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Kinetic and thermodynamic investigation of the (dien)Pd(II)-polycytidylic acid system

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The reaction between (dien)$PdCl^+$ and polycytidylic acid was studied using spectroscopic and stopped-flow methods. In neutral solution, the palladium complex binds at the N3 site of the cytosine base and causes a noncooperative disruption of the ordered helical structure of poly(C). Interaction at the phosphate group of the polynucleotide was also demonstrated by using the dye acridine orange as an indicator. The results of this study show that the mechanism previously proposed for cytidine and CMP can be applied to poly(C), taking into account particular features of the polymer (polyelectrolytic nature, structure, etc.). In particular, electrostatic effects seem to play a major role in the interaction with metal ion complexes like (dien)Pd(II).

1. Introduction

Our laboratory is interested in the interaction between nucleic acids and metal ion complexes. Although the determination of thermodynamic parameters is of interest, the main goal of our studies is to contribute to the knowledge of dynamic and kinetic aspects of these systems. Palladium was chosen as metal ion because its behavior is similar to that of the well-known antitumor platinum complexes. Furthermore, it presents the advantage of reacting 10^5-times faster than the very slowly reacting Pt(II) complexes [1,2].

Since DNA is a rather complex molecule with numerous possible structures and binding sites, the interaction of metal ions with the simpler constituents, nucleosides and nucleotides, has been mostly studied [3–7]. In a previous paper, we reported the results of our study on the (dien)Pd(II) interaction with cytidine (C) and cytidine 5′-monophosphate (CMP) [8]. The reaction mechanism has been established and implies the participation of the solvent in the process (via a (dien)$Pd(H_2O)^{2+}$ intermediate) as well as an intermediate formed between (dien)Pd(II) and the phosphate group of CMP. Interaction of poly(C) with Pt(II) complexes has been studied previously with emphasis being placed on the determination of its thermodynamic parameters [9–11]. The present work describes the interaction of (dien)Pd(II) with poly(C) from both thermodynamic and kinetic points of view. Since poly(C) is a homopolynucleotide of the cytosine base, comparison with cytidine and CMP will elucidate the effects arising from the polymeric nature and the structure of poly(C) on both the kinetics and mechanism.

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2. Experimental

2.1. Materials

Polycytidylic acid (potassium salt) was a high-purity product from Sigma and was used without further purification. The minimum molecular weight was estimated at 100000. The palladium complexes [(dien)PdCl]Cl, [(dien)Pd(H_2O)]-(ClO_4)$_2$ and [(dien)Pd(H_2O)](NO_3)$_2$ were prepared as described previously [8,12]. Acridine orange (3,6-bis(dimethylamino)acridine), obtained as the hydrochloride from Eastman Kodak, was purified by repeated crystallization in a methanol/ether mixture.

Stock solutions of poly(C) and [(dien)PdCl]Cl were kept at 6°C and used shortly after preparation. Concentrations of the polynucleotide solutions are given in terms of mononucleotide units. Since acridine orange is photosensitive and can be adsorbed on glass, stock solutions of the dye were kept in opaque teflon bottles. Experiments involving acridine orange were performed using plastic labware, including polypropylene spectrophotometric cells from Sarstedt Canada. Ionic strength was adjusted using $NaClO_4$ (prepared from high-purity $HClO_4$ (Biopharm) and NaOH (Fisher Scientific). No buffer was used in this study due to its possible interaction with the palladium complex. The temperature was maintained at 25.0 ± 0.1°C throughout the experiments, except for the circular dichroism measurements.

2.2. Methods

Absorption spectra were recorded using a Perkin-Elmer model 552 spectrophotometer equipped with a digital temperature controller. Circular dichroism (CD) was used to investigate conformational changes of poly(C). CD spectra were obtained, at room temperature (21–23°C), using a Cary model 61 spectropolarimeter. Kinetics were studied by means of a Dionex model D-130 stopped-flow apparatus, interfaced with an Apple II plus computer. The experimental set-up has been described earlier [8]. Since low concentrations of reactants were used, reversibility of the reactions was taken into account in order to obtain reliable values of the second-order rate constants.

3. Results

3.1. Equilibrium measurements

Due to the limited solubility of poly(C) and especially of its product formed upon reaction with (dien)Pd(II), the absorption band of (dien)Pd(II) cannot be used to study this system. However, due to the higher molar absorptivity of poly(C), its absorption band could be used at much lower concentrations (5×10^{-5} M). Even under these conditions, a precipitate was frequently observed when in the presence of sufficient amounts of (dien)Pd(II) to neutralize the negative charges of poly(C). Therefore, experiments could not be carried out using an excess of the metal.

