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Interaction of aromatic ionen oligomers with DNA

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Abstract. A novel polycationic ionen was synthesized and fractionated on carboxymethyl-Sephadex using a salt gradient in 7M urea. A series of oligomers of discrete length were characterised by ultraviolet spectra. The ultraviolet spectra of oligomers revealed a new band centred at 232.5 nm which was probably due to exciton splitting. Thermal denaturation studies indicated both stabilization of the helix conformation and a higher degree of cooperativity in the melting of DNA $(oligomers)_n$ complex as compared to native calf thymus DNA. Ionen oligomers exhibited large extrinsic Cotton effect at 232.5 nm which could be attributed to exciton interaction.

Keywords. Ionen-oligomers; DNA; melting temperature; circular dichroism; exciton splitting.

Introduction

It is well known that various types of polycationic ionens interact with nucleic acids which are polyanions (Mulimani *et al*., 1977; Day *et al*., 1978; Mulimani and Day, 1980, 1981). Polycationic ionen was synthesized and its interaction studied with DNA. Ionen exhibited an extrinsic Cotton effect attributable to the ordered arrangement of the aromatic chromophore along the DNA helix (Bhat *et al*., 1977). The interaction between DNA and ionen polymers was examined to determine the binding of cationic polymers with DNA dependent on the charge density of the polycation (Mita *et al*., 1977). Interaction of oligopeptides with DNA showed that the peptides which contain aromatic amino acids at the C terminus markedly decreased the specific visocity of DNA. Based on these as well as proton magnetic resonance studies of oligopeptide-DNA complexes, ''nonclassical intercalation'' was proposed (Gabbay, 1978) whereby the aromatic residues of oligopeptides were partially inserted between base pairs of DNA, leading to bending of the helix at the point of intercalation.

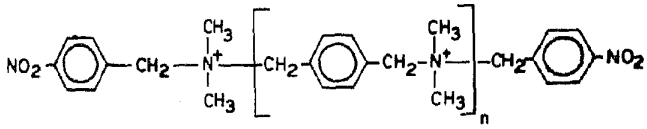
This paper describes the synthesis, fractionation and ultraviolet (uv) characterization of polycationic ionen and the interaction of ionen oligomers with DNA by thermal denaturation and circular dichroism (CD).

Abbreviations used: uv, ultraviolet; CD, circular dichroism.

Materials and methods

Synthesis of p-nitrophenyl blocked polymer

Dimethyl *p*-nitrobenzylamino bromide methylene *p*-phenylmethylene dimethyl amine bromide *p*-nitrotoluene was made by blocking the ends of polymer with a



chromophore that absorbs light at longer wavelengths than the polymer, so that the length could be determined from the absorption spectrum. The ionen was synthesized according to the procedure of Rembaum *et al.*, (1967, 1968). Dimethylamine passed through a solution of *α*-bromo *p*-nitrotoluene (0.01 mol) and 0.01 mol of α, *a*'-dibromo *p*-xylene were dissolved in approximately 50 ml of dimethylformamide. The pale yellow precipitate which appeared almost immediately was filtered, washed with dimethyl formamide and acetone and dried. The product was characterised by ultraviolet and nuclear magnetic resonance (nmr).

Polycationic ionen (170 mg) was applied to CM Sephadex column (4.3×3.5 cm) equilibrated with 0.3 M NaCl and eluted with a gradient of 0.3 M to 0.8 M NaCl (figure 1). Peak fractions in the elution profile were characterised by their uv spectra (figure 2 and 3). It is clearly evident from a comparison of the uv spectra of oligomer fractions that the absorbance at 220 nm was due to phenylene chromophore. A new band centered at 230.5 nm appeared in the case of oligomers (n>1) and increased in relative intensity with each successive oligomer fraction and became a sharp spike at n>3 (figure 3). The absorbance at 220 nm divided by the absorbance at 263 nm gave the proportional relative numbers (n) of phenylene and *p*-nitrophenyl groups in the oligomer fractions.

