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Studies on Reactivity of Deoxynucleosides with *cis*-$Pt(NH_3)_2Cl_2$ and the Related Complexes by High Performance Liquid Chromatography

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Interaction of *cis*-$Pt(NH_3)_2Cl_2$ with DNA constituents has been widely investigated because *cis*-$Pt(NH_3)_2Cl_2$ is effective against various tumor systems and DNA is the main target attacked by *cis*-$Pt(NH_3)_2Cl_2$. Robins [1] examined the reactivity of Pt(ethylenediamine)Cl_2 with DNA constituents by paper chromatography, in which overnight development was required for the separation of the reaction mixtures. He used ^{14}C-labeled Pt(ethylenediamine)-Cl_2 and ^{3}H-labeled nucleosides to detect the reactants. Tobias *et al.* [2] have studied the reaction of *cis*-$Pt(NH_3)_2(OH_2)_2^{2+}$ with a mixture of the four nucleotides at pH 7 by means of Raman difference spectrometry.

In order to clarify the reactivity of the platinum complexes (*cis*-$Pt(NH_3)_2Cl_2$, *trans*-$Pt(NH_3)_2Cl_2$, and $[Pt(NH_3)_3Cl]Cl$) with nucleosides, we have employed high performance liquid chromatography as a rapid and simple method. In this work, a reaction mixture of the platinum complexes with an equimolar mixture of deoxyguanosine (dG), deoxyadenosine (dA), deoxycytidine (dC), and thymidine (T) was separated by a strong cation exchange resin (Zipax SCX, 0.21 × 50 cm column) and was detected at 260 nm by using a UV detector. The reactivity of the platinum complexes with deoxynucleosides was monitored by quantification of the unreacted nucleosides.

Figure 1-d shows an elution pattern obtained when a reaction mixture of $[Pt(NH_3)_3Cl]Cl$ with an equimolar mixture of the four deoxynucleosides was injected onto the Zipax SCX column and was eluted with 0.02 *M* ammonium formate (pH 3.0, adjusted with H_2SO_4) at a flow rate of 3 ml/min. The first peak, which emerges at the void volume of the column, is due to thymidine. In all of the chromatograms obtained in this work, the peak height of thymidine did not change at all. This means that thymidine did not react with the platinum complexes under the reaction conditions employed in this work. The elution pattern of the standard mixture containing equimolar amounts of the four deoxynucleosides was the same as that of Fig. 1-d. It only took 7 minutes to separate the standard mixture. The chromatograms obtained for the reaction

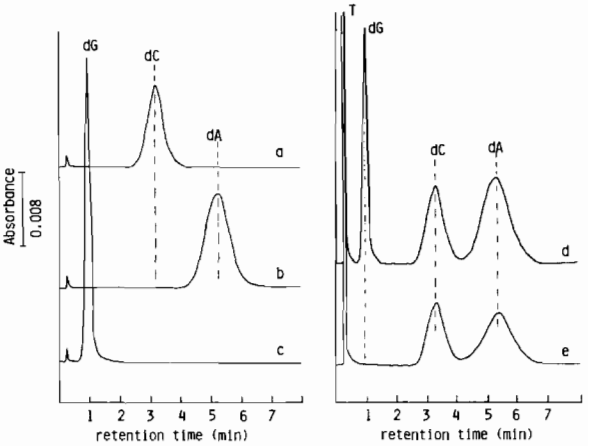

Fig. 1. Elution patterns obtained for the reaction solutions of $[Pt(NH_3)_3Cl]Cl$ with deoxynucleosides. Conditions: eluent, 0.02 *M* ammonium formate (pH = 3.0); flow rate, 3 ml/min; sample injection, 10 μl. A reaction mixture in 0.02 *M* phosphate buffer was incubated at 40 °C for 9 days. [each base] = 0.3 m*M*. r = Pt/base. a) dC + $[Pt(NH_3)_3Cl]Cl$, r = 1.0, pH = 6.8; b) dA + $[Pt(NH_3)_3Cl]Cl$, r = 1.0, pH = 6.8; c) dG + $[Pt(NH_3)_3Cl]Cl$, r = 1.0, pH = 6.8; d) dG + dA + dC + T + $[Pt(NH_3)_3Cl]Cl$, r = 0.5, pH = 6.8; e) dG + dA + dC + T + $[Pt(NH_3)_3Cl]Cl$, r = 0.5, pH = 4.68.

mixture of $[Pt(NH_3)_3Cl]Cl$ with dG, dA, or dC are shown in Fig. 1-a, -b, and -c. These chromatograms have shown only the peaks of each deoxynucleoside, and the reaction products do not seem to be eluted under the conditions. Figure 1-e is a chromatogram obtained for the reaction mixture (pH = 4.68) of $[Pt(NH_3)_3Cl]Cl$ with the equimolar mixture of the four deoxynucleosides. The peak of dG disappeared completely and the areas of the peaks of dA and dC showed a considerable reduction. The peaks due to the reaction products could not be observed anywhere on this chromatogram. These reaction products are eventually eluted when ionic strength of the eluant is allowed to increase by using Na_2SO_4. Consequently, the reaction products do not interfere with the quantification of the unreacted deoxynucleosides, so that the reactivity of $[Pt(NH_3)_3Cl]$-Cl with deoxynucleosides can be seen by the quantification of the unreacted deoxynucleosides, which was carried out by measuring the areas under each peak.

