Secondary Conformational Polymorphism of Nucleic Acids as a Possible Functional Link between Cellular Parameters and DNA Packaging Processes†

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ABSTRACT: Circular dichroism and electron microscopy studies of various in vitro DNA packaging systems indicate that all the factors which induce and modulate the *secondary* conformation of DNA molecules are capable of eliciting nucleic acids condensation processes into tight, highly ordered *tertiary* structures as well as altering the extent of order and compactness within the resulting species. Specifically, such factors include the ionic strength, the presence of particular dehydrating agents and polyamines, as well as the pH values. It is proposed that slight alterations of these parameters induce the formation of short non-B-DNA segments that propagate as a perturbation along the B-DNA double helix. The structural fluctuations of the dsDNA molecules that result from the conformational discontinuities formed at the junction sites between the B motif and the conformationally altered segments alter the elastic response of the nucleic acids and facilitate cooperative condensation processes. Moreover, the type and frequency of the structurally modified clusters interspersed within the B conformation and determined by the environmental parameters are shown to provide a means for continuous regulation of the extent and mode of DNA packaging. The ionic strength and hydrophobic environment in the close vicinity of the DNA molecules are controlled and modulated in vivo by DNA-binding proteins such as histones and protamines; similarly, pH values and polyamine concentrations are constantly regulated in living systems. It is suggested, therefore, that the secondary structural polymorphism which characterizes the DNA molecules might display a regulatory role by acting as a functional link between cellular parameters and the extent, mode, and timing of nucleic acid packaging processes.

The polymorphism that characterizes the secondary structures of DNA molecules is well established and documented [Saenger (1983) and references cited therein]. Yet, while a wealth of data on the nucleic acids' various structural families as well as on the factors responsible for conformational transitions between these secondary structural motifs has been accumulated, the biological consequences, if any, of such polymorphism remain elusive. The physiological significance of the tertiary and quaternary structural modulations of nucleic acids is, on the other hand, straightforward: such modulations must accompany all processes where reversible DNA condensation is involved (Kellenberger, 1987)—encapsulation of nucleic acids into preassembled viral proheads, the organization of chromatin in sperm cells, spores, and eukaryotic chromosomes, as well as replication and transcription. The factors that control and modulate these high-order conformational modifications of the mechanisms responsible for such processes are, however, far from being fully understood. Equally unclear are issues pertaining to the detailed structural features of the DNA condensed phases, namely, their hydration states and their secondary conformation.

The notion that packaging processes of DNA molecules might involve secondary structures other than the common B motif has been proposed (Eickbush & Moudrianakis, 1978; Shin & Eichhorn, 1984). The implications of this notion are far-reaching: they might point toward a potential mechanism by means of which biological processes that depend on the high-order organization of DNA molecules can be regulated. According to such mechanism, alterations of the nucleic acid secondary structure, which are, in turn, induced by slight, specific changes of various cellular parameters, may affect the extent of DNA local and overall compactness. In order to assess this possibility, the various factors involved in DNA packaging processes have been examined. The results and arguments presented in this study point toward a functional link between the two levels of conformational modulations revealed by dsDNA molecules: cooperative packaging of nucleic acids into highly ordered tertiary structures is shown to correlate and, presumably, depend upon segmental transitions between the DNA secondary structural motifs. Notably, the ordered packaging involves both DNA compaction, corresponding to linear reduction, and condensation, associated with the three-dimensional organization of the packed fibers. As in the in vitro DNA packaging systems the two processes are believed to be interrelated, both terms are used interchangeably.

