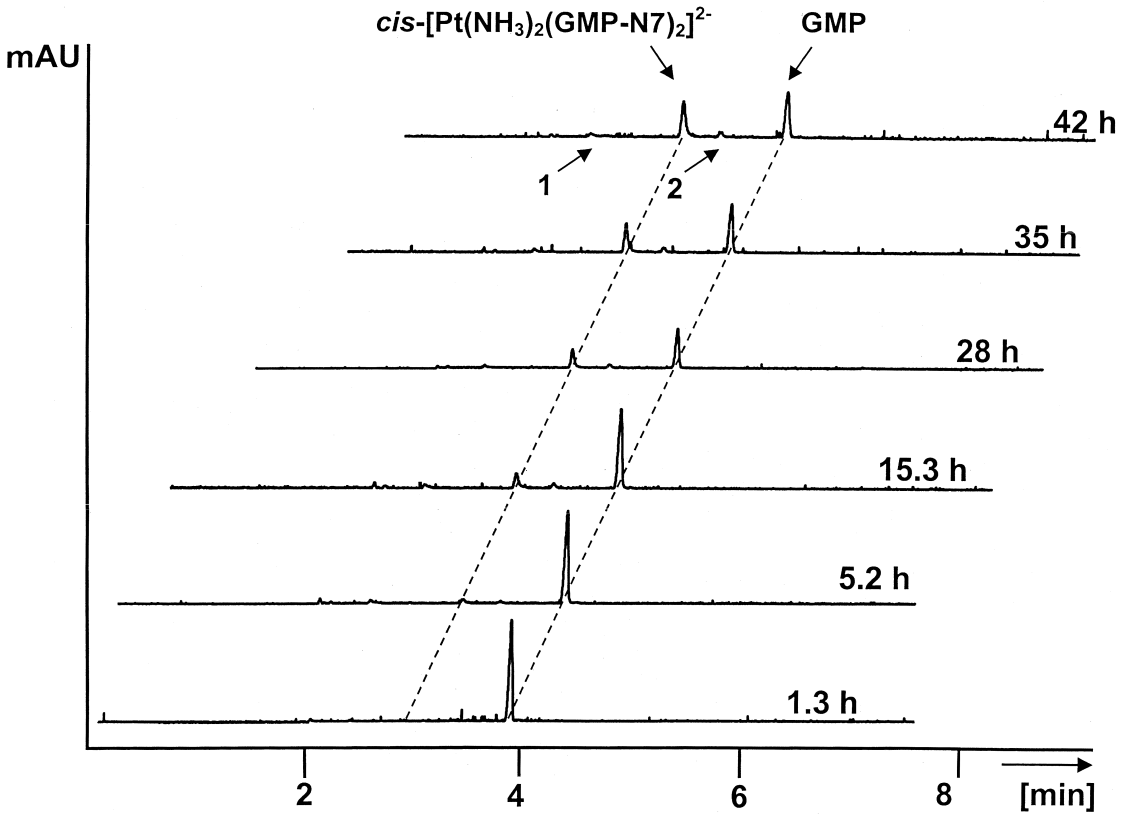

Fig. 3. Electropherogram of the reaction between GMP and cisplatin in equimolar amounts; λ=260/10 nm, λ_{ref}=380/80 nm.

and GTT (Fig. 5a, peak 5) also exhibits an absorption maximum characteristic for an adduct but was not identified.

