SPECTROSCOPIC EVIDENCE FOR *cis*-[$PtCl_2(NH_3)_2$]-ERYTHROCYTE MEMBRANE INTERACTION

Oswald L. HÖRER and Claude NICOLAU
Stefan S. Nicolau Institute of Virology, Bucharest, Romania

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1. Introduction

cis-Platinum (II) diammino dichloride shows a strong inhibition effect on DNA, RNA and protein synthesis and also a considerable oncostatic activity [1–3]. It emerged that the cell membrane may play a role in the biological response of the cell to this type of compounds [4]. We report here spectrofluorimetric and spectrophotometric investigations on erythrocyte ghosts reacted with this platinum compound.

2. Method

Erythrocyte ghost fragments were prepared using the Dodge, Mitchell and Hanahan method [5] with minor modifications. *cis*-[$PtCl_2(NH_3)_2$] was a gift from Prof. B. Rosenberg (State University of Michigan, East Lansing, Mich.). Control experiments were carried out with tryptophan and phenylalanine (Merck, analytical grade).

Erythrocyte ghost suspensions in distilled water were mixed with aqueous *cis*-[$PtCl_2(NH_3)_2$] solutions, the concentration of ghosts was maintained constant at 0.5 mg dry weight/ml, and stored at room temperature. The pH was kept constant at 5.5 in all systems, with platinum-complex concentrations ranging between 0 and 0.83 mM.

The absorption and fluorescence spectra were obtained in a Shimadzu QV-50 machine with the GF-16E excitation unit and U-100 Pantos Unicorder, chart speed 2.5 cm/min.

The high turbidity of the ghost suspensions required the use of the reflection method in spectrofluorimetry, and the correction of the absorption values for light-scattering calculated by the equation: $k=\tau\lambda^{-n}$, where τ represents the turbidity, k a turbidimetric constant dependent upon concentration, shape, dimensions and optical efficiency of the scattering particles, n being the wavelength exponent, found equal to 2.0 ± 0.1 from transmission data in the 330–390 nm wavelength range in the ghost suspensions, in agreement with the theoretical value corresponding to particles much larger than the wavelength. The k values were calculated, taking into account the absorption of the platinum complex in the range 330–390 nm.

3. Results and discussion

Fig. 1 shows the quenching effect of the *cis*-[$PtCl_2(NH_3)_2$] on the fluorescence of erythrocyte ghost suspensions after 10 min of contact (curve 1) and 24 hr later (curve 2). These data indicate a slower reaction rate than with ovalbumin (curve 3) and the k values have a negative correlation (curve 4) with the relative emission intensity corresponding to curve 2. Comparison of the emission and excitation spectra with the maxima as the same wavelengths (figs. 2 and 3) shows that significant spectral changes occur only below 250 nm. A blue shift in the platinum-complex spectrum in the presence of the membranes is more likely than would be a change in the protein spectrum, tryptophan does not show any quenching of its fluorescence in the same platinum-complex concentration range.

The quenching effect must be therefore attributed to interactions of the platinum complex with amino acid residues in the polypeptide chain which are not

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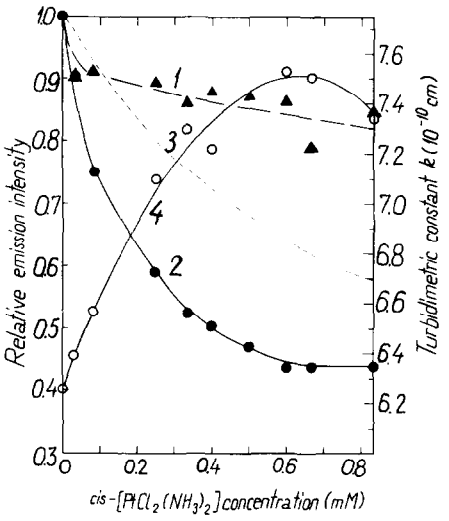

Fig. 1. Fluorescence emission at 350 nm (λ_{exc} = 285 nm) versus *cis*-$[Pt(NH_3)_2Cl_2]$ concentrations of:
(1) Aqueous suspension of erythrocytes after 10 min, contact with *cis*-$[Pt(NH_3)_2Cl_2]$;
(2) the same suspension after 24 hr contact;
(3) an ovalbumin solution after 10 min contact;
(4) turbidimetric constant ($k = \tau\lambda^{-n}$) of the membrane suspension after 24 hr contact (n = 2.0 ± 0.1).

