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INTERACTION OF PALLADIUM (II) WITH DNA

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Summary

The nature of interaction of palladium(II) with calf thymus DNA was studied using viscometry, ultraviolet, visible and infrared spectrophotometry and optical rotatory dispersion and circular dichroism measurements. The results indicate that Pd(II) interacts with both the phosphate and bases of DNA. The ORD/CD data indicate that the binding of Pd(II) to DNA brings about considerable conformational changes in DNA.

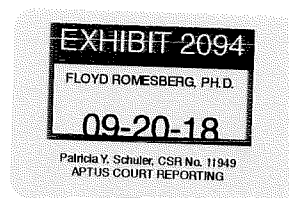
Metal ions play a significant role in biological systems involving nucleic acids and a good volume of literature exists on the subject. Heavy metals like Hg(II) [1–4], Ag(I) [5,6], Cu(II) [7,9], Pt(II) [10–12] and Au(III) [13–15] have been studied in some detail but work on Pd(II) is hardly reported except that by Shishniashvili et al. [16]. It is reported that Pd(II) complexes are anti-tumour active and they bind to the bases of DNA and degrades DNA. Since this type of metal binding is believed not to bring about degradation of DNA, we thought it worthwhile to investigate the nature of interaction of Pd(II) with nucleic acids.

Experimental

Calf thymus DNA was obtained from Swarch Bioresearch Inc. and was used without further purification. The dissolution of DNA was carried out by the addition of small amounts of buffer to DNA at intervals of 4–6 h for 3–4 days at 4°C with gentle stirring of the solution. The stock solution was diluted to the desired concentration when necessary. The concentration of DNA was determined by measuring the absorbance (*A*) at 258 nm.

PdCl₂ was a product of Johnson Matthey Chemicals Ltd., (London). Mainly NaClO₄ · 2H₂O (Fluka, GR) was used as the salt medium. KCN(May and Baker) was used for complexing palladium.

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All measurements were carried out at pH 5.6 using citric acid/NaOH buffer (0.01 M) containing 0.01 M NaClO_4 .

Preparation of PdCl_2 solution in the buffer itself was found to be suitable for the present study. The solutions were kept for 24 h before use. Since PdCl_2 solution absorbs in the ultraviolet region, an equal amount of PdCl_2 was added to the reference solution also to cut off its absorption [20] and the difference spectra were measured.

A UNICAM SP 700A spectrophotometer was used for ultraviolet and visible spectral measurements. The optical rotatory dispersion (ORD) and circular dichroism (CD) measurements were carried out using Model J-20 spectropolarimeter of Japan Spectroscopic Co. Ltd., Tokyo, Japan. All viscosity measurements were carried out at 30°C using a Ubbelohde viscometer.

Results and Discussion

The effect of PdCl_2 on the viscosity of calf thymus DNA (pH 5.6, citrate buffer (0.01), 0.01 M NaClO_4) is shown in Fig. 1. It can be seen from Fig. 1a, that the viscosity (η_{sp}/c value) of DNA upon addition of PdCl_2 decreases with time as in the case of the Au(III) -DNA system [13–15]. The reaction of PdCl_2 with DNA seems to be faster than the Au(III) -DNA system since it takes only about 1 h for the η_{sp}/c vs. time curve to level off in the case of the Pd(II) -DNA system whereas it takes about 8–10 h in the case of the Au(III) -DNA system. Fig. 1b shows the decrease in the η_{sp}/c value of DNA with respect to the value of r ($r = \text{mol of PdCl}_2 \text{ added/mol of DNA-(P)}$), the η_{sp}/c values for each value of r measured after attainment of equilibrium. Addition of KCN (pH adjusted to 5.6 with HClO_4) reversed the viscosity values almost to the original value of DNA, but not completely. A similar situation has been encountered in the