Experimental Procedures

Highly polymerized calf thymus (%GC = 42), *Micrococcus lysodeikticus* (%GC = 71) and *Clostridium perfringens* (%GC = 31) DNA species, as well as spermine and cadaverine, were purchased from Sigma. DNA was dissolved in 20 mM Tris buffer, pH 7.5, and sonicated for 2 × 30 s by using an Ultratip labsonic sonicator operating at 20W. The DNA fragments were loaded on a Sephacryl S-400 (Pharmacia LKB Biotechnology Inc.) column and eluted with 20 mM Tris buffer and 0.25 M NaCl, pH 7.5. Fractions of 5 mL were collected and the size distribution of the DNA fragments in each fraction was determined by 0.75% agarose gel electrophoresis. The samples were extensively dialyzed against 5 mM Tris buffer, pH 7.5, and concentrated by ultrafiltration. Nucleic acid concentrations were determined by measuring the absorption at 260 nm, with extinction coefficients of 6600, 6840,

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and 6440 M^{-1} cm^{-1} for the calf thymus, *M. lysodeikticus*, and *Cl. perfringens* DNA species, respectively, on a Hewlett-Packard 8450A diode array spectrophotometer. The size of the DNA molecules used in the present study ranged from 2500 to 4000 base pairs.

Circular dichroism (CD) spectra were recorded on a Jasco J-500 spectropolarimeter equipped with a DP-500N data processor. Spectra were taken at room temperature, in 1-cm light path cells. The conditions used to prepare the condensed species are described in the figure legends. It should be emphasized that each point on the given graph represents a CD maximum that was obtained from a separate, independent experiment and not from a continuous titration. Error bars represent the distribution of CD maxima values obtained from three experiments that were conducted on three identical, separately prepared mixtures.

Electron microscopy samples were prepared by applying 10 μL of the various DNA solutions on a carbon-coated, glow-discharged grid for 30 s, followed by removal of the excess with filter paper. For the study of the DNA–NaCl–PEG (of MW 8000) mixtures, glow-discharged grids were coated with poly-D-lysine prior to the application of the sample by floating the grid on a drop of 1 μg/mL poly-D-lysine (Sigma) for 1 min; excess liquid was removed and the grid was air dried (Laemmli, 1975). In order to allow a systematic assessment of the differences in shape and size of the various condensed species, five solutions of each class of DNA condensates were independently prepared and three grids from each solution were thoroughly scanned. The specimens shown in Figures 3 and 7 represent, consequently, typical structures exhibited by given DNA composition and packaging conditions. Specimen were stained with 1% (w/v) aqueous uranyl acetate and examined on a Philips EM-400T at 100 kV.

Results

Under appropriate conditions DNA molecules undergo an extensive compaction process. The resulting structures are characterized by nonconservative circular dichroism (CD) spectra whose magnitudes are significantly larger than those revealed by dispersed nucleic acid species. The conspicuous optical anomalies, and consequently the structures responsible for such phenomena, were coined "psi", for polymer- and salt-induced ellipticities and condensed phases (Jordan et al., 1972). Both the nonconservative shape (defined as a CD spectrum for which $\sum R_K \neq 0$, where R stands for the rotational strength of the absorption band K) and the unusual size of the CD bands were assigned to long-range, interhelical couplings between nucleotide chromophores that are induced within the tightly packed species (Keller & Bustamante, 1986). The magnitude of the signals was shown to reflect the extent of the compactness and order assumed by the condensed DNA molecules, as well as the existence of differential scattering, which is due to the fact that the resulting particles are larger than the wavelength of the incident light (Tinoco et al., 1980). The sign of the nonconservative bands has been related to the sense of the long-range twist that characterizes the organization of the contiguous double helices in the condensed species; specifically, positive CD signals were related to a long-range right-handed organization, whereas negative bands were interpreted as originating from a left-handed interhelical arrangement (Maestre & Reich, 1980; Kim et al., 1986). The salient modifications of the nucleic acid optical properties that accompany the packaging process were used in this study as a sensitive means to evaluate the roles and mutual effects of the various factors that are known or expected to be involved in the DNA condensation process: the ionic strength, dehydrating agents, polyamines, pH, and DNA composition.