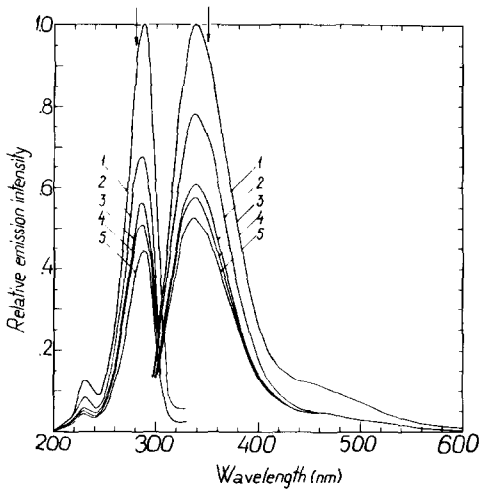

Fig. 2. Fluorescence spectra of erythrocyte membranes in aqueous solutions (λ_{exc} = 280 nm; λ_{emiss} = 350 nm) versus *cis*-$[Pt(NH_3]_2Cl_2]$ concentration (mM): (1) 0; (2) 0.083; (3) 0.25; (4) 0.40; (5) 0.80.

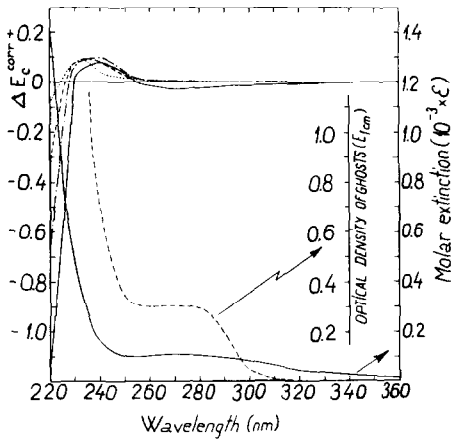

Fig. 3. The optical density ($E_{1\ cm}$) of erythrocyte ghosts (- - - -), the molar extinction coefficient (ϵ) of *cis*-$[Pt(NH_3)_2Cl_2]$ (——) and the corrected difference spectra ΔE_c^{corr} of the erythrocyte membrane–platinum complex mixtures after 24 hr contact. *cis*-$[Pt(NH_3)_2Cl_2]$ concentrations (mM): 0.083 (. . . . .); 0.25 (- - - - -); 0.50 (-.-.-.-.-); 0.83 mM (——).

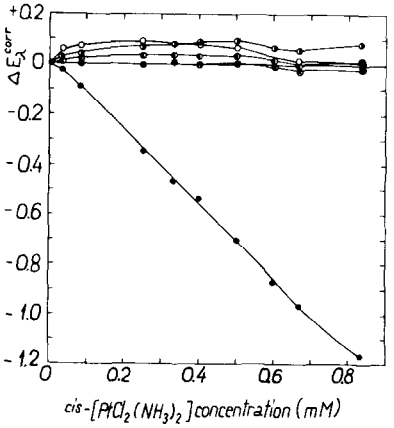

Fig. 4. Corrected differences of the optical density (ΔE_λ^{corr}) of the erythrocyte membranes–*cis*-$[Pt(NH_3)_2Cl_2]$ mixtures after 24 hr contact, in terms of Pt-complex concentrations at several wavelengths (nm): 220, •; 230, ○; 240, ◉; 250, ◐; 260, ⊗.

fluorochromes, but are involved in the secondary and tertiary structures of the proteins.

The approximate linear dependence of the absorption difference at 220 nm, contrary to the values corresponding to higher wavelengths, in terms of the *cis*-$[PtCl_2(NH_3)_2]$ concentration, shown in fig. 4, suggests a specific reaction of the platinum compound, following Bear's law, as related to the blue shift.

The data presented suggest that *cis*-$[PtCl_2(NH_3)_2]$ is to a great extent bound to the cell membrane, before acting inside the cell. This explains the inhibition of active transport in terms of the platinum-complex concentration, especially after a longer contact with this substance [4, 6], due to the slow reaction rate observed.

Acknowledgements

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References

[1] B.Rosenberg, L.Van Camp, J.E.Trosko and V.H.Mansour, Nature 222 (1969) 385.
[2] B.Rosenberg, L.Van Camp, E.B.Grimley and A.T.Thomson, J. Biol. Chem. 242 (1967) 1347.
[3] B.Rosenberg and L.Van Camp, Cancer Res. (1970), in press.
[4] H.C.Harder and B.Rosenberg, (1970) in press.
[5] J.T.Dodge, C.Mitchell and D.T.Hanahan, Arch. Biochem. Biophys. 100 (1963) 119.
[6] Cl.Nicolau, O.Burducea, M.Weiner and N.Mocanu, (1970) in preparation.