DNA was purchased from Sigma Chemical Co., St. Louis, Missouri, USA as its sodium salt (sodium deoxynucleotide or calf thymus DNA highly polymerised). Sodium deoxynucleate (1 mg/ml) was added to sodium chloride 0.01 M and sodium citrate 0.001 M pH 7 buffer) over a few drops of carbon tetrachloride hereafter referred to as buffer A. DNA was estimated spectrophotometrically assuming $\varepsilon=6.6\times10^{3}cm^{-1}M^{-1}$ at 260 nm. The A_{260}/A_{330} ratio of this solution was 2.3. The components of the reaction mixture were added in the following order: DNA (7.5×10^{-5}), sodium chloride (1 M), EDTA (0.001 M) and ionen oligomer. The total volume of the mixture was made upto 4 ml by diluting with SSC buffer A. The ionen oligomer and DNA ratio is represented by r. Before determining the melting temperature (T_m) the uv absorbance of the mixture was measured using a Gilford spectrophotometer model 240. Thermal denaturation studies were carried out in microcuvettes using a Gilford spectrophotometer equipped with thermo-programmer, model 2527. The samples were heated from room temperature to 100°C at a rate of 1°C/min.No correction for thermal expansion was made to the absorbance readings in routine experiments. CD spectra of DNA-ionen oligomers at different r in buffer A was obtained using Cary 60 spectrophotometer with model 6002 CD attachment.

Results and discussions

Separation of oligomers with polycationic ionen by CM sephadex was complete for all oligomers (figure 1). It is evident from the elution profile that nine main peaks

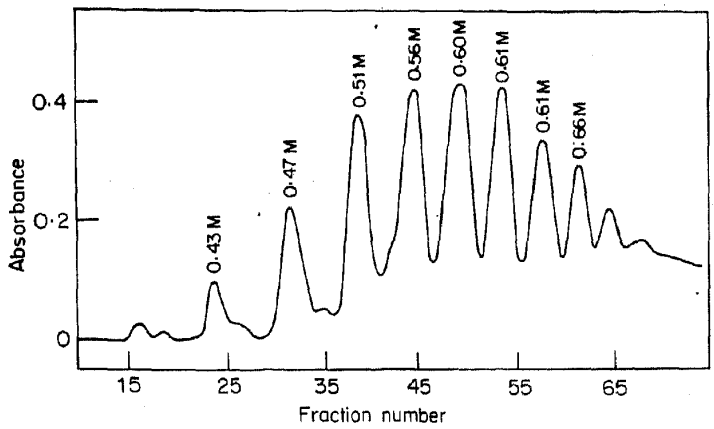


Figure 1. Separation of oligomers from ionen on CM-Sephadex with salt gradient. Numbers above the peaks are the concentrations of sodium chloride.

were well separated and two were not. The uv spectra of the oligomers revealed a new peak at 232.5 nm that did not appear in the spectrum of monomers (figures 2 and 3). The melting profiles of the complexes formed between DNA and oligomer (n=7) at different r are shown in figure 4.$(Oligomers)_n$ contribute towards raising the T_m values of all molecules or every portion of DNA. The melting band at