3.6. Interaction of the trinucleotide 5′-GTG-3′ with cisplatin

In contrast to the trinucleotide investigated above 5′-GTG-3′ has two potential binding sites for cisplatin. Next-neighboring guanines react with cisplatin and the so-called GNG chelates are formed. The development of an intrastrand crosslink should therefore be expected. The time course of the reaction is shown in Fig. 6. From the initial analysis one major adduct (Fig. 6, peak 2) is preferentially formed. The area of this component increased during the study and we identified it as the major adduct *cis*-[$Pt(NH_3)_2$(G-N7-TG-N7)]. The last peak corresponds to free GTG (peak 1), which was added in excess. Beside these two components several minor adducts could be separated. One of them is *cis*-$[Pt(NH_3)_2(GTG)_2]^{2-}$. The monoadducts *cis*-$[Pt(NH_3)_2Cl(GTG)]^-$ and *cis*-$[Pt(NH_3)_2(OH)(GTG)]^-$ might be formed also but could not be identified due to the small amounts.

4. Conclusions

In this work the versatility of CE–DAD for separation and identification of major nucleotide adducts formed by reaction with cisplatin is demonstrated. A simple sodium phosphate buffer system was sufficient to separate all four common nucleotides and their major adducts, *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)_2]^{2-}$ and *cis*-$[Pt(NH_3)_2(AMP\text{-}N7)_2]^{2-}$ in a single run. The formation of *cis*-$[Pt(NH_3)_2$(G-N7-G-N7)$]^+$ was shown and could be separated from free

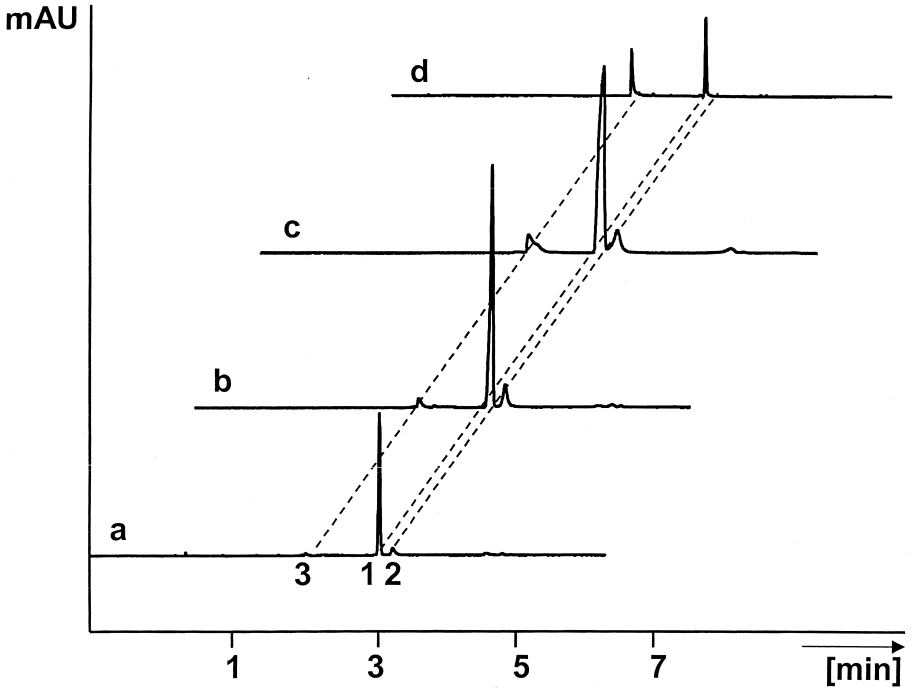

Fig. 4. Electropherogram of the dinucleotide d(GpG) incubated with cisplatin after (a) 20 h, (b) 40 h, (c) 70 h and (d) 190 h; electrophoretic conditions of (d): separation buffer (Tris–borate buffer solution), pH 7.4 with 1.5% HEC; λ=260/10 nm, λ_{ref}=380/80 nm.