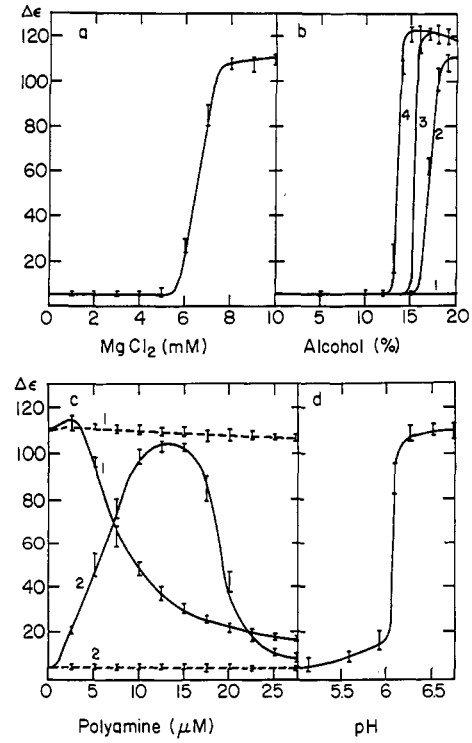

FIGURE 1: Effects of ionic strength, dehydrating agents, polyamines, and pH upon the CD maxima of calf thymus DNA molecules in $MgCl_2$–alcohol solutions. DNA concentration was 5×10^{-5} M in base pairs. (a) DNA in H_2O–EtOH (20% v/v). (b) DNA + $MgCl_2$ (10 mM) + the following alcohols: (1) MeOH, (2) EtOH, (3) *i*-PrOH, (4) *t*-BuOH. (c) DNA + $MgCl_2$ + spermine (solid line) or cadaverine (broken line) in H_2O–EtOH (20% v/v) + $MgCl_2$ at (1) 10 mM or (2) 5 mM. (d) DNA + $MgCl_2$ (10 mM) in H_2O–EtOH (20% v/v) at various pH values. In all samples, the alcohols were the last components to be added. Each point on the graph represents a CD maximum obtained from an independently prepared mixture. Error bars represent the distribution of CD maxima from three experiments conducted on identical mixtures.

Three systems in which DNA packaging has been shown to occur were scrutinized: a DNA–$MgCl_2$–EtOH system where a right-handed interhelical twist is induced under all experimental conditions, as evidenced by the positive nonconservative CD bands; a DNA–NaCl–poly(ethylene glycol) (PEG) system, characterized by a long-range left-handed organization of the condensed nucleic acid species (Shin & Eichhorn, 1984; Cheng & Mohr, 1975), and a DNA–NaCl–EtOH system where both right- and left-handed twists in between the contiguous DNA helices can be affected, depending upon the ionic strength (Huey & Mohr, 1981; Weinberger et al., 1988). The results presented in Figure 1 are concerned with the various factors that induce and modulate the packaging of DNA molecules by $MgCl_2$, in conjunction with a dehydrating agent. As indicated in Figure 1a, the transition of extended DNA species into fully condensed tertiary structures occurs at a critical ionic strength, below which no optical and, hence, no long-range structural modifications can be detected. Similarly, for a given ionic strength, a critical, well-defined concentration of a dehydrating agent is required to elicit a full-scale DNA condensation (Figure 1b). Evidently, this specific concentration depends upon the hydrophobicity of the agent—the higher the hydrophobic character of the alcohol is, the lower is the concentration that is required to affect the packaging process. Titration of the DNA–Mg^{2+}–EtOH system with increasing amounts of the polyamine spermine results in an initial increase of the magnitude of the nonconservative CD bands, suggesting a higher

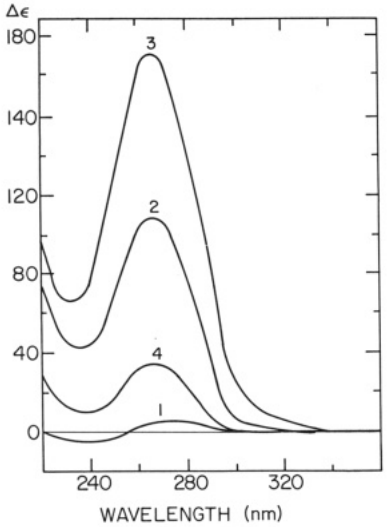

FIGURE 2: Effects of the base composition upon the CD signals exhibited by DNA molecules of three different GC base pair percentages, in H_2O–EtOH (20% v/v). DNA concentration was 5×10^{-5} M in base pairs. (1) Calf thymus DNA; (2) calf thymus DNA + 10 mM $MgCl_2$; (3) *M. lysodeikticus* DNA + 10 mM $MgCl_2$; (4) *Cl. perfringens* DNA + 10 mM $MgCl_2$.