The absorption band maximum of poly(C) is located at 268 nm. Addition of (dien)PdCl$^+$ leads to a small increase in absorbance and a shift of the maximum to higher wavelengths. These variations are similar to those observed for cytidine and CMP [8]. Since in these cases complexation is known to occur at the N3 site of cytosine [8,13–16], the result with poly(C) suggests that the same site is involved. The reaction can be represented by eq. 1.

$$\text{poly(C)} + \text{(dien)PdCl}^+ \overset{K_1}{\rightleftharpoons} \text{(dien)Pd-poly(C)}^{2+} + \text{Cl}^- \qquad (1)$$

The equilibrium constant for eq. 1, K_1, has been determined by monitoring changes in absorbance due to progressive dissociation of the poly(C) complex upon addition of Cl^- to a (dien)Pd^{2+}-poly(C) solution. By taking into account the presence of (dien)Pd(H_2O)$^{2+}$ at low Cl^- concentrations, a value of $K_1 = 800 \pm 150$ was obtained. This value compares well with those obtained for cytidine and CMP (300 and 500, respectively).

In neutral solution, the phosphate groups are deprotonated ($pK_a \simeq 1$ [17]) and poly(C) adopts

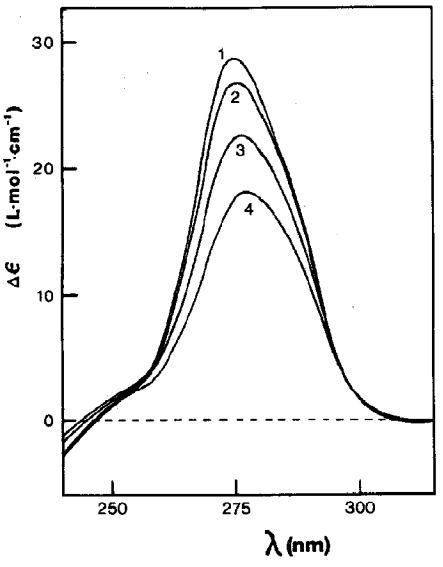


Fig. 1. CD spectra of poly(C) in the presence of various (dien)Pd(II) concentrations. Conditions: 0.100 mM poly(C), 0.10 mM NaCl, 0.20 M $NaClO_4$, pH 7. [(dien)PdCl]Cl concentration: (1) 0, (2) 0.010 mM, (3) 0.030 mM, (4) 0.050 mM.

an ordered single-stranded helical structure [18–21]. This is shown by the presence of a strong positive band at 275 nm in the CD spectrum (fig. 1). A decrease in intensity, with a shift of the maximum to 277 nm, is observed when (dien)Pd (II) is added to the solution. The shift of the maximum is due to the effect of (dien)Pd(II) fixation on the absorption band of poly(C). The intensity decrease of the CD band indicates that metal complexation disturbs the stacking of cytosine bases and disrupts the secondary structure of poly(C). A similar effect was observed for the interaction of poly(C) with *cis*- and *trans*-$(NH_3)_2PtCl_2$ [9]. The decrease in intensity with increasing (dien)Pd(II) concentration has shown no cooperative disruption of the ordered structure of poly(C).

The effects of pH on absorbances of poly(C) and (dien)Pd(II)-poly(C) systems are shown in fig. 2. In the case of poly(C), two transitions are observed. At pH ≈ 5.6, cooperative formation of a semi-protonated double-stranded structure is