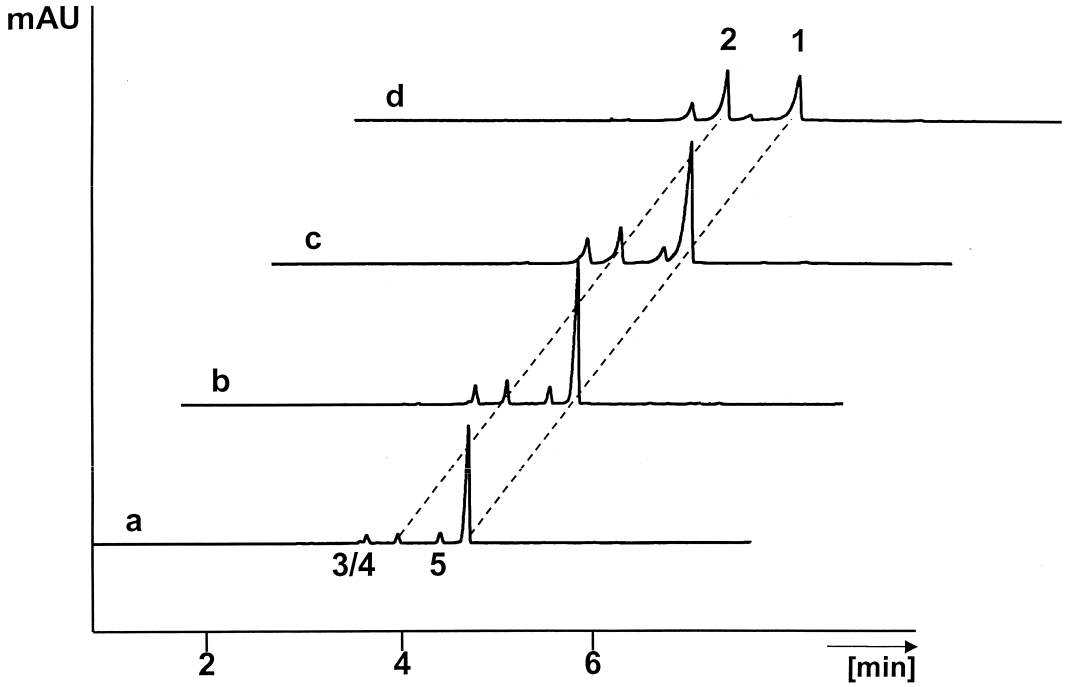

Fig. 5. Electropherogram of the trinucleotide d(GpTpT) incubated with cisplatin after (a) 20 h, (b) 40 h, (c) 70 h and (d) 190 h; λ=260/10 nm, λ_{ref}=380/80 nm.

