NOTE

[^{82}Br]Cisplatin derivative: A potential biological model for cisplatin.

N.D. TINKER, H.L. SHARMA, & C.A. McAULIFFE[1]

Dept. of Medical Biophysics, Univ. of Manchester, Manchester, M13 9PT;
[1]Dept.of Chemistry, UMIST, P.O. Box 88, Manchester, M60 1QD

Abstract

The complex cis-[$Pt(NH_3)_2{}^{82}Br_2$] has been prepared as a possible model for cisplatin, cis-[$Pt(NH_3)_2Cl_2$]. The stability of cis-[$Pt(NH_3)_2{}^{82}Br_2$] was investigated in 0.9% saline solution and sterile water. In both cases the ^{82}Br label was rapidly displaced in the first hour ($t_{½}$ = 73 min and 96 min respectively). Thus, cis-[$Pt(NH_3)_2{}^{82}Br_2$]cannot be used for determining the fate of the labile ligands of cisplatin.

Key words: bromine-82, cisplatin, bromo-derivative (complex)

Introduction

Cisplatin, cis-[$Pt(NH_3)_2Cl_2$], is an established anti-tumour drug, principally used for the treatment of testicular, ovarian, and head and neck tumours.

The aquation of this complex has been previously studied under conditions of different ambient chloride concentrations,(1) however, the rate of chloride displacement has not been studied in biological fluids, eg. plasma, urine, etc. Where in the body the majority of this displacement occurs (whether in the blood stream, at cell membranes, or in cells) is still unclear. The use of a chlorine radiolabel would be ideal for such studies; unfortunately, although ^{36}Cl can be used for in-vitro experiments involving β liquid scintillation counting, its long half-life (3×10^5 years) and decay characteristics (98.1% β-decay, $E_{\beta max}$ = 0.714 MeV) would make it quite unsuitable for human investigations. Other radionuclides of Cl, such as ^{38}Cl, are very short lived and would not allow time for the compound synthesis and subsequent biological investigations.

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To overcome this problem we have considered the use of radioactive bromine as a substitute for chlorine, since bromine has several reasonably long lived, γ emitting radionuclides (^{76}Br, $t_{½}$ = 16.2 h, ^{77}Br, $t_{½}$ = 56.0 h, and ^{82}Br, $t_{½}$ = 35.3 h). ^{82}Br has been chosen since it can be produced by neutron irradiation of natural bromine.

To use the ^{82}Br labelled complex as a model for cisplatin, the chemical and biological reactivity of cis-$[Pt(NH_3)_2{}^{82}Br_2]$ must be similar to that of cis-$[Pt(NH_3)_2Cl_2]$. Chemically, the Pt-Br bond is of similar strength to that of the Pt-Cl bond, leading to similar rates of reaction. However, Cis-$[Pt(NH_3)_2Br_2]$ has been shown to be more toxic than cisplatin, showing anti-tumour activity at a higher molar concentration.(2)

Experimental

Synthesis of labelled complex:

^{82}Br was produced by an (n,γ) irradiation of a natural sample of NH_4Br, sealed in a silica ampule, at a neutron flux of 1 x 10^{12} n cm^{-2} s^{-1}. The sample was used after 64 hours decay, by which time the other bromine radioisotopes had decayed to levels below detection by a Ge(Li) detector.

Cis-$[Pt(NH_3)_2{}^{82}Br_2]$ was produced according to the analogous synthesis of cisplatin (3) by adding 1.04 g (10.6 mmol) $NH_4{}^{82}Br$ to a solution of cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ (1.06 mmol); yield = 81% (specific activity of complex = 2.1 μCi/mg). An unlabelled sample of the complex prepared under identical conditions gave the analysis: Found (expected) %H 1.5 (1.6), %N 7.2 (7.2), %Br 40.4 (41.1).

HPLC analysis of cis-$[Pt(NH_3)_2{}^{82}Br_2]$ stability:

Cis-$[Pt(NH_3)_2{}^{82}Br_2]$ was incubated in water and in 0.9% saline solution, as outlined below, in order to determine the stability of the complex in these solvents.

a) The labelled complex (8.1 mg, 0.021 mmol) was dissolved in 9.0 ml of 0.9% saline (pre-equilibrated to 37 ^{o}C) prior to immediate incubation at 37 ^{o}C in the dark. Samples (20 μl) were then taken at 5, 16, 30, 45, 60, 90, 120, 150 and 180 minutes after the start of the incubation. The samples were immediately injected onto a C_{18} ODS column (BioRad, 25 cm) which had been pre-equilibrated with

150 ml of 2.7 mM HTAB (Hexadecyltrimethylammonium bromide) solution, followed by 30 ml of 1 x 10^{-4} mM HTAB solution. Samples were eluted with 1 x 10^{-4} mM HTAB solution, using a flow rate of 1 ml/min; column eluant was collected as 0.2 ml fractions (10 ml in total). Samples were monitored using a LKB/Wallace Ultrogamma counter which automatically corrected for background and isotopic decay during counting (window set at 513.9 to 842.9 keV). Areas of the peak(s) present in the elution profile obtained were integrated and converted to nmoles ^{82}Br by comparing with a standard.

b) The labelled complex (6.2 mg, 0.016 mmol) was dissolved in 7.0 ml of sterile water (at 37 ^{o}C) and incubated in the dark at 37 ^{o}C. Samples (20 μl) were removed at 3, 15, 30, 45, 60, 90, 240 and 300 minutes. The samples were then eluted through the HPLC column and treated as above.