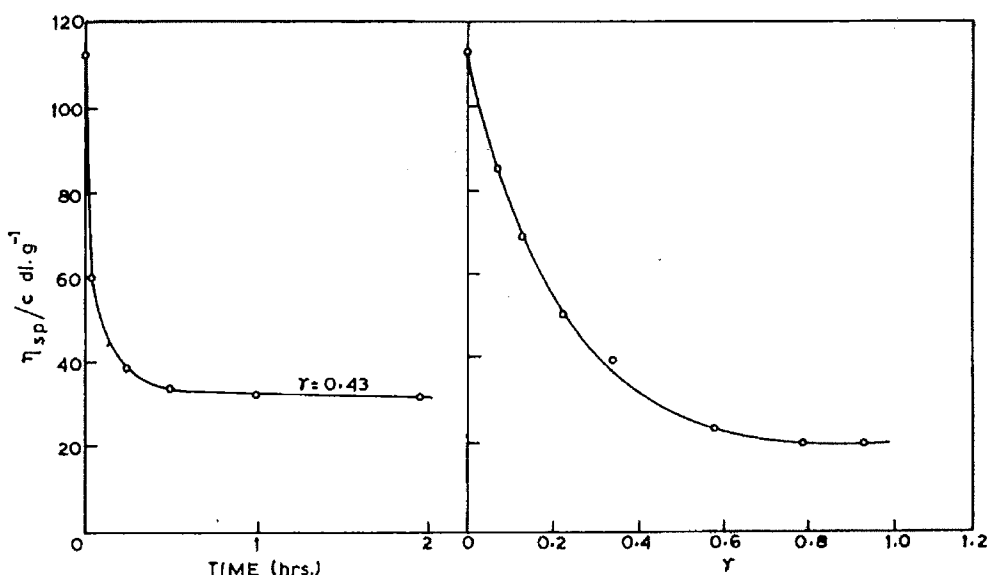


Fig. 1. Effect of Pd(II) on the viscosity of native calf thymus DNA. Concn. DNA $2.84 \cdot 10^{-4} \text{ M(P)}$, citrate buffer (0.01 M), 0.01 M NaClO_4 , pH 5.6. (a) Variation of viscosity with time and (b) Variation of viscosity with r ($r = \text{mol of PdCl}_2 \text{ added/mol of DNA-(P)}$).

Au(III)-DNA system also [13]. This incompleteness might be due to ionic effects of excess KCN and palladium cyanides formed during the reaction. No increase in viscosity was observed when an equivalent amount of KCN was added to PdCl_2 solution in citrate buffer at pH 5.6. This indicates that the reversal of viscosity might be due to the removal of bound Pd(II) from DNA. This type of behaviour is quite general in the case of metal-DNA systems and has been observed in the cases of Hg(II) [1], Ag(I) [5], Pt(II) [11], etc.

Shishniashvili et al. [16] report that PdCl_2 decreases the viscosity of DNA (saline citrate buffer, 0.15 M NaCl) at pH 5.0 and 3.0 and the decrease is due to the result of the degradation of DNA. In the present case, a reversal of the viscosity was observed on addition of KCN and hence denaturation or degradation of DNA cannot be accounted for, because the decrease of viscosity brought about by the addition of PdCl_2 to DNA. The decrease in viscosity might be due to a reaction similar to that of Hg(II) [1] or Au(III) with DNA [13–15].

The ultraviolet spectra of DNA was found to be very much altered on addition of PdCl_2 to DNA (see Fig. 2). There is an increase in absorbance and a shift in the λ_{max} of DNA at 258 nm to 270 nm ($r = 0.62$). Since the spectra change slightly with time upon addition of PdCl_2 , the spectra reported here are

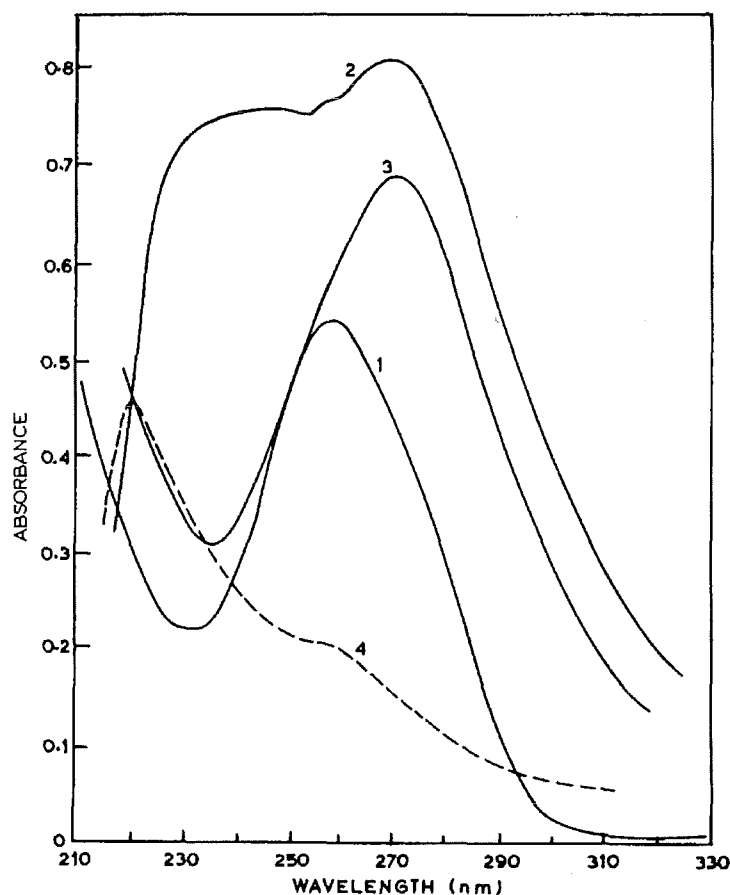


Fig. 2. Ultraviolet spectra of DNA-Pd(II) system. Concn. DNA $8.06 \cdot 10^{-5}$ M(P) citrate buffer (0.01M), 0.01 M NaClO_4 , pH 5.6, (1) DNA alone, (2) with PdCl_2 in the sample alone ($r = 0.62$), (3) with PdCl_2 in both sample and reference ($r = 0.62$), (4) PdCl_2 alone in citrate buffer.

