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Interaction of (dien)Pd(II) with Double-stranded Poly(I)·poly(C): a Kinetic and Structural Investigation

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Single and double-stranded homopolynucleotides are useful models for elucidating the interaction of DNA with metal ions. The (dien)Pt(II)–poly(I)·poly(C) system was studied by Guschlbauer *et al.* and has shown a stabilizing effect of the metal complex on the polymer, while (en)PtCl$_2$ led to destabilization [1–3]. In this paper it will be shown, using (dien)Pd(II), that the effect of the metal complex depends largely on the experimental conditions. The effects of the structure of poly(I)·poly(C) on the kinetics of complexation are also discussed.

Experimental

Stock solutions of poly(I)·poly(C) (Sigma Chemical Co.) were prepared by dissolving known amounts of the polymer in a 0.1 M NaClO$_4$ solution. Concentration of poly(I)·poly(C) is given in terms of mononucleotide units. [(dien)PdCl]Cl was prepared as described previously [4]. All solutions were kept in the dark at 6 °C. Absorbance measurements and melting temperature experiments were done using a Perkin-Elmer model 552 spectrophotometer equipped with a programmable digital temperature controller. Melting temperatures (T_m) were determined by monitoring the absorbance changes at 250 nm with increasing temperature (1 °C/min). T_m was evaluated as the maximum in the differential curve (dAbs/dT). Circular dichroism (CD) spectra were recorded on a CARY model 61 spectropolarimeter at room temperature (21–23 °C). Experimental set-up and methods for kinetic studies have been previously described [4].

Results and Discussion

The kinetic study of the (dien)Pd(II)–poly(I)·poly(C) system shows that at least two consecutive reactions are present. The first has a rate constant of $(2.8 \pm 0.4) \times 10^5$ M^{-1} s^{-1} at low chloride concentration (0.001 M) and is attributed to initial binding at N7 of hypoxanthine, similar to the case of Pt(II) complexes [1, 2, 5, 6]. The N7 site of poly(I) was shown to be kinetically favored over the N3 site of poly(C) [7, 8]. The rate of this reaction decreases with increasing chloride ion concentration, in agreement with the mechanism established using model compounds [4, 7, 8]. The second reaction is about ten times slower, with a first order rate constant of 0.52 s^{-1}, and is independent of chloride ion concentration in the range 0.001 M to 0.10 M. This slower reaction is attributed to the redistribution of the metal to other sites on the polymer (N3 site of cytosine, deprotonated N1 site of hypoxanthine) with opening of the hydrogen-bonded base pairs. However, conformational changes could also produce these observed changes in absorbance. At [Cl$^-$] > 0.005 M, signals of the two reactions interfere, which makes the determination of the rate constants more difficult even though the decreasing trend of the rate constant for the first reaction is clearly observed.

The rate constant for the reaction of (dien)Pd(II) with poly(I) ($(2.1 \pm 0.2) \times 10^5$ M^{-1} s^{-1}) is slightly lower than for poly(I)·poly(C) ($(2.8 \pm 0.4 \times 10^5$ M^{-1} s^{-1}). This can be explained by the stronger electrostatic field due to higher density of negatively charged phosphate groups in the double-stranded poly(I)·poly(C). Its N7 site, being oriented towards the outside of the double helix in its major groove, is readily accessible, which should minimize unfavorable steric effects. This explains the dominance of the electrostatic effects whose importance was previously shown by the higher rates of reaction of (dien)Pd(II) with polynucleotides as compared with the corresponding monomers [7, 8].

Figure 1 shows the influence of (dien)Pd(II) on the melting temperature of poly(I)·poly(C) under various conditions. In the presence of 0.2 M NaClO$_4$ (curve 2) a small increase in T_m is observed at low r (= [(dien)Pd(II)]/[poly(I)·poly(C)]) values. This stabilization is attributed to partial neutralization of negative charges of the phosphate groups upon complexation of the positively charged (dien)Pd(II) to the neighbouring hypoxanthine bases. The electrostatic repulsions between strands are thus decreased and the double-stranded structure stabilized. In the presence of Mg^{2+} ions at the same ionic strength, poly(I)·poly(C) is more stable (T_m = 72.2 °C) than in presence of only Na$^+$ (T_m = 64.8 °C), due to the greater affinity of Mg^{2+} for the polynucleotide phosphate [9]. In this case (curve 1), addition of (dien)Pd(II) cannot greatly improve the neutralization of the negative charges (in fact, it may even