Experiments were performed in duplicate. The HPLC column was washed after each experiment with 100 ml of water, followed by 100 ml of acetone, 100 ml of methanol and finally with 200 ml of 50% methanol solution.

Results and Discussion

A sample of cis-[$Pt(NH_3)_2{}^{82}Br_2$] was found to elute from the column between 4.6 and 6.2 minutes, with the peak maximum at 5.2 min (fractions 23-31). Even after 30 min post injection, over 90% of $^{82}Br^-$, injected as $NH_4{}^{82}Br$, remain on the column.

Fig. 1 shows the three ^{82}Br containing peaks obtained in the HPLC elution profile. How the ^{82}Br concentration of each peak varies with time is shown in Fig. 2 (incubation in saline) and Fig. 3 (incubation in water).

In both cases the activity associated with the cis-[$Pt(NH_3)_2{}^{82}Br_2$] peak (peak C) decreases with time (biphasically), most rapidly in the 0-90 minutes period and indicates the displacement of the ^{82}Br label. The rates of decrease in cis-[$Pt(NH_3)_2{}^{82}Br_2$] activity give fast phase $t_{\frac{1}{2}}$ values of 73 min in saline and 96 min in water (5-60 and 3-60 min periods respectively).

The amount of ^{82}Br associated with peak B following incubation in saline increased very rapidly in the first few minutes (0-17% of the 402 nmol ^{82}Br

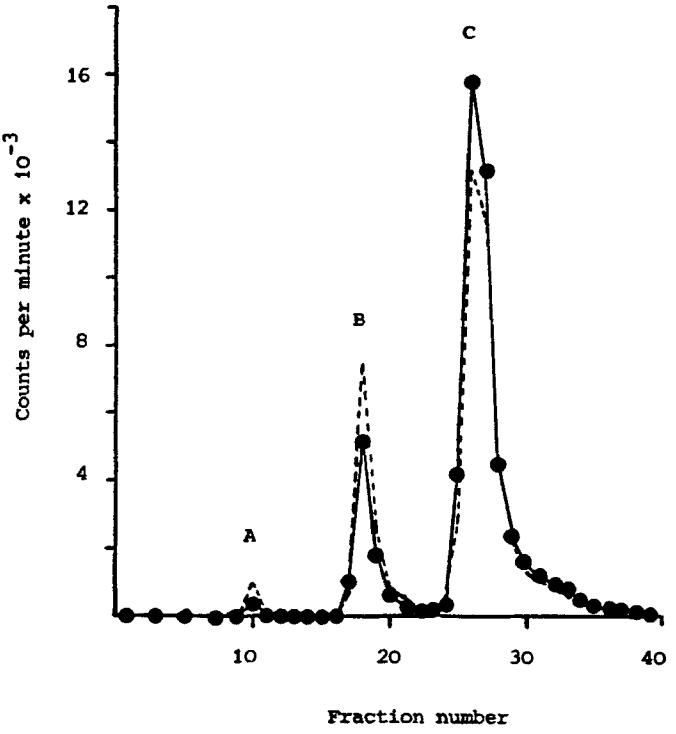

Fig. 1. HPLC elution profile of cis-$[Pt(NH_3)_2{}^{82}Br_2]$ incubated in 0.9% saline at T = 5 minutes (●—●) and T = 30 minutes (- - - -). Peak A = fractions 9-12; peak B = fractions 17-22; and peak C = fractions 24-36.

injected onto the column, in the first 16 minutes) and then more slowly, until it reached a maximum around 90 minutes (25% of the total ^{82}Br injected), after which time there was a slight decline. However, in contrast, on incubation in water, peak B reached its maximum ^{82}Br level by three minutes, and then decreased with time, in a manner similar to cis-$[Pt(NH_3)_2{}^{82}Br_2]$.

The fact that peak B elutes off the HPLC column before cis-$[Pt(NH_3)_2{}^{82}Br_2]$ indicates the presence of a more polar, or positively charged, compound than the parent complex. For the incubation in saline, the fall in ^{82}Br activity associated with the cis-$[Pt(NH_3)_2{}^{82}Br_2]$ peak (100% to 76% in the first 16 minutes) is mirrored by the rise in activity associated with peak B (0-17% in the first 16 minutes). These observations would suggest that the complex giving rise to this peak could be the mono-bromo complex (either cis-$[Pt(NH_3)_2{}^{82}BrCl]$ or cis-$[Pt(NH_3)_2(H_2O){}^{82}Br]^+$).