degree of DNA compactness and order. Notably, the addition of spermine is found to elicit ordered packaging of the nucleic acids even at those Mg^{2+} concentrations in which no DNA condensation is induced in the absence of the polyamine (i.e., 5 mM). As the concentration of the polyamine is further increased, a sharp decrease of the CD magnitude is observed. In clear contrast, no optical effects accompany the titration of the condensation system with another polyamine, cadaverine (Figure 1c). In addition, nucleic acid packaging is found to reveal a sharp pH dependence (Figure 1d); a gradual increase of the pH results in minor optical, and hence structural, modifications until a critical value is reached, whereupon a sharp transition from dispersed into fully condensed particles is induced.

An additional factor found to affect the properties of the packed DNA states is related to the base composition of the nucleic acids (Cheng & Mohr, 1975). The largest CD signals that characterize the condensed species of DNA molecules from three different sources and of three different base compositions are presented in Figure 2. A clear correlation between the percentage of guanine–cytosine base pairs and the magnitudes of the nonconservative ellipticities is indicated. It should be noted, in this context, that long repetitive motifs, known to characterize eukaryotic DNA molecules, might present a difficulty in comparing the properties exhibited by the condensed calf thymus DNA species to those revealed by the condensates obtained from prokaryotic sources. It has been shown, however (Cheng & Mohr, 1975), that the magnitudes of the ellipticities exhibited by *Escherichia coli* condensed DNA species (%GC = 50) are slightly larger than those characterizing calf thymus condensates, in agreement with our suggested correlation. Electron microscopy studies of the compact particles obtained from the three different DNA species point toward a significant difference in the overall morphology of these condensates. Whereas the condensed states of *M. lysodeikticus* DNA (%GC = 71) appear as regular, separated toroids, the packed forms of *Cl. perfringens* (%GC = 31) are detected as bent stems that lack a defined, regular morphology. Condensed specimen obtained from calf thymus DNA molecules (%GC = 42) appear as both aggregated toroids and irregular, bent stems (Figure 3, panels a, b, and c, respectively).

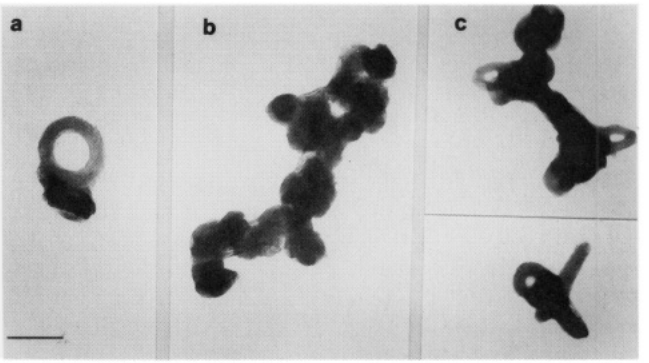

FIGURE 3: Electron microscopy of condensed DNA structures obtained in the presence of $MgCl_2$ and EtOH. Samples were prepared by applying 10 μL of the DNA (1 μg/mL)–$MgCl_2$ (10 mM)–EtOH (20% v/v) solution on a carbon-coated, glow discharged grid. Scale bar is 100 mm. (a) *M. lysodeikticus* DNA. (b) *Cl. perfringens* DNA. (c) Calf thymus DNA.

