INTERACTIONS BETWEEN POLYNUCLEOTIDES AND PLATINUM(II) COMPLEXES

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SUMMARY
Reaction of either cis or trans $Pt(NH_3)_2Cl_2$ with poly(A) in dilute aqueous solution leads to quantitative precipitation of the polymer at Pt/nucleotide ratios above 0.5. It is proposed that at ratios less than this, intramolecular binding of one Pt to two bases is favored; at higher ratios, intermolecular cross-linking becomes important and precipitation results. The absence of isomer selectivity in precipitation implies that the biological specificity of the cis form results from a process other than cross-linking of polynucleotide strands. Other observations suggest that the coordinated ammonia of nucleotide-platinum(II) ammine complexes may be unusually labile.

INTRODUCTION

Square planar platinum(II) complexes of the type PtA_2B_2 exist in two isomeric configurations; the cis form has A ligands adjacent to each other, while the trans form has A ligands diagonally opposed. The biological activity of cis substituted platinum(II) compounds (especially the effectiveness of cis-$Pt(NH_3)_2Cl_2$ against tumors), relative to the inactivity of the trans isomers, is of current interest (1-4). Our concern with the nature of the platinum-polynucleotide interactions which may underlie these biological phenomena stems from the observation that Pt(II) is absorbed from solution by a crystal of methionine transfer RNA to yield an insoluble, glassy substance. This suggests that Pt(II) cross-links polynucleotide strands within the crystal, a mode of action similar to that proposed for the anti-tumor activity of cis platinum(II) complexes (1-3,5). In order to test this hypothesis we initiated studies on the reactions of Pt(II) complexes with homopolynucleotides in dilute aqueous solution.

EXPERIMENTAL

Materials. Water was twice distilled. Unless otherwise specified, all chemicals were of reagent grade. Homopolynucleotides (mol. wt.

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> 10^5) and oligoadenylates were from Miles Labs or P-L Biochemicals Inc. and were used without further purification. K_2PtCl_4 was obtained from D. F. Goldsmith Corp. and other platinum complexes were synthesized from it according to published procedures (6,7) or, in the case of adenine derivatives, by procedures analogous to those given for bipyridyl complexes (8,9). Anal. Calcd for (adenine)$PtCl_2 \cdot 1.5H_2O$: C, 14.06; H, 1.65; N, 16.40. Found: C, 14.02; H, 1.53; N, 16.38.

Assays. Concentrations of polynucleotide and oligoadenylate solutions were determined spectrophotometrically using extinction coefficients supplied by the manufacturers (error estimated as ±5%). Stock platinum solutions were prepared by weight. Unknown platinum concentrations as low as 10^{-5} M were determined spectrophotometrically (10) (error estimated as ±10%). The hydrolysis of poly(A) was monitored by the increase in acid soluble absorbance (11).

Conditions. Experiments were performed in unbuffered solutions because of the affinity of common buffers for metal ions (12). Whenever measured, the pH of reaction solutions fell in the range 5-8; there was no discernible correlation between measured pH and the phenomena being monitored. One series of experiments using the weakly coordinating buffer N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (13) (HEPES, 0.01 M, pH = 7.5) showed no difference, other than a slightly decreased platinum effectiveness, from an equivalent unbuffered series.

RESULTS

Hydrolysis. The effect of Zn^{2+} and cis-$Pt(NH_3)_2Cl_2$ on the hydrolysis of poly(A) is shown in Figure 1. Whereas Zn^{2+} is an effective hydrolysis catalyst (11), the platinum complex actually retards degradation of the polymer. This demonstrates an interaction between cis-$Pt(NH_3)_2Cl_2$ and poly(A), and also establishes that the phenomena described below are not due to hydrolysis.

Precipitation. For either cis or trans-$Pt(NH_3)_2Cl_2$, if the ratio

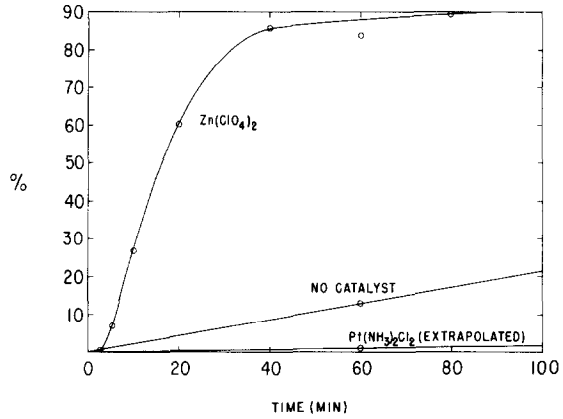

Figure 1. Hydrolysis of poly(A) as a function of time. Conditions: 10 mM $NaClO_4$, 64°, 0.63 mM P, 0.22 mM metal.

of moles platinum to moles nucleotide phosphorus present in the reaction solution (hereafter referred to as Pt/P) is greater than 0.5, quantitative precipitation of poly(A) occurs. If Pt/P $\leqq$ 0.5, no precipitation occurs but shifts in the adenylate absorption maximum (largest shift from 257 to 263 nm at Pt/P = 0.5) indicate that platinum still complexes the polymer. Figure 2 shows a titration of poly(A) with cis-$Pt(NH_3)_2Cl_2$ as monitored by the total platinum concentration in the supernatant. The conspicuous discontinuity at Pt/P = 0.5 should be expressly noted; up to this point no precipitation occurs, and after this point all the poly(A) is precipitated.

Polymer dependence. When adenine, adenosine, 3'- and 5'-adenosine monophosphates, and the oligoadenylate series dimer to hexamer, are incubated with cis-$Pt(NH_3)_2Cl_2$ (conditions of Figure 2, Pt/P = 1.0), no precipitation occurs although the characteristic spectral shift is observed in all cases.

Base dependence. Poly(A), poly(dA), poly(G), poly(I) and poly(C) all precipitate with cis-$Pt(NH_3)_2Cl_2$ (conditions of Figure 2, Pt/P = 1.0). Poly(U) does not precipitate under these conditions, although a spectral shift is observed.

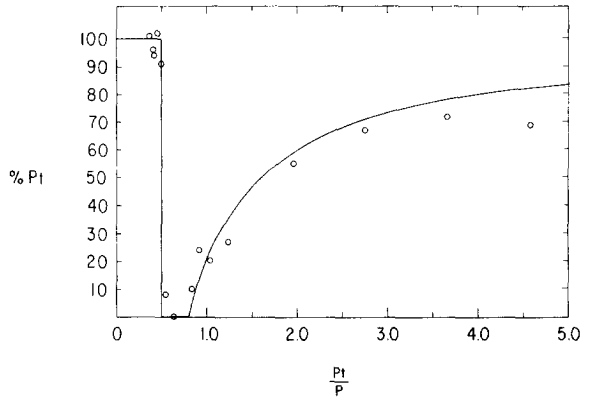

Figure 2. Titration of poly(A) with cis-$Pt(NH_3)_2Cl_2$: percent platinum left in solution vs. Pt/P. Continuous line drawn assuming no precipitation up to 0.5 Pt/P and then 0.8 moles Pt bound per P in precipitate. Conditions: 8.0 mM $NaClO_4$, solutions incubated at 45° for 24 hours, 0.088 mM P, 0.02 - 0.37 mM Pt. Equivalent curves showing complete precipitation of poly(A) at Pt/P > 0.5 can be obtained by spectrophotometrically monitoring the concentration of poly(A) in solution.

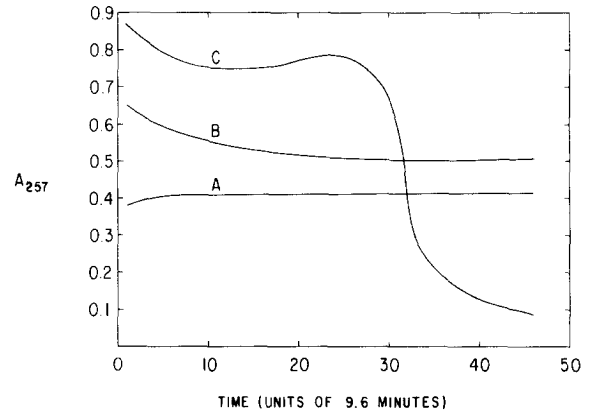

Figure 3. Kinetics of precipitation of poly(A) by cis-$Pt(NH_3)_2Cl_2$: absorbance at 257 nm vs. time. Conditions as in Figure 2. Curve A: no platinum. Curve B: 0.5 Pt/P, displaced 0.2 absorbance units for clarity. Curve C: 1.0 Pt/P, displaced 0.4 absorbance units for clarity.

Ligand dependence. In addition to cis and trans-$Pt(NH_3)_2Cl_2$, (ethylenediammine)$PtCl_2$ and $[Pt(NH_3)_3Cl]Cl$ also precipitate poly(A) (conditions of Figure 2, Pt/P = 1.0). K_2PtCl_4 is ineffective when the background electrolyte ($NaClO_4$) concentration is 0.0086 M, but precipitates poly(A) when this concentration is raised to 0.035 M or

higher*. $[Pt(NH_3)_4]Cl_2$ has no effect (does not even cause spectral shifts) when incubated with poly(A) for several days at 45°, Pt/P = 17.