measured when the system reached equilibrium, say after about 24 h. PdCl_2 in citrate buffer also absorbs in the same region and has a small peak near 258 nm. Fig. 2(2) was obtained by adding PdCl_2 to DNA alone whereas Fig. 2(3) obtained when equal amounts of PdCl_2 was added to both the sample and reference compartments. When the absorbance of PdCl_2 solution alone (mainly at 260 nm and 270 nm) (Fig. 2(4)) was subtracted from Fig. 2(2), Fig. 2(3) was obtained. Hence the spectrum 2(2) could be a real representation of the effect of PdCl_2 on DNA and perturbation of the electron dense regions of the bases by Pd(II) is thus indicated.

PdCl_2 in citrate buffer containing 0.01 M NaClO_4 exhibits a peak at 381 nm (Fig. 3). Addition of NaCl shifts the band to higher wavelength. For example, addition of NaCl equivalent to 0.01 M NaCl shifted the band to 391 nm. A number of species of chlorocomplexes are reported to exist in solution depending upon the chloride ion concentration and pH of the solution [18]. Only one species PdCl^{2-} exists in the absence of excess NaCl i.e., when only NaClO_4 is present in solution. Addition of DNA to this solution was found to shift the peak to 369 nm. The peak became almost a shoulder and the absorbance decreased. Addition of DNA to the higher chlorocomplexes of Pd(II) for example, to the system at 391 nm, also brought the peak back to 369 nm indicating that the binding of DNA is independent of the species of Pd(II) present in solution. This type of a blue shift is reported to be quite general when one passes from a chloro complex to an amine complex [19–21]. A blue shift was observed when DNA was added to Cu(II) in the visible region and was interpreted as due

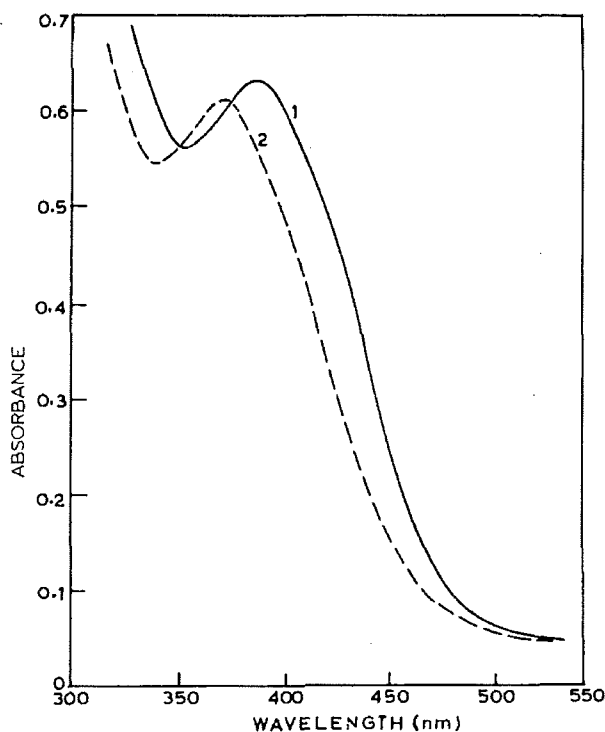


Fig. 3. Visible spectra of DNA- Pd(II) system. Conc. of PdCl_2 $3 \cdot 10^{-3}$ M, citrate buffer (0.01 M), 0.01 M NaClO_4 , pH 5.6, (1) PdCl_2 alone, (2) with DNA, $r = 0.62$.