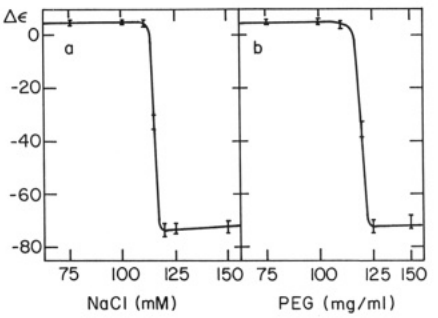

FIGURE 4: Effects of ionic strength and dehydrating agents upon the CD maxima of calf thymus DNA molecules in NaCl–PEG solutions. DNA concentration was 5×10^{-5} M in base pairs. (a) DNA + PEG (150 mg/mL). (b) DNA + NaCl (150 mM). In all samples, the PEG was the last component to be added.

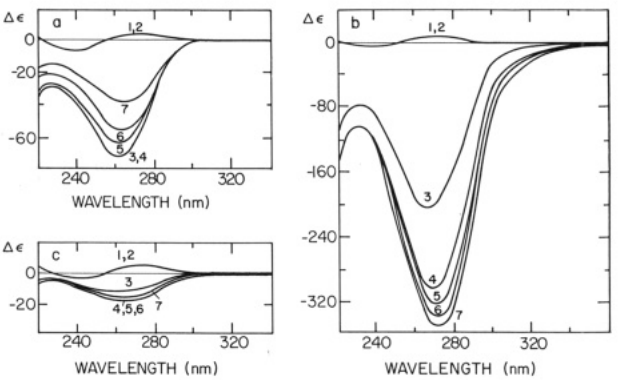

FIGURE 5: Effects of ionic strength and base composition upon the CD signals exhibited by DNA molecules of three different GC base pair percentages, in NaCl–PEG (150 mg/mL) solutions. (a) Calf thymus DNA. (b) *M. lysodeikticus* DNA. (c) *Cl. perfringens* DNA. NaCl concentrations were (1) 0, (2) 100, (3) 125, (4) 150, (5) 175, (6) 200, and (7) 225 mM. The different ellipticity scale in (b) should be noted.

Similar trends are displayed by the NaCl–PEG packaging system (Figure 4). Specifically, condensation of the DNA molecules into compact, ordered structures occurs at a very narrow range of both salt and polymer concentrations. The dependence of the optical properties of the condensed species upon the DNA base composition, observed in the DNA–Mg^{2+}–EtOH systems, is even more pronounced when packaging is induced by NaCl and PEG (Figure 5). Whereas the magnitudes of the nonconservative CD signals exhibited by the packed AT-rich structures are small, the GC-rich species

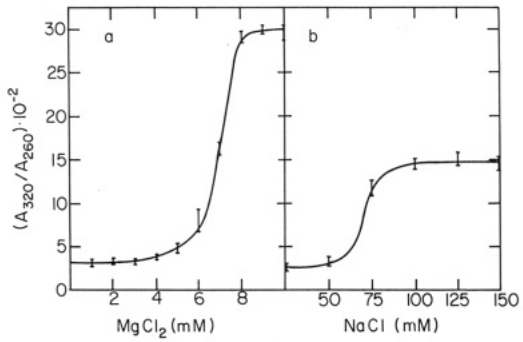

FIGURE 6: Tyndall scattering of DNA condensates, measured as a function of the ionic strength. As virtually identical scattering values were obtained for the three types of DNA molecules (specified in Figure 5), a representative curve is provided. DNA concentration was 5×10^{-5} M in base pairs. (a) DNA + EtOH (20% v/v). (b) DNA + PEG (150 mg/mL).

are characterized by extremely large ellipticities.

An additional difference between the AT-rich condensed species and the GC-rich packed structures is concerned with the modifications of their optical properties as a function of the ionic strength (Figure 5). Following the initial large and sharp increase of the magnitude of the CD spectra that occurs at the critical salt concentration, the ellipticities exhibited by the AT-rich condensed particles gradually diminish as the ionic strength is further augmented, while those displayed by the GC-rich species continue to rise. Two structural effects could account for such dissimilarity: a salt-mediated aggregation of the DNA molecules into particles that are large enough to manifest severe perturbation of their CD spectra, through either substantial differential scattering or a Duysens-type flattening effect known to characterize very large aggregates such as membrane-incorporated proteins (Tinoco et al., 1980). Such optical distortions might be more pronounced for AT-rich DNA condensates if these particles reveal a higher tendency for an extensive salt-mediated aggregation. Alternatively, as the magnitudes of the nonconservative CD bands reflect the extent of compactness and long-range order of the condensed particles, the results presented in Figure 5 might indicate that whereas the increase of the ionic strength causes a tighter packaging and higher order within the GC-rich species, such an increase is accompanied by a gradual disruption of the tight, long-range organization of the AT-rich particles.