In the chromatogram obtained for the reaction mixture of *cis*-$Pt(NH_3)_2Cl_2$ with the four deoxynucleosides, a peak which could not be observed on the chromatogram of the standard mixture appeared in the vicinity of the peak of dG (Fig. 2-a). The new peak, which may be considered to be due to the reaction products, is also observed on the chromatograms obtained for the reaction mixtures of *cis*-$Pt(NH_3)_2Cl_2$ with each deoxynucleoside (Fig. 2-b, -c, and -d).

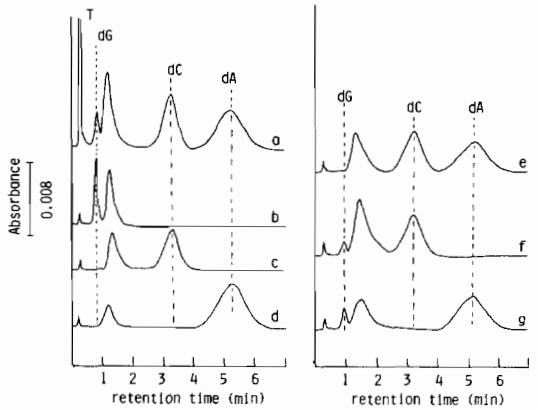

Fig. 2. Elution patterns obtained for the reaction solutions of *cis*-$Pt(NH_3)_2Cl_2$ with deoxynucleosides. Conditions are the same as in Fig. 1. a) dG + dA + dC + T + *cis*-$Pt(NH_3)_2Cl_2$, r = 0.5, pH = 6.8; b) dG + *cis*-$Pt(NH_3)_2Cl_2$, r = 1.0, pH = 6.8; c) dC + *cis*-$Pt(NH_3)_2Cl_2$, r = 1.0, pH = 6.8; d) dA + *cis*-$Pt(NH_3)_2Cl_2$, r = 1.0, pH = 6.8; e) dC + dA + *cis*-$Pt(NH_3)_2Cl_2$, r = 1.0, pH = 6.8; f) dG + dC + *cis*-$Pt(NH_3)_2Cl_2$, r = 1.0, pH = 6.8; g) dG + dA + *cis*-$Pt(NH_3)_2Cl_2$, r = 1.0, pH = 6.8.

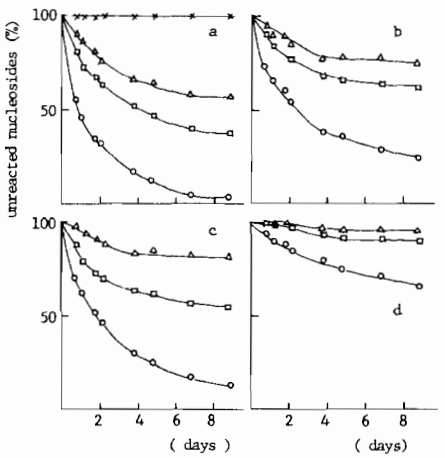

Fig. 3. Changes of the unreacted deoxynucleosides as a function of time for the reaction solutions of the platinum complexes with the equimolar mixture of the four deoxynucleosides. [each base] = 0.3 m*M*, pH = 6.8, incubation temperature = 40 °C. a) dG + dA + dC + T + *cis*-$Pt(NH_3)_2Cl_2$, r = 0.5; b) dG + dA + dC + T + *cis*-$Pt(NH_3)_2Cl_2$, r = 0.25; c) dG + dA + dC + T + *trans*-$Pt(NH_3)_2Cl_2$, r = 0.5; d) dG + dA + dC + T + $[Pt(NH_3)_3Cl]Cl$, r = 0.5. —×—, T; —△—, dC; —□—, dA; —○—, dG.

Since *cis*-$Pt(NH_3)_2Cl_2$ is a bifunctional reagent, it may be possible to form a mixed ligand complex containing two kinds of deoxynucleoside.