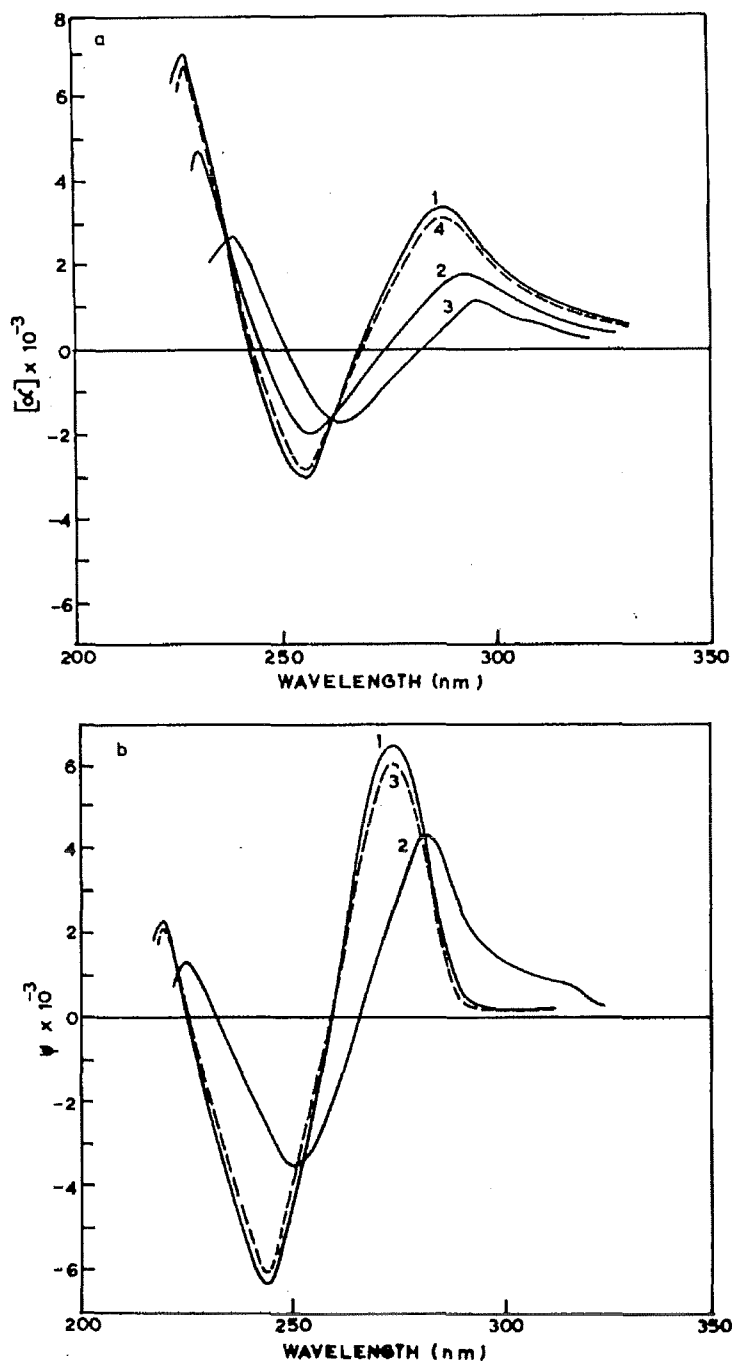


Fig. 4. (a) The optical rotatory dispersion spectra of native calf thymus DNA in the presence of Pd(II) ions: (1) DNA alone, concn. $1 \cdot 10^{-4}$ M(P), acetate buffer (0.01 M), 0.01 M NaClO_4 , pH 5.6; (2) With PdCl_2 , $r = 0.40$; (3) with PdCl_2 , $r = 0.62$ and (4) after addition of KCN to (3). (b) Circular dichroism spectra of native calf thymus DNA in the presence of PdCl_2 : (1) DNA alone, conditions same as (a); (2) with PdCl_2 , $r = 0.62$; (3) after addition of KCN to (2).

to the formation of copper-nitrogen bonds [22]. The infrared spectra of Pd(II)-DNA complex ($r = 0.2$) obtained by precipitating with alcohol showed a decrease of the band at 1242 cm^{-1} . This might give some indication as to the binding of Pd(II) to the phosphate [30], but a more definitive statement can only be made after T_m and X-ray studies are carried out.

The ORD and CD curves of DNA were also altered considerably on addition of PdCl_2 (Fig. 4). The measurements in this case were carried out in sodium acetate/acetic acid buffer (0.01 M) containing 0.01 M NaClO_4 . The magnitude of the ORD and CD maxima are considerably reduced with a red shift of the higher wave lengths peaks. A shift of about 7–8 nm has been noticed. Addition of KCN reverses these effects and the original spectra of DNA were obtained.

The ultraviolet spectrum (Fig. 1) indicate binding of Pd(II) to the bases of DNA. This is expected to modify the electronic state of the bases and will be reflected in the interaction of their dipoles in the polymer thus affecting the shape and magnitude of the cotton effects [6]. Unstacking of the bases of polynucleotides is thought to result in diminished magnitudes of cotton effects [24]. Similarly disruption of hydrogen bonds between the base pairs of double stranded polynucleotides may give rise to a red shift in the position of the maxima [24]. Fig. 4a and 4b show that binding of Pd(II) to DNA results in the reduction of ORD and CD bands accompanied by red shifts. These results suggest that stacking interactions are reduced considerably and the Watson and Crick hydrogen bonded double helical system has been disturbed at least in the regions where binding of Pd(II) takes place.

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