In order to distinguish between these two potential effects, the A_{320}/A_{260} ratio revealed by the condensed structures was examined for both the Mg^{2+}–EtOH and PEG–NaCl condensation systems as function of the ionic strength (Figure 6, panels a and b, respectively). In both systems a sharp increase of the A_{320}/A_{260} ratio is observed at salt concentrations that are just below those required to elicit DNA packaging. Such an increase, being a clear manifestation of Tyndall scattering, indicates that the condensation processes involve aggregation. However, an increase of the ionic strength beyond the specific values required to induce the initial aggregation is not accompanied by a further change in the scattering intensity. Thus, once formed, the overall size of the condensed, scattering particles remains constant, insensitive to modifications of the ionic strength within the studied range. Moreover, the Tyndall scattering patterns, and hence the overall size of the compact DNA aggregates, are found to be insensitive to the base composition of the nucleic acids. In order to assess the possibility that the different response of the AT- and GC-rich species to the increase of the ionic strength is related to a different mode of *differential* scattering, the ellipticities of the condensates were studied at various solid angles of CD detection. We find that, in those cases where DNA condensation is induced in the absence of proteins, the shapes and magnitudes of the CD bands revealed by the packed species are not significantly affected following the increase of the solid angle of detection (Reich et al., 1990a; see below). These observations indicate that the DNA condensates devoid of polypeptides do not reveal a tendency to form large, asymmetric quaternary aggregates, and consequently, their ellipticities are not substantially affected by differential light scattering. On the basis of these findings, it may be suggested that the different optical properties revealed by the GC- and AT-rich DNA condensates reflect a difference in the extent of compactness and order within the tertiary structure of the various particles, being larger for the GC-rich species, as opposed to a significant dissimilarity in their tendency to aggregate or in their asymmetric quaternary organization. This assumption is further supported by electron microscopy studies of GC- and AT-rich DNA condensates whose packaging has been induced by $MgCl_2$ and EtOH as well as by NaCl and PEG (Figure 3 and 7, respectively). The shapes of the packed particles obtained from nucleic acids of different base compositions are conspicuously different, appearing either as toroids for the GC-rich species or as a mixture of both irregular and elongated stem-shaped forms for the AT-rich structures. The variability in the overall sizes is, however, found to be rather limited; specifically, no extensive aggregation is observed for either species. Notably, substantially larger DNA condensates are obtained when the packaging is induced in the presence of DNA-binding proteins of polypeptides.

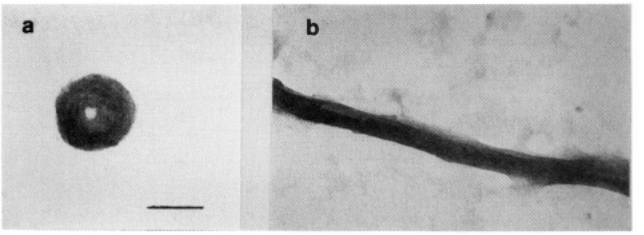

FIGURE 7: Electron microscopy of condensed DNA structures obtained in the presence of NaCl and PEG. Samples were prepared by applying 10 μL of the DNA (1 μg/mL)–NaCl (150 mM)–PEG (150 mg/mL) solution on a polylysine-coated grid (see Experimental Procedures). Scale bar is 100 mm. (a) *M. lysodeikticus* DNA. (b) *Cl. perfringens* DNA.

Of particular interest are the phenomena observed upon titration of the DNA–NaCl–PEG system with increasing amounts of polyamