INTERACTION OF NOVEL POLYCATIONS WITH NUCLEIC ACIDS

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ABSTRACT

Polycations are linear and contain aromatic and quaternary salt units. It is well known that various types of polycations interact strongly with nucleic acids which are polyanions. Thermal denaturation studies indicated both stabilization of the helix conformation and a high degree of cooperativity in the melting of DNA-polycation complex as compared to native calf thymus DNA. Tm values of DNA-polycation complex decreased linearly with increasing urea concentration. The stability of complex is influenced by both hydrophobic forces and electrostatic interactions. Two polycations are used as pair of CD structural probes for aiding in the characterization of DNA and chromatin. The probes can provide both quantitative and qualitative data with respect to secondary structure and may reflect the amount of available binding sites in the major and minor groove regions of the DNA. One of the polycation is used as potential probe for telestability in DNA. A series of oligomers are isolated from novel polycation. Polycation oligomers exhibited large extrinsic Cotton effect at 232.5 nm which could be attributed to exciton interactions.

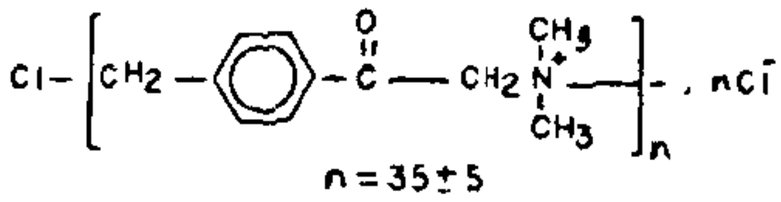
INTRODUCTION

THE recognition of nucleic acid structures and base sequences by proteins play a very fundamental role in all living cells[1-6]. It is recognised that the interactions specificity between the two macromolecules is a problem of immense complexity and involves numerous types of forces operating at several sites along the nucleic acids and protein chains. To reduce the complexity of native systems, extensive use of models has been made. In recent years several basic polypeptides containing lysine, arginine and different neutral aminoacids have been probed to elaborate the properties of proteins that interact with DNA[5]. A number of simple cationic compounds have been shown to stabilise native DNA against thermal denaturation presumably by preferential binding to native DNA helix. These compounds include spermine, spermidine, diamines, polylysine and polyarginine[7, 8]. Thermal denaturation studies have been widely utilised to probe the interactions of basic proteins and other polycations with DNA[9, 10]. Circular dichroism studies have been used extensively to investigate the secondary structure of DNA and to correlate CD data with x-ray diffraction patterns of DNA fibres[11, 12].

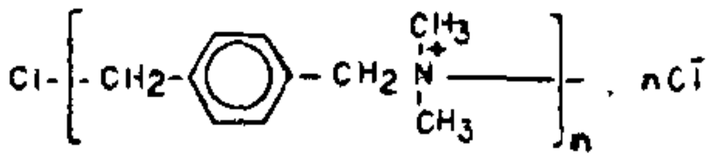
This review is primarily based on the work in our laboratory on the interaction specificities of novel polycations (*i e.* polycationic ionens) with DNA, chromatin and polynucleotides. It is shown that considerable information can be obtained concerning the dynamic and structural aspects of Watson-Crick helix.

The following novel polycations were synthesised according to the procedure of Rembaum and coworkers[13, 14] and Hoover[15].

Bhat *et al*[16] have reported the preliminary investigation of the CD and melting temperature behaviour of novel DNA-polycation complex formed by the interaction of Calf thymus DNA and the polycation, 2-bromo-poly [-methylene 1,4-phenylenecarbonyloxyethylene-(dimethylamino)-chloride] (I). Thermal denaturation studies indicated both stabilization of the helix conformation (Δtm 21°C) and a high degree of cooperativity in the melting of DNA-polycation complex as compared to native Calf thymus DNA. The extrinsic Cotton effect observed is attributed to the ordered arrangement of the aromatic chromophores along with the DNA helix. Mita *et al*[17] have examined the interaction between DNA and polycation (VI) in order to know that the binding with DNA depends on the charge density of polycation. 3-3 polycationic ionens bind cooperatively with DNA, while 6-6, and 6-10 polycations bind noncooperatively. The cooperative binding of 3-3 polycations and noncooperative binding of 6-6 polycations were also supported by thermal melting studies. They have concluded that the charge density at DNA-phosphate is a critical value in determining whether the polycations bind to the DNA by cooperative or by noncooperative binding, since the distance between successive cationic charges of 3-3 polycation is shorter than that between successive phosphate charges on DNA double helix and those of 6-6 and 6-10 polycations are longer. Melting temperature behaviour of a novel DNA-polycation (III) formed



(I)



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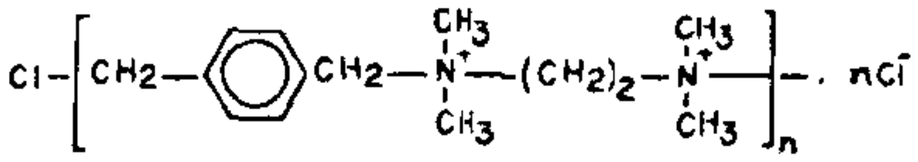
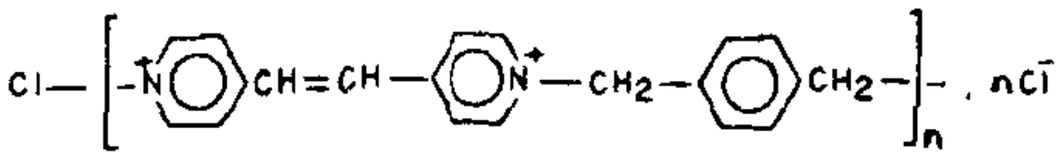


(III) n = 10



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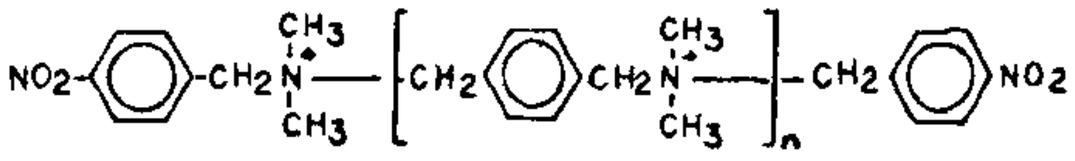

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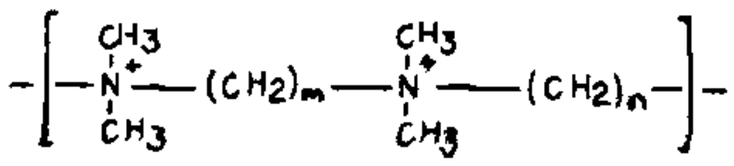
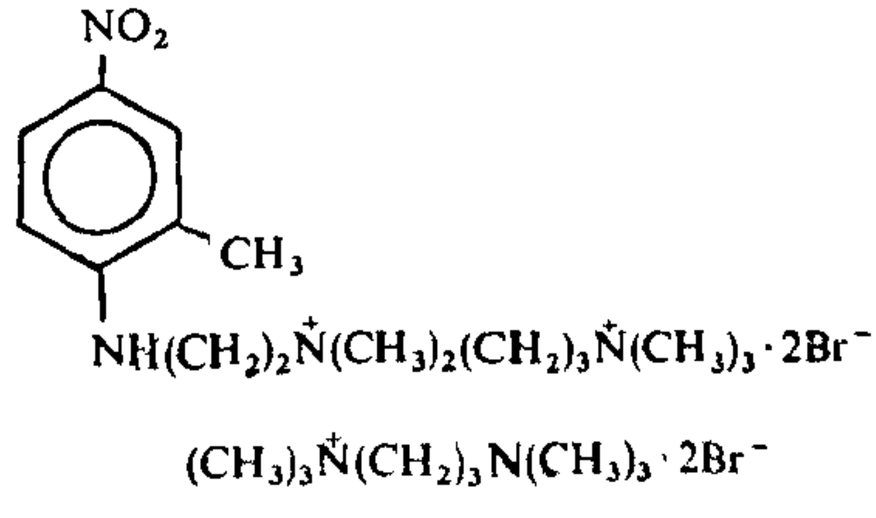