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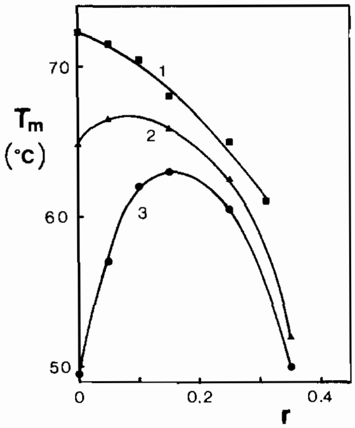

Fig. 1. Influence of (dien)Pd(II) on the melting temperature of poly(I)•poly(C). [Mg^{2+}] = 0.01 M, μ = 0.2 M (1); μ = 0.2 M (2); μ = 0.01 M (3). [poly(I)•poly(C)] = 1.00 × 10^{-4} M, [Cl^-] = 10^{-4} M.

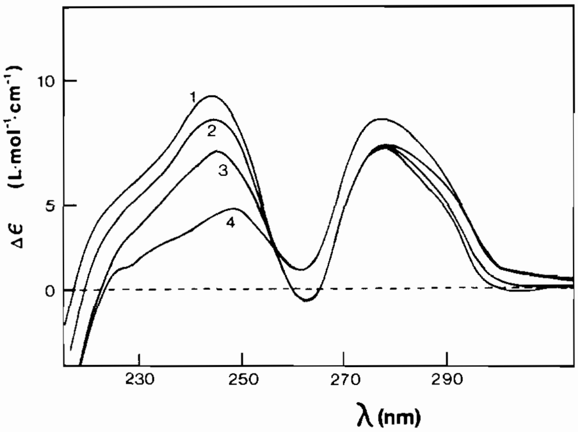

Fig. 2. CD spectra of poly(I)•poly(C) at various r values: 0 (1); 0.05 (2); 0.15 (3); 0.25 (4). [poly(I)•poly(C)] = 2.00 × 10^{-4} M.

cause the release of some Mg^{2+} ions), and binding of the metal complex has only a destabilizing effect. Finally, in conditions where the polymer is inherently less stable (0.01 M $NaClO_4$, T_m = 49.5 °C at r = 0), a strong stabilizing effect by (dien)Pd(II) can be observed, as shown by curve 3 in Fig. 1 (ΔT_m = 13.5 °C at r = 0.16).

Stabilization of double-stranded polymers by analogous (dien)Pt(II) has been observed previously [1, 2, 10]. For poly(I)•poly(C), this effect was attributed to hydrogen bonding between the dien ligand and hypoxanthine bases adjacent to the binding site of the metal complex [2]. Our results suggest that the nature of the stabilization is mainly of the electrostatic type. Hydrogen bonding with neighbouring bases as well as with the phosphate groups may, however, have a small contribution to the overall effect.

Circular dichroism spectra of poly(I)•poly(C) show a decrease in the poly(I) portion of the spectra (positive band near 244 nm) upon addition of (dien)-Pd(II) (Fig. 2). This implies a disruption of hypoxanthine base stacking due to steric factors and repulsions between doubly charged (dien)Pd(II)s on adjacent bases. Rupture of hydrogen bonds between bases is also possible. These perturbations have a destabilizing contribution to the overall stability of the polymer. The ellipticity changes observed are slightly more important than those obtained with (dien)Pt(II) [2] but less than those with $enPtCl_2$ which causes greater disruption [3]. At low r values, the disruption of the nucleic base orientations is small and the electrostatic factors predominate. With increasing amounts of (dien)Pd(II) complexed to poly(I)•poly(C), the perturbation of the double-helical structure becomes stronger and wins over the potential electrostatic stabilization. This explains decreases in T_m at higher r values for all curves in Fig. 1. In these conditions, where significant fractions of sites are occupied by the Pd(II) complex, a partial dissociation of the two strands leading to the formation of free poly(C) loops is evidenced by a small increase in the positive band at 277 nm in the CD spectrum for r = 0.25. Such local openings without total dissociation of the two strands have been proposed for the binding of Pt(II) complexes to DNA and poly(I)•poly(C) [11, 12].

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