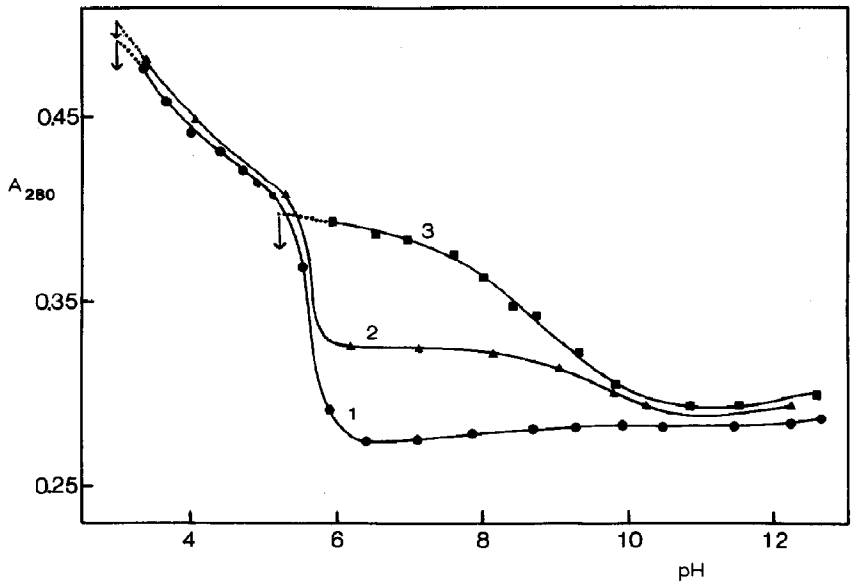


Fig. 2. Effect of pH on the absorbance at 280 nm of the (dien)Pd(II)-poly(C) system. Conditions: (1) 0.070 mM poly(C), 0.10 M NaCl, 0.10 M $NaClO_4$; (2) 0.050 mM poly(C), 0.050 mM [(dien)PdCl]Cl, 0.10 M NaCl, 0.10 M $NaClO_4$; (3) 0.050 mM poly(C), 0.025 mM $[(dien)Pd(H_2O)](ClO_4)_2$, 0.20 M $NaClO_4$. Arrows indicate the pH at which precipitation occurred.

observed while at pH 4, complete protonation leads to dissociation of the two strands, in accordance with literature data [18,22,23]. At low pH, precipitation occurs due to charge neutralization.

In the presence of (dien)Pd(II) (curves 2 and 3 in fig. 2), the behavior both above and below pH 5.6 is affected. When 23% of N3 sites of poly(C) are covered by (dien)Pd(II) (calculated using K_1), double-stranded helix formation is still possible by lowering the pH (curve 2). The presence of 0.1 M Cl^- facilitates proton displacement of (dien)Pd(II) from the N3 sites and allows formation of the semi-protonated poly(CH^+) · poly(C). If no Cl^- is added to the sample, 47% of N3 sites are complexed at pH near 7 and the net charge of the (dien)Pd(II)-poly(C) complex is already close to zero. Addition of a few protons to metal-free N3 sites is sufficient to neutralize the polymer and precipitation occurs at a relatively high pH (curve 3). In both cases, formation of thermodynamically stable (dien)Pd(II)$(OH)^+$, with dissociation of the metal-polynucleotide complex, leads to a decrease in absorbance at high pH.

3.2. Kinetic measurements

3.2.1. Medium effects

The effect of pH and Cl^- concentration on the rate constant of the reaction between the palladium complex and poly(C) is illustrated in fig. 3. A decrease in the reaction rate with increasing Cl^- concentration is observed. This suggests that the mechanism proposed for cytidine and CMP, implying the formation of a more reactive (dien)Pd$(H_2O)^{2+}$ intermediate [8], is also valid in the case of poly(C).

The behavior of the system upon variation of the pH is also very similar to that encountered with the nucleoside and nucleotide analogues. Outside the region pH 6–9.5, the absorbance changes during the reaction are very small, indicating that small amounts of the Pd-N3 complex are formed under these conditions. The decrease in rate constant at pH > 7 can be attributed to the presence of the less reactive (dien)Pd$(OH)^+$ species at high pH. Buth at pH < 6, the decrease is too significant to be caused only by protonation of the N3 sites of the bases. This protonation leads to a decrease of electrostatic interaction which can also cause a decrease in rates. Furthermore, as discussed before, formation of the double-stranded poly(CH^+) · poly(C) also occurs in this pH region and can affect the kinetics.

Interesting effects are observed by varying the ionic strength of the solution. In fig. 4, rate constants are shown along with the results obtained with CMP and cytidine (curves 2 and 3) [8]. First, we observe that at low ionic strength, reaction with poly(C) is much faster than with cytidine or CMP. Poly(C) is a polyelectrolyte and thus possesses a very strong electrostatic field. Since palladium complexes are positively charged, this leads to a strong increase in the reaction rate as compared to the monomer analogues. For the same reason, the rate of reaction is strongly influenced