(VI) m-n = 3-3, 6-6

Figure 1. The derivative melting profile of DNA-polycation III ($r = 0.461$) complex at varying concentrations of sodium chloride. Closed circle represents 0.05 N NaCl, closed triangle for 0.1 N NaCl and closed square for 0.2 N NaCl.

by the interaction of Calf thymus DNA and polycationic ionen has been studied by us[18]. Both stabilization of helix conformation and a high degree of cooperativity in the melting of DNA-polycation complex as compared to native Calf thymus DNA have been observed. The stability of the complex is influenced by the electrostatic interactions.

Studies in our laboratory have shown that the polycation, chloro -poly- (methylene -*p*- phenylene-methylenedimethylaminoethylene-diethylaminedichloride) (III) and DNA give melting profile sensitive to the variation in r (polycation/DNA phosphate), salt strength and urea concentration[19–26]. Biphasic melting profiles were observed at $r = 0.231$ and 0.1 M sodium chloride while monophasic profiles were observed at $r = 0.231$ at higher/lower concentrations of sodium chloride in the presence of urea. In the absence of urea only monophasic melting profiles were observed. The two bands in the profiles were ascribed to complexed and free regions of DNA. The maximum cooperativity in melting was observed at 0.4 M urea. At higher value of $r = 0.461$, the profile was monophasic under all conditions studied, where all of the DNA was complexed effectively. The low concentration of urea was assigned the role of catalysing the rearrangement of a more random, kinetically determined complex to a thermodynamically favoured less random structure characterised by two discrete regions. Thermal denaturation of DNA in the presence of novel polycations (I & III) have been studied at varying concentration of sodium chloride leading to sharpened differential profiles which reflect higher degree of cooperativity in the melting of DNA-polycation complex (figure 1). Higher concentrations of urea decrease Tm of DNA-polycation complex (figure 2). This decrease in Tm may be due to the fact that urea functions by destroying intramolecular hydrogen bonds and hydrophobic interactions which may be involved in maintenance of tertiary structure of DNA-polycation[27]. Both electrostatic and hydrophobic interactions probably influence the stability of DNA-polycation complex. 5 M urea treated DNA-polycation complex gives the melting of free DNA regions[16]. The addition of 5 M urea to the complex resulted in the elimination of extrinsic band without affecting the CD, thereby suggesting that hydrophobic interactions influence the stability of DNA-polycation complex. Frishman *et al*[23] have shown that urea functions as the structure breaker even in extremely low concentrations possibly due to their competition for hydrogen bonding. Aslanyan *et al*[28] observed during their study on the effects of urea on



Reporter Molecules

Figure 2. The derivative melting profile of DNA-polycation III ($r = 0.461$) in 0.15 M NaCl in the presence of various concentrations of urea. Closed triangle represents 7 M urea, closed square for 5 M urea and closed circle for 0.5 M urea.