In order to know whether such complexes may interfere with the quantification of the unreacted deoxynucleosides, reaction mixtures of *cis*-$Pt(NH_3)_2Cl_2$ with a mixture of the two kinds of deoxynucleosides were prepared and their separation was carried out (Fig. 2-e, -f, and -g). The chromatograms thus obtained are similar to those obtained by the sum of the corresponding b, c, and d in Fig. 2. Although such mixed ligand complexes were formed, they were not eluted by 0.02 *M* ammonium formate. The peak of the products, which emerges in the vicinity of the peak of dG, has been observed in all of the chromatograms in Fig. 2, but it almost does not interfere with the quantification of the unreacted deoxynucleosides. The products seem to be minor components from measuring the area under the peak. The major products, which contain the mixed ligand complexes, are eventually eluted when ionic strength of the eluant is allowed to be increased by using Na_2SO_4. Elution patterns obtained for the reaction mixtures of *trans*-$Pt(NH_3)_2Cl_2$ with each deoxynucleoside or the equimolar mixture of the four deoxynucleosides are similar to those obtained with *cis*-$Pt(NH_3)_2Cl_2$. In all cases, the reaction products are not entirely involved with the quantification of the unreacted dA and dC.

Figure 3 shows a change of the unreacted deoxynucleosides as a function of time. The rates of these reactions seem to be very slow. In every case, the reactivity of the platinum complexes (*cis*-$Pt(NH_3)_2Cl_2$, *trans*-$Pt(NH_3)_2Cl_2$, and $[Pt(NH_3)_3Cl]Cl$) with the deoxynucleosides is in the order of dG > dA > dC. This order is in good agreement with that reported

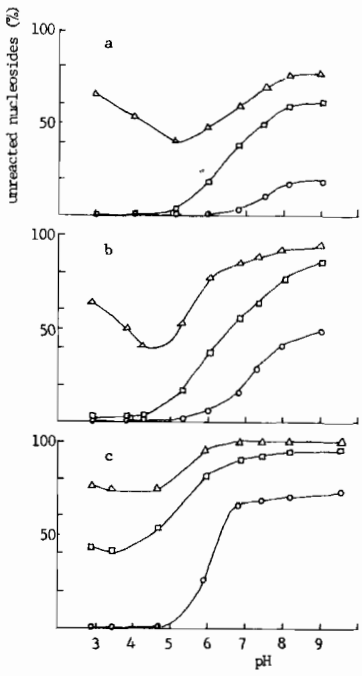

Fig. 4. Changes of the unreacted deoxynucleosides as a function of pH for the reaction solutions of the platinum complexes with the equimolar mixture of the four deoxynucleosides. [each base] = 0.3 m*M*, r = 0.5, incubation = 40 °C for 9 days. a) dG + dA + dC + T + *cis*-$Pt(NH_3)_2Cl_2$; b) dG + dA + dC + T + *trans*-$Pt(NH_3)_2Cl_2$; c) dG + dA + dC + T + $[Pt(NH_3)_3Cl]Cl$. —△—, dC; —□—, dA; —○—, dG.

by Tobias *et al.* [2]. They have reported that the order of the nucleotides in terms of their nucleophilicity towards *cis*- or *trans*-$Pt(NH_3)_2(OH_2)_2^{2+}$ was GMP > AMP ≫ CMP ≫ UMP. It seems that the reactivity of the platinum complexes with dC in this work is somewhat greater than that reported by Tobias *et al.* The results obtained with the reaction solution at r(Pt/base) = 0.1 and r = 0.25 indicated a similar behavior to those obtained for r = 0.5.

Figure 4 shows a change of the unreacted deoxynucleosides as a function of pH. The reactivity of the platinum complexes to dG and dA increased with decreasing pH in the reaction solutions. The reactivity seems to be influenced primarily by pH, especially at the pH in the vicinity of the pKa values of the aqua species of these platinum complexes. The curve of the unreacted dC *vs.* pH showed a minimum at pH 4–5. When binding with *cis*-$Pt(NH_3)_2Cl_2$ occurs through the N(3) site in dC, there should be some competition between *cis*-$Pt(NH_3)_2Cl_2$ and the proton because the pKa value of the N(3) in dC is 4.2. At pH < 5, the curve for the unreacted dC *vs.* pH seems to reflect the competition with the proton. In every case, it is also apparent from Fig. 4 that the order of the deoxynucleosides in the reactivity is of dG > dA > dC, and that the order of the platinum complexes in the reactivity is of *cis*-$Pt(NH_3)_2Cl_2$ > *trans*-$Pt(NH_3)_2Cl_2$ > $[Pt(NH_3)_3Cl]Cl$ under the physiological pH.

In conclusion, i) high performance liquid chromatography is a very useful tool for examining the reaction of the platinum complexes with deoxynucleosides; ii) the reactivity of the platinum complexes with the four deoxynucleosides is in the order of dG > dA > dC ≫ T; iii) the reactivity is affected by the pH of the reaction solutions, especially in the pH range of 4–7.

References

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2 S. Mansy, G. Y. H. Chu, R. E. Duncan and R. S. Tobias, *J. Am. Chem. Soc.*, *100*, 607 (1978).