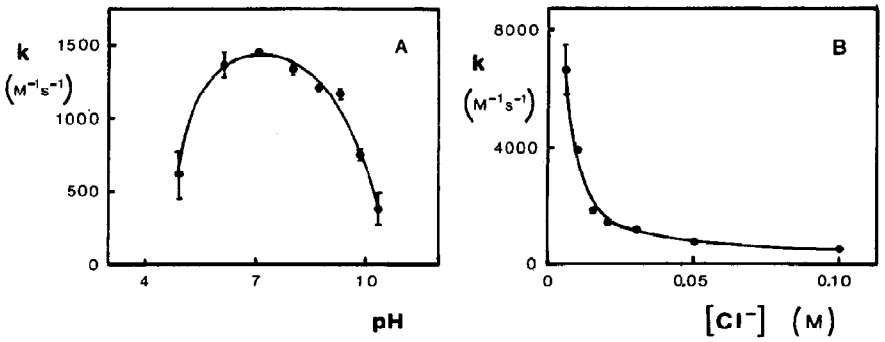


Fig. 3. Effect of pH (A) and of Cl^- concentration (B) on the second-order rate constant for the reaction between (dien)Pd(II) and poly(C). Conditions: (A) 0.050 mM poly(C), 0.050 mM [(dien)PdCl]Cl, 0.02 M NaCl, 0.18 M $NaClO_4$; (B) 0.050 mM poly(C), 0.050 mM [(dien)Pd(H_2O)]$(ClO_4)_2$, ionic strength constant (0.20 M NaCl + $NaClO_4$), pH 7.

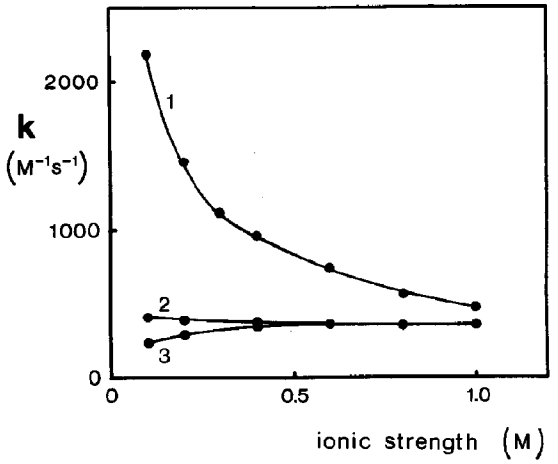


Fig. 4. Ionic strength effect on the second-order rate constant for the reaction between (dien)Pd(II) and poly(C) (1), CMP (2) and cytidine (3). Conditions: (1) 0.050 mM poly(C), 0.050 mM [(dien)PdCl]Cl; (2) 5.00 mM CMP, 0.500 mM [(dien)PdCl]Cl; (3) 5.00 mM cytidine, 0.500 mM [(dien)PdCl]Cl. In all three cases, 0.020 M NaCl, pH 7, was present. The ionic strength was adjusted with $NaClO_4$.

by the presence of various ions in solution. The shielding effect of Na^+ and its condensation on poly(C) contribute to the decrease of the rate constant when the ionic strength of the solution is increased. These effects result from long-range electrostatic attraction without actual fixation of ions to the phosphate sites.

3.2.2. Interaction with the phosphate group

Interaction of (dien)Pd(II) at the phosphate group of cytidine 5′-monophosphate has been demonstrated in a previous work [8]. To verify the presence of such an interaction with poly(C), acridine orange (AO) was used as an indicator. This dye, which is positively charged at pH 7, aggregates on the polynucleotide chain and is released when (dien)Pd(II) binds to poly(C) [12]. Therefore, it can be used to monitor the reaction between (dien)Pd(II) and poly(C). As shown in fig. 5, a decrease in AO concentration leads to a gradual increase of the rate constant k_{AO}, except when the ratio [AO]/[poly(C)] falls below 0.5, where a very strong increase of the rate of AO release is observed. However, extrapolation of the data at high AO concentration to [AO] = 0 leads to the same rate constant as for the reaction in the absence of AO. Similar effects were observed for other experiments under various conditions, but the strong increase in reaction rate always occurs at low dye-to-polymer ratios. This result is explained by considering the reactions depicted in scheme 1.