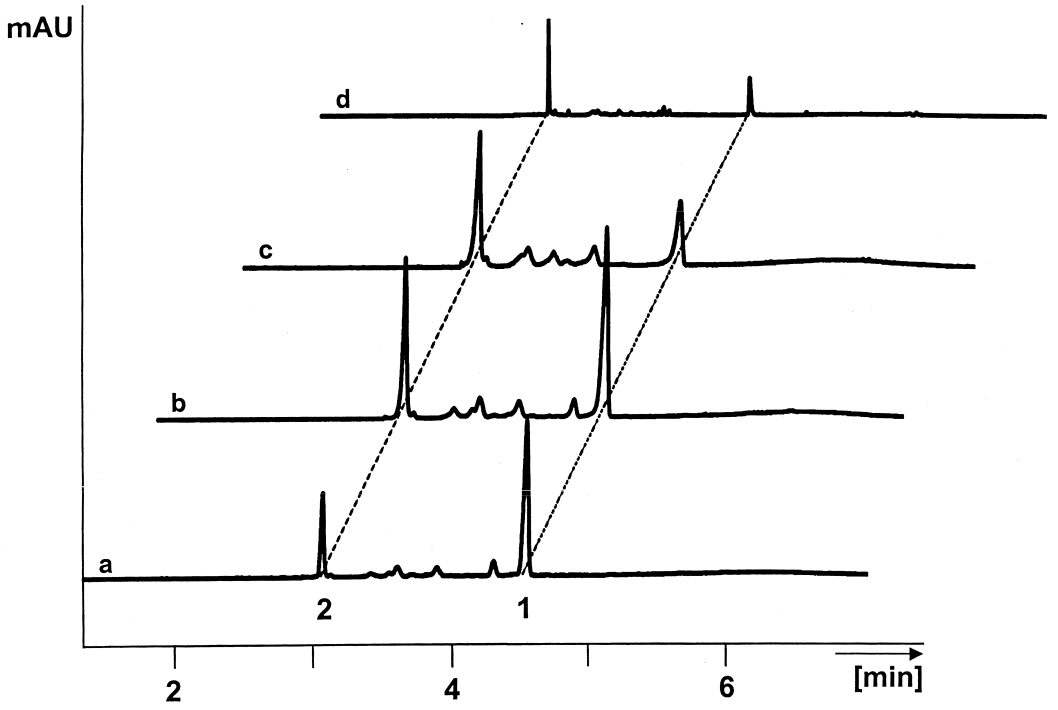

Fig. 6. Electropherogram of the trinucleotide d(GpTpG) incubated with cisplatin after (a) 20 h, (b) 40 h, (c) 160 h and (d) 190 h; electrophoretic conditions of (d): separation buffer (Tris–borate buffer solution), pH 7.4 with 1.5% HEC; λ=260/10 nm, λ_{ref}=380/80 nm.

GG. The investigation of the trinucleotide GTG with cisplatin led to the bifunctional adduct *cis*-[$Pt(NH_3)_2$(G-N7-TG-N7)] in contrast to GTT, where two nucleotides were bound to one cisplatin and the adduct *cis*-$[Pt(NH_3)_2(G\text{-}N7\text{-}TT)_2]^{2-}$ was formed. Preliminary kinetic studies were also undertaken and the results confirmed CE as a very promising analysis method although calibration and quantification must be done very carefully. Finding new compounds with similar or even better activity compared to cisplatin demand for an efficient, fast and simple method, which was found with this electrophoretic technique. The versatility of CE will lead to a wide use in areas of question like study of kinetic behavior and preferred interactions under simulation of physiological parameters. The major disadvantage of CE in combination with DAD is the lack of directly obtained structural information of DNA adducts. This problem could be solved by CE coupled to mass spectrometry.

References

[1] H.M. Pineto, J.H. Schornagel, Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy 2, Plenum Press, New York, 1996.
[2] J.J. Roberts, A.J. Thomson, Progr. Nucl. Ac. Res. Mol. Biol. 22 (1979) 71.
[3] S.J. Lippard, Science 218 (1982) 1075.
[4] R.W. Gellert, R. Bau, in: H. Sigel (Ed.), Metal Ions in Biological Systems, Vol. 8, Marcel Dekker, New York, 1979, p. 1.
[5] A.M.J. Fichtinger-Schepman, J.L. van der Veer, J.H.J. den Hartog, P.H.M. Lohman, J. Reedijk, Biochemistry 24 (1985) 707.
[6] A. Eastman, Biochemistry 21 (1982) 6732.
[7] H.C. Harder, B. Rosenberg, Int. J. Cancer 6 (1970) 207.
[8] S.J. Berners-Price, T.A. Frenkiel, U. Frey, J.D. Ranford, P.J. Sadler, J. Chem. Soc., Chem. Commun. (1992) 789.
[9] A.T.M. Marcelis, J. Reedijk, Recl. Trav. Chim. Pays-Bas 102 (1983) 121.
[10] R.E. Duncan, R.S. Tobias, Inorg. Chem. 16 (1977) 2625.
[11] J.Y. Lallemand, J. Soulié, J.-C. Chottard, J. Chem. Soc., Chem. Commun. (1980) 438.