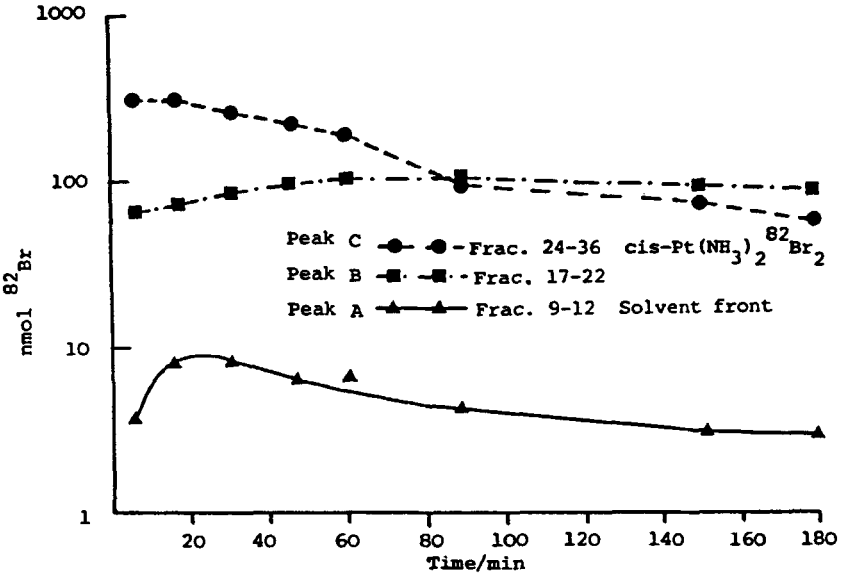

Fig. 2. Variation over 3 hours of the ^{82}Br concentration associated with the three ^{82}Br containing peaks seperated by HPLC (A, B, C), following incubation of cis-[Pt(NH$_3$)$_2$^{82}Br$_2$] in 0.9% saline at 37 ^{o}C.

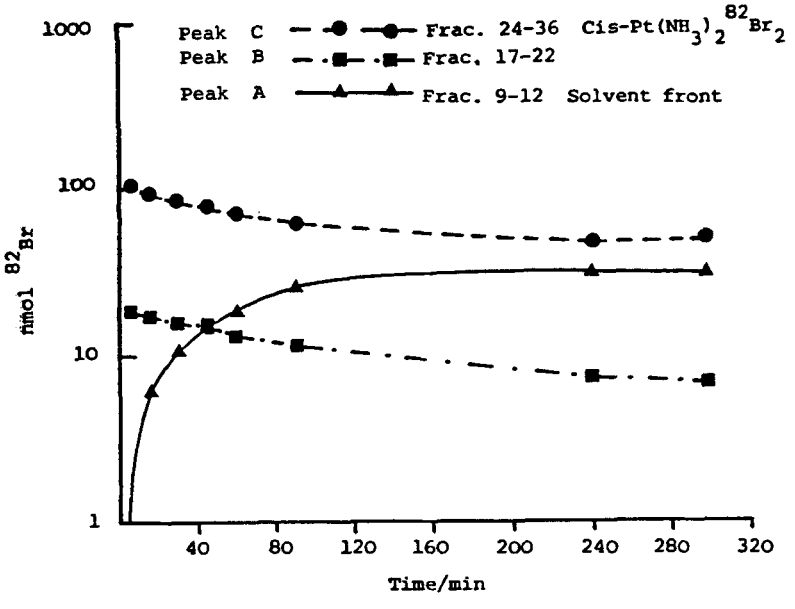

Fig. 3. Variation over 5 hours of the ^{82}Br concentration associated with the three ^{82}Br containing peaks seperated by HPLC (A, B, C), following incubation of cis-[Pt(NH$_3$)$_2$^{82}Br$_2$] in sterile water at 37 ^{o}C.

The amount of ^{82}Br associated with the solvent front was never > 3% of that injected onto the column.

Clearly, there are differences in the rate at which cis-$[Pt(NH_3)_2{}^{82}Br_2]$ reacts in the two solutions, as well as the rate of product formation. The faster rate of ^{82}Br displacement in saline solution indicates that chloride ions are better at displacing Br^- than H_2O, or that solvolysis is aiding this displacement by Cl^-.

Conclusion

This communication reports the synthesis of cis-$[Pt(NH_3)_2{}^{82}Br_2]$ and the subsequent investigation of the possible chloride or water displacement of $^{82}Br^-$, to determine whether or not cis-$[Pt(NH_3)_2{}^{82}Br_2]$ is a usable model for cisplatin.

Cis-$[Pt(NH_3)_2{}^{82}Br_2]$ clearly aquates when incubated in water at 37 °C, and is also unstable in saline solution (and hence biological fluids), unlike cisplatin which is stable in solutions of chloride concentrations greater than 0.9%. Thus, in any biological work the ^{82}Br label will be rapidly displaced and cis-$[Pt(NH_3)_2{}^{82}Br_2]$ cannot be used for determining the fate of the labile ligands of cisplatin.

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