Calf-thymus DNA and phage T_2 by CD and thermal denaturation, a linear decrease in Tm values of DNA with increase in the urea concentration and thus concluded that urea destabilises A-T and G-C base pairs to different extents. Since urea has not been shown to bind directly to DNA, the observed effects are probably due to the disruption of hydration shell of DNA by urea[29]. The helical secondary structure of DNA has been reported to be destabilised by increasing concentration of urea[30]. A novel polycation was synthesised and fractionated on carboxymethyl sephedex using a salt gradient in 7 M urea. A series of oligomers of discrete length were characterised by ultraviolet spectra[31]. A new band centered at 230.5 nm appeared in the case of oligomer ($n > 1$) and increased in relative intensity with each successive oligomer fraction and became a sharp spike at $n > 3$. The absorbance at 220 nm divided by the absorbance at 263 nm gave the proportional relative number (n) of phenylene and *p*-nitrophenyl groups in the oligomer fractions. The ultraviolet spectra of oligomers revealed a new band centered at 232.5 nm which was probably due to exciton splitting. Thermal denaturation studies indicated both stabilization of the helix conformation and high degree of cooperativity in the melting of DNA-$(\text{oligomer})_n$ complex as compared to native Calf thymus DNA. Polycation oligomers exhibited large extrinsic Cotton effect at 232.5 nm which could be attributed to exciton splitting[31, 32]. The changes caused by oligomer binding in the long wavelength portion of poly d(AT) and DNA are indicative of conformational change in the polynucleotide form B to C[33]. The polycation (III) formed a complex with DNA. The complex was stabilised to thermal denaturation by millimolar concentration of $LiClO_4$, in the range of 0.01-0.1 N $LiClO_4$, no hyperchromicity was observed in the thermal profiles and instead hypochromicity was observed. The complex showed no significant extrinsic CD band but showed $LiClO_4$ dependent alterations in the DNA intrinsic bands. It was found that Li^+ was primarily responsible for the stabilization of the complex. It is proposed that Li^+ stabilization arises through ternary complex formation and that the chaotropic ClO_4^- catalyses conversion of less stable DNA-polycation complex into a more stable form[34, 35].

More useful information is obtained in our laboratory about the use of aromatic polycationic iones as probes for chromatin structure[36–38]. Two polycations (II & IV) formed complexes with DNA and chromatin with extrinsic CD bands and reduced intrinsic bands. Purified *n*-mers of II provided UV spectroscopic evidence that the CD band arose from exciton splitting The induced chirality in II & IV are consistent with the organization of the chromophores in the major and minor grooves respectively, with chromatin II gave a negligible extrinsic band, IV gave $\Delta\epsilon$ 345 upto 70% of the DNA induced value. The complex extrinsic band pattern was altered. In conclusion we have described a pair of CD structural probes for aiding in the characterization of DNA and chromatin. The probes can provide both quantitative and qualitative data with respect to secondary structure and may reflect the amount of available binding sites in the major groove of the DNA.

Lin *et al*[39] have reported polycation as the potential probe for telestability in DNA. Polycationic polymer poly-[methylene-*p*-phenylene methylene dimethylaminoethylene dimethylamine dibromide] (III), interacts with DNA in SSC buffer and gives two kinds of useful CD data: (1) the DNA-III complex exhibits an intense extrinsic band at 232.5–233 nm which is near UV maximum of III. (2) The intensity of both intrinsic and extrinsic CD band vary with ratio (r = DNA/III) in a way suggestive of B → C transition at low value of r. The intense extrinsic band provides evidence that the III chromophores are stacked, models are compatible with this, and further show that the phenylenes of III readily pack against the bases and the quaternary ammonium groups are in good proximity to the phosphates. Thus it would appear that there is one/one base pair/III residue stacking. The data indicates that when less than 40% of the DNA length is associated with III, all of it has undergone B → C transition. The complex transition can only occur if the change in the secondary structure has been transmitted across the unoccupied regions of DNA.

The interactions of several aromatic substituted diammonium cations (Reporter molecules) with DNA have been investigated by NMR, viscometric and melting temperature studies[40–43].

Reporter molecules

The aromatic ring of the dication is postulated to be partially or fully inserted between the base pairs of DNA. This "nonclassical intercalation" is quite different from intercalation observed with polynuclear aromatic compounds. Extrinsic CD effects have been reported with a few DNA dication complexes[42]. The aromatic residue in the peptide was considered to be partially inserted between the base pairs of DNA[43]. NMR studies of aromatic aminoacids containing peptides binding with DNA have indicated stacking interactions between the aromatic residues of the peptides and nucleic acid bases[44].