Rate dependence. Plots of A_{257} vs. time for solutions of cis-$Pt(NH_3)_2Cl_2$ and poly(A) at three different Pt/P ratios are shown in Figure 3. The absorbance rise just before precipitation in curve C is characteristic for the precipitation phenomenon and is not due to light scattering by particulate matter. In Figure 4, the Pt/P ratio is plotted vs. the time required for onset of precipitation, and it is seen that at high concentrations of Pt this time becomes independent of the platinum concentration. Also, it should be expressly noted that there is no discernible rate difference between the cis and trans isomers.

DISCUSSION

The above results manifest a strong,polymer specific, interaction between platinum(II) complexes and polynucleotides even at the dilute concentrations used in this study. Placement of this interaction at the base of the polynucleotide is strongly implied by (a) the evidence from recent spectrophotometric binding studies (14-16), (b) the spectral shifts observed prior to precipitation, (c) the synthesis of (adenine)$PtCl_2$ reported herein, and (d) the non-precipitation of poly(U) which mitigates against interaction at the phosphate or sugar. The sharp break in Figure 2 presumably results from saturation of the coordination sites within a polynucleotide chain at two bases per platinum (Pt/P = 0.5). At higher Pt/P ratios intermolecular bonding occurs, leading to cross-linking and precipitation. This model is consistent with the fact that the time required for precipitation becomes independent of Pt/P at high values of Pt/P since the rate of cross-linking of two chains, each intramolecularly

*High ionic strength presumably counteracts the electrostatic repulsion between $PtCl_4^{2-}$ and the phosphate backbone.

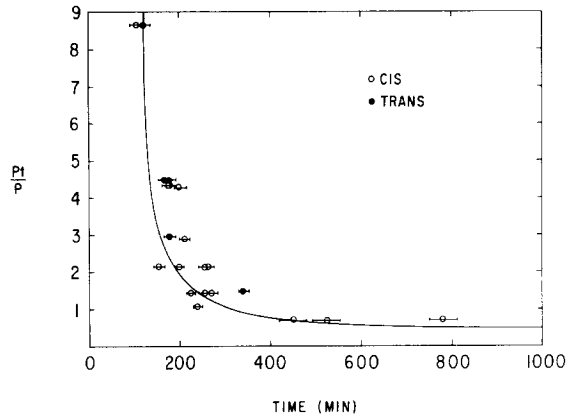

Figure 4. Pt/P vs. time required for precipitation of poly(A) with cis and trans-$Pt(NH_3)_2Cl_2$. Time of precipitation taken as first drop in absorbance after original absorbance decrease due to coordination (see Figure 3, curve C). Conditions: 10 mM $NaClO_4$, 45°, 0.09 mM P.

saturated with platinum, is not expected to be dependent upon the concentration of free platinum in solution.

Our results raise two points of particular relevance to platinum(II) complexes as anti-tumor agents. First, on the basis of the established inorganic chemistry of platinum(II) complexes (17,18), it is generally assumed that chloride ligands may be relatively easily displaced from $[Pt(NH_3)_n(Cl)_{4-n}]^{-2+n}$ complexes (n = 0 to 3), but ammonia ligands are not subject to displacement (1). However, $[Pt(NH_3)_3Cl]Cl$ which cannot cross-link polynucleotide chains without losing a coordinated ammonia precipitates poly(A). Therefore either ammonia ligands are not absolutely inert, or precipitation does not require cross-linking. Ammonia release seems to be the more likely alternative since all observed effects of platinum(II) on polynucleotides in solution, in mammalian cells (5), and in the crystal (vide supra), are consistent with the cross-linking hypothesis Also, our attempts to synthesize $[(adenine)Pt(NH_3)_2]^{2+}$ salts from $Pt(NH_3)_2Cl_2$ have led to products which do not contain ammonia. Since $[Pt(NH_3)_4]Cl_2$ does not cause precipitation even under stringent

conditions, it appears that at least one easily displaced ligand, here chloride (17), is required to allow coordination of a nucleotide which then labilizes the more tightly bound ammonia. Second, the apparantly equivalent reactivities of cis and trans-$Pt(NH_3)_2Cl_2$ towards poly(A) is in marked contrast to the biological specificity for the cis isomer (1-4), but is consistent with the nucleotide-induced ammonia loss proposed above since both cis and trans isomers would lead to the same nucleotide-Pt(II) product. This implies that the physiological distinction between these stereochemical isomers results from a process other than the cross-linking of polynucleotide strands.

ACKNOWLEDGMENT

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