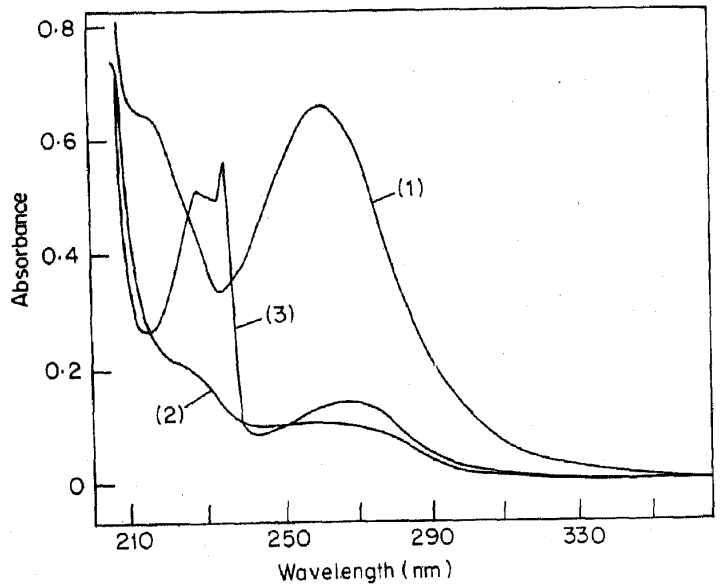


Figure 2. 1) UV spectrum of fraction no 25 (n=1).
2) UV spectrum of fraction no 34 (n=2).
3) UV spectrum of fraction no 49 (n=3).

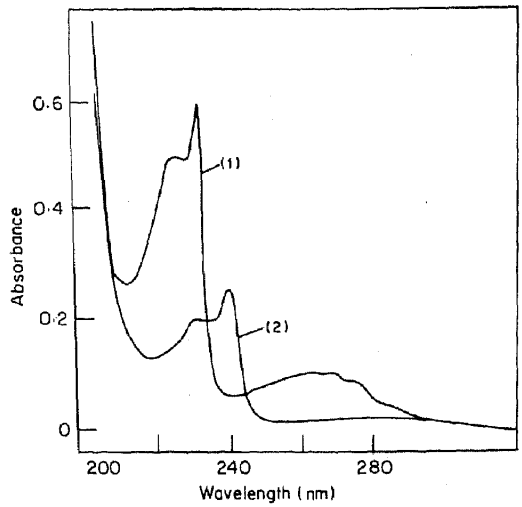


Figure 3. 1) UV spectrum of fraction no 54 (n=4)
2) UV spectrum of fraction no 63 (n=7).

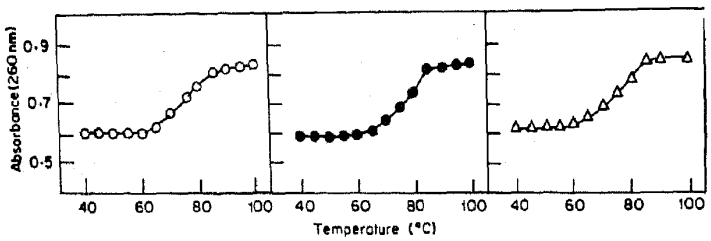


Figure 4. Melting profile of DNA oligomer n=7.
O=0.05, ●=0.1, Δ=0.2.

70°C (T_m) corresponds to the melting of native DNA. The melting band appears at 75°C when oligomer (n=7) is added at the different ratio. Addition of oligomers (n=4 and 7) to DNA gave an extrinsic band at 232.5 nm and lowered the positive lobe centred at 275 nm until at higher r values it became slightly negative (figures 5 and 6).

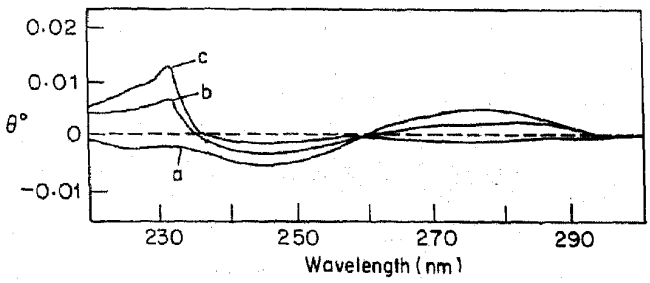


Figure 5. CD- spectra of oligomer n =4 with calf thymus DNA.
a) DNA; b) r=0.21; c) r=0.35.