The phosphate groups impart the greatest negative charge density in the minor groove[45]. Therefore interactions of polycation is likely to be in this region. The chromophores of polycation may be readily stacked along the phosphate backbone. A similar chromophore stacking has been proposed in DNA-dye complex[46]. Polycation as probe provides valuable information concerning the dynamic and structural aspects of DNA.

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ANNOUNCEMENT

INDIAN ACADEMY OF SCIENCES, GOLDEN JUBILEE MEETING OF THE ACADEMY 6–8 FEBRUARY 1985

The postponed Golden Jubilee Meeting of the Indian Academy of Sciences, will now be held at Bangalore from 6 to 8 February 1985. The programme is as follows:–

6 Feburary 1985 (Wednesday) (*Chowdaiah Memorial Hall*) **Inaugural function** including release of volume 'Indian Academy of Sciences—The first 50 years' Introduction and garlanding of Foundation Fellows, Presidential Address. Golden Jubilee Inaugural Lecture by Prof. S. Chandrasekhar, (Nobel Laureate and Foundation Fellow) on 'The pursuit of science: its motivations' Special lecture (*Venue: Faculty Hall, Indian Institute of Science*) M. G. K. Menon, Planning Commission, New Delhi – 'Cosmic ray research and cosmic ray physicists over the past fifty years in India'. Unveiling of Ramanujan bust (*Venue: Raman Research Institute*).

7 February 1985 (Thursday) (*Venue: Faculty Hall, Indian Institute of Science*) **Symposium on Animal Communication** (a) Madhav Gadgil, Indian Institute of Science, Bangalore—'On the Communication of well being', (b) V. Nanjundiah, Tata Institute of Fundamental Research, Bombay—'Communication, Social behaviour and patterning in the cellular slime moulds', (c) A. S. Rand, Smithsonian Tropical Research Institute, Panama—'Trade-offs in the evolution of frog vocalizations', (d) R. Gadagkar, Indian Institute of Science, Bangalore—'Communication of kinship in social insects', (e) M. K. Chandrashekaran, Madurai Kamaraj University, Madurai—'Communication and synchronization of biological rhythms in insectivorous bats'. Lecture by (a) S. R. Gadre, University of Poona, Pune—'Electron density in chemistry', (b) T. N. Guru Row, National Chemical Laboratory, Pune—'Structure, conformation and charge density studies by x-ray diffraction', (c) L. C. Padhy, Tata Institute of Fundamental Research, Bombay—'Oncogenes: and their function'. Special lecture by O. Siddiqi, Tata Institute of Fundamental Research, Bombay—'Neurogenetics of smell'.

8 February 1985 (Friday) (*Venue: Faculty Hall, Indian Institute of Science*) **Symposium on 'Monsoons'** (a) Introduction by R. Narasimha, Indian Institute of Science, Bangalore (b) Sulochana Gadgil, Indian Institute of Science, Bangalore—'The Phenomenon', (c) P. J. Webster, Department of Meteorology, Pennsylvania State University, USA—'The dynamics', (d) J. Shukla, Department of Meteorology, University of Maryland, USA—'Predictability'. Special lecture by (a) M. M. Sharma, University of Bombay, Bombay—'Excursions into multiphase reactions' (b) D. Lal, Physical Research Laboratory, Ahmedabad—'The role of atmospheric carbon dioxide in controlling the earth's climate'. Lectures by (a) V Krishnan, Indian Institute of Science, Bangalore—'Biomimetic model reactions in photosynthesis' (b) G. Padmanabhan, Indian Institute of Science, Bangalore—'Gene basis for drug metabolism', (c) G. Srinivasan, Raman Research Institute, Bangalore—'The most rapidly rotating star known'.