[12] A. Eastman, Biochemistry 25 (1986) 3912.
[13] D.P. Bancroft, C.A. Lepre, S.J. Lippard, J. Am. Chem. Soc. 112 (1990) 6860.
[14] N.P. Johnson, A.M. Mazard, J. Escalier, J.P. Macquet, J. Am. Chem. Soc. 107 (1985) 6376.
[15] A. Eastman, Pharmacol. Ther. 34 (1987) 155.
[16] A.M.J. Fichtinger-Schepman, P.H.M. Lohman, J. Reedijk, Nucl. Acid Res. 10 (1982) 5345.
[17] J.A. Howle, G.R. Gale, Biochem. Pharmacol. 19 (1970) 2757.
[18] J. Reedijk, Inorg. Chem. Acta 198–200 (1992) 873.
[19] A.W. Prestayko, S.T. Crooke, S.K. Carter, Cisplatin – Current Status and New Developments, Academic Press, New York, 1980.
[20] M.P. Hacker, E.B. Douple, I.H. Krakoff, Platinum Coordination Compounds in Cancer Chemotherapy, Martinus Nijhoff, Boston, MA, 1984.
[21] P.J. Loehrer, K.H. Einhorn, Ann. Intern. Med. 100 (1984) 704.
[22] E. Wiltshaw, Plat. Met. Rev. 23 (1979) 90.
[23] M. Sharma, R. Jain, E. Ionescu, H.K. Slocum, Anal. Biochem. 228 (1995) 307.
[24] H. Troujman, J.-C. Chottard, Anal. Biochem. 252 (1997) 177.
[25] S.E. Miller, D.A. House, Inorg. Chim. Acta 166 (1989) 189.
[26] N.P. Johnson, J.P. Hoeschele, R.O. Rahn, Chem. Biol. Interact. 30 (1980) 151.
[27] A.T.M. Marcelis, C.G. van Kralingen, J. Reedijk, Inorg. Biochem. 13 (1980) 213.
[28] A.T.M. Marcelis, J.H.J. den Hartog, J. Reedijk, J. Am. Chem. Soc. 104 (1982) 2664.
[29] A.T.M. Marcelis, C. Erkelens, J. Reedijk, Inorg. Chim. Acta 91 (1984) 129.
[30] J.-C. Chottard, J.-P. Girault, G. Chottard, J.-Y. Lallemand, D. Mansuy, J. Am. Chem. Soc. 102 (1980) 5565.
[31] W.M. Scovell, T. O'Conner, J. Am. Chem. Soc. 99 (1977) 120.
[32] S.C. Dhara, Indian J. Chem. 8 (1970) 193.
[33] S. Mansy, G.Y.H. Chu, R.E. Duncan, R.S. Tobias, J. Am. Chem. Soc. 100 (1978) 607.
[34] K. Inagaki, N. Tamaoki, Y. Kidani, Inorg. Chim. Acta 46 (1980) L93.
[35] S.E. Sherman, D. Gibson, A.H.-J. Wang, S.J. Lippard, Science 230 (1985) 412.
[36] F. Gonnet, F. Reeder, J. Kozelka, J.-C. Chottard, Inorg. Chem. 35 (1996) 1653.
[37] J.-C. Chottard, J.-P. Girault, G. Chottard, J.-Y. Lallemand, D. Mansuy, Nouv. J. Chim. 2 (1978) 551.
[38] A.T.M. Marcelis, G.W. Canters, J. Reedijk, Recl. Trav. Chim. Pays-Bas 100 (1981) 391.
[39] J.-P. Girault, G. Chottard, J.-Y. Lallemand, J.-C. Chottard, Biochemistry 21 (1982) 1352.
[40] J.-P. Caradonna, S.J. Lippard, M.J. Gait, M. Singh, J. Am. Chem. Soc. 104 (1982) 5793.
[41] F. Reeder, Z. Guo, P. Murdoch, A. Corazza, T.W. Hambley, S.J. Berners-Price, J.-C. Chottard, P.J. Sadler, Eur. J. Biochem. 249 (1997) 370.