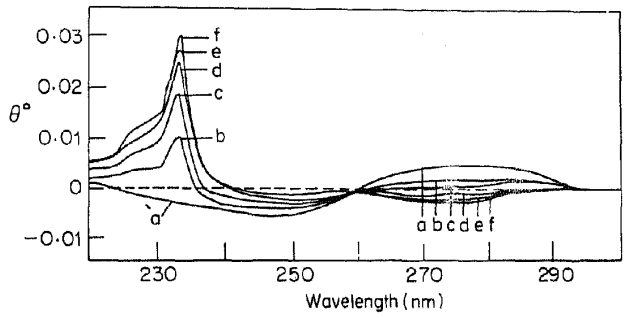


Figure 6. CD spectra n=7 with calf thymus DNA.
a) DNA; b) r=0.077; c) r=0.144; d) r=0.216; e) r=0.288; f) r=0.36.

The uv spectra of oligomers revealed a new band centred at 232.5 nm that did not appear in the spectrum of monomers. This can be explained by the phenomenon of exciton splitting due to resonance interactions between excited states of weakly coupled aggregate systems (Kasha 1963). Interaction between the transition dipoles of identical unit molecules in a regular array yields discrete exciton states, only a very few of which can be reached by allowed transitions. This gives rise to striking effects in the absorption spectrum of aggregates as compared to a unit molecule such as band splitting or shifts and hyper or hypochromism. This concept has been applied to molecular crystals dye aggregates in solution as well as polymers (Kasha 1963, Davydov 1962, Levison *et al.,* 1957). Exciton splitting was predicted for helical polypeptides and it was subsequently shown that α helix is hypochromic as compared to the random coil and exhibits a shoulder to the side of the peptide band absorbance at 190 nm (McRae and Kasha 1958, Moffitt 1956). The uv spectra of nucleic acids have also been analyzed in terms of exciton splitting. According to generalised selection rules for helical polymer the light absorption due to the component of the transition dipoles of the interacting chromophores polarised parallel to the helix axis will be red shifted in comparison to monomer (Kasha 1963). The new band at 230.5 nm and its shoulder must arise from transitions of the phenylene chromophore below 210 nm. The oligomers and polymer would be expected to maintain a regular conformation in solution due to intramolecular hydrophobic interactions of the aromatic groups.

The melting band at 70°C represents the melting of free base pairs of DNA not bound by oligomers. The melting temperature of the free base pair increases after addition of oligomers. Thus thermal denaturation indicated both stabilization of the helix conformation and higher degree of cooperativity in the melting of DNA—$(oligomer)_n$ complex as compared to native calf thymus DNA. The data presented here are consistent with the hypothesis (Gabbay, 1978) which lead to an understanding of various aspects of DNA-polycation interactions as reflected in melting profiles. Previous reports of thermal denaturation of $(Arg)_n$ (Kawashima *et al.,* 1969) and $(Ornithine)_n$ (Kawashima and Toshio, 1978) with DNA complexes also have noted an increase of T_m of free base pairs when basic oligorhers were added to DNA. The large extrinsic Cotton effect induced by the oligomers at 232.5 nm is attributable to exciton interaction. The chiral interaction of two or

more isolated but spatially close chromophores give rise to Davydove split (Harada *et al.,* 1975). The sign of the first Cotton effect is a consequence of the dihedral angle between the interacting electric dipole vectors. If the angle is less than 180°C (in a clockwise sense) the sign of the first Cotton effect is positive and if it is greater than 180° (clockwise) the sign of the first Cotton effect will be negative. The intensity of the Cotton effect is inversely proportional to the square of the interchromophoric distances if the angle between the chromophores is constant. Change in dihedral angle affects the CD amplitude which is zero at 0° and 180° and for vicinal dibenzoate reaches a maximum at 70°C. The second Cotton effect is quite often minimal or absent which is attributed to the asymmetric pattern of the corresponding electronic absorption band (Harada *et al.,* 1975). The exciton chirality method would predict a positive extrinsic band if the oligomer were aligned in one of the grooves of the polynucleotide and this is exactly what is observed. The free oligomers in solution show no CD signal. They are in helical conformation but without the constraint of the right handed polynucleotide helix the oligomers will exist as a racemic mixture of right and left handed helical conformation.

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