When the ratio [AO]/[poly(C)] is high (reaction A), all phosphate sites can be considered as being occupied by AO molecules. Reaction of the Pd(II) complex occurs at the N3 site of poly(C) and the introduction of positive charges in the

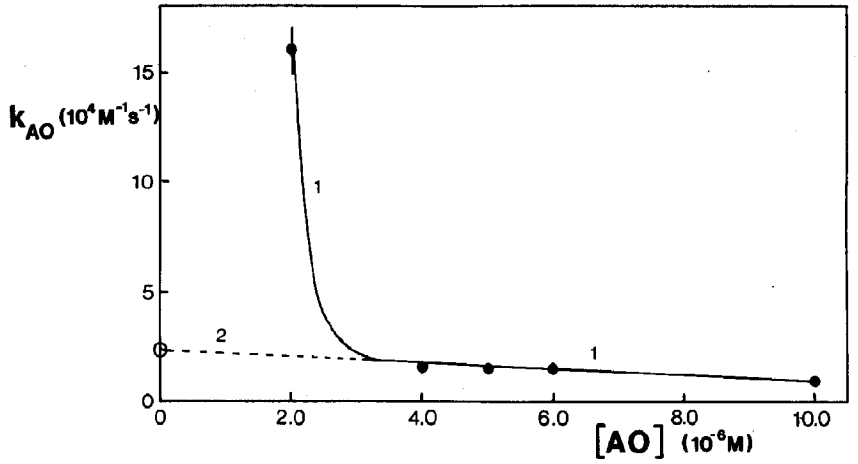
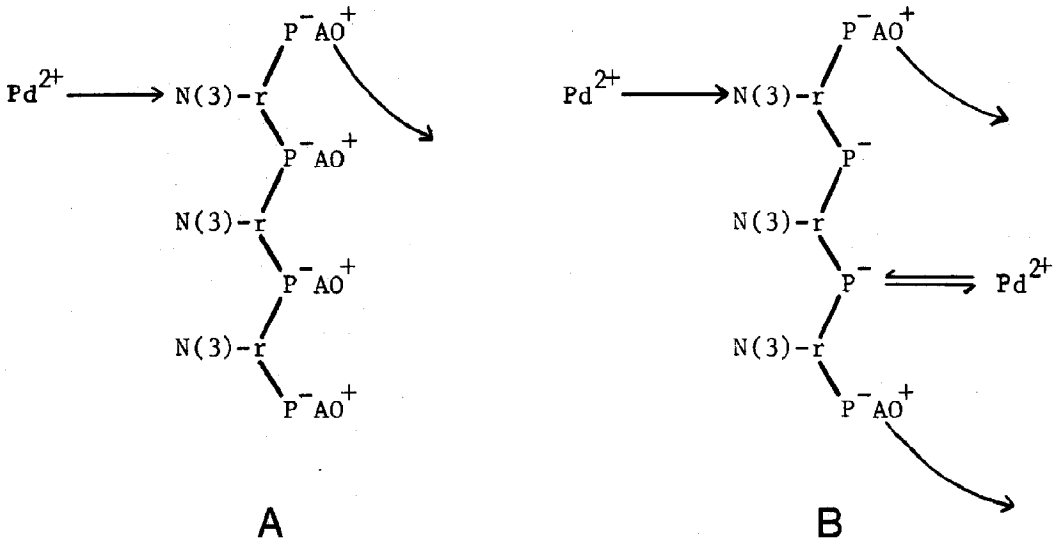


Fig. 5. Influence of acridine orange concentration on the second-order rate constant for the reaction between (dien)Pd(II) and the AO-poly(C) complex. Conditions: (1) 0.0050 mM poly(C), 0.0025 mM $[(dien)Pd(H_2O)](NO_3)_2$, 0.001 M NaCl, 0.10 M $NaNO_3$, pH 7. (2) Extrapolation of the data to [AO] = 0. The circle at [AO] = 0 was obtained for the following conditions: 0.050 mM poly(C), 0.025 mM $[(dien)Pd(H_2O)](ClO_4)_2$, 0.001 M NaCl, 0.10 M $NaClO_4$, pH 7 and no AO.



Scheme 1.

vicinity of the dye-binding site causes the release of AO molecules. This release can be followed spectrophotometrically (at 492 nm) and the results indicate that the rate of AO release corresponds to the rate of (dien)Pd(II) binding to the cytosine base [12]. In the presence of low amounts of AO (reaction B in scheme 1), free phosphate groups are available for direct interaction with the Pd(II) complex. The strong increase in observed rate can be explained by this very rapid interaction of (dien)Pd(II) with the phosphate site, which also leads to liberation of adjacent AO molecules.

Further evidence of an interaction at the phosphate group was obtained in a similar study with sodium polyphosphate. This polyelectrolyte, containing only phosphate units, also binds AO and addition of (dien)Pd(II) leads to the release of dye with observable spectral changes. The rate constant for this process is very high as expected (1.5×10^7 M^{-1} s^{-1} at $\mu = 0.001$ M and in the absence of Cl^-).

4. Discussion

The results of this study illustrate the effects of the macromolecular nature of polynucleotides on their interactions with metal ion complexes. The general behavior of the (dien)Pd(II)-poly(C) system is very similar to that observed with the monomers cytidine and CMP [8]. The results of equilibrium measurements and the influence of pH and Cl^- concentration on the reaction rate indicate that the mechanism described for cytidine also applies in the case of the polymer. However, significant effects, which are specific to poly(C), can be attributed to its polyelectrolyte nature.

The thermodynamic study shows that the complex formed between (dien)$PdCl^+$ and poly(C) is more stable than those with cytidine and CMP. The values of the equilibrium constants K_1 are, in order: poly(C) (800) > CMP (500) > C (300), and reflect the increasingly negative electrostatic contribution to the ΔG^0 of product formation when going from cytidine to poly(C). This is due to the strong electrostatic field of the polyelectrolyte. These effects are already significant at relatively high ionic strength (0.20 M) and an even stronger stabilization of the complex with poly(C) is expected at lower ionic strength.

The kinetic studies also illustrate the importance of electrostatic factors, as shown by the 5.5-times higher reactivity of (dien)Pd(II) with poly(C) than with CMP (at $\mu = 0.10$ M). A similar increase in reactivity for a polynucleotide was also observed for the reaction of a Pt(II) complex with

various adenine derivatives [24]. For CMP, electrostatic attraction already caused an increase in rate as compared to cytidine [8]. This effect is much greater with poly(C) (fig. 4) due to the much stronger electrostatic field created by the high density of negative charges on the polymer chain. Consequently, the presence of other ions has a strong influence on the reaction rates with poly(C), as demonstrated by the effect of ionic strength in fig. 4.

CD measurements have shown that metal binding produces a noncooperative disruption of the regular structure of poly(C), an effect which was observed previously for the binding of Pt(II) complexes [9]. This can be attributed partly to steric factors and partly to repulsions between positively charged Pd(II) complexed to neighboring cytosine bases. Furthermore, metal fixation partially neutralizes the negative charges of the phosphates which increases the flexibility of the polymer. It is less clear, however, whether the helical structure of poly(C) has an influence on the kinetics of reaction with (dien)Pd(II). The presence of ribose, phosphate and other nucleotide units in the neighborhood of the binding site for (dien)Pd(II) could slow down the reaction. The results show, on the contrary, that the reaction is faster with poly(C) than with CMP. This does not rule out, however, the existence of a steric effect but only indicates that the rate-increasing electrostatic effect is the dominant one. Moreover, the influence of the structure should not be too important considering that poly(C) is a single-stranded polynucleotide where the binding site, N3, is still very accessible for the Pd(II) complex.

Evidence of interaction with the phosphate group of poly(C) was obtained using the dye AO as an indicator. In the absence of dye, the reaction at this site cannot be monitored due to the lack of spectral change in the absorption band of poly(C). The fact that phosphate interaction is present does not mean that the mechanism proposed for CMP still applies quantitatively in the case of poly(C). With CMP, the phosphate complex ((dien)Pd-phosphate) can be substituted by another CMP molecule to give the more stable Pd-N3 product. This latter reaction is most probably absent in the case of poly(C) due to steric factors hindering the approach of two polynucleotide chains or two parts of a polynucleotide chain. Transfer to the N3 site is more likely to proceed via dissociation of the phosphato complex.

The influence of AO binding on the kinetics of the (dien)Pd(II)-poly(C) reaction is, by itself, significant. Even though AO and (dien)Pd(II) do not bind at the same site, the presence of increasing amounts of dye bound to poly(C) leads to a decrease in the rate of attack by the metal, due to charge neutralization by protonated AO molecules. Thermodynamically, binding of the Pd(II) complex at the cytosine base is not prevented by the presence of AO and leads to the release of AO molecules. The results underline further the importance of electrostatic factors in poly(C) interactions. These considerations are very important, since within the cell nucleus, DNA is associated with basic proteins (histones) [25]. The anionic character of nucleic acids is thus partly neutralized by positively charged amino groups of these proteins.

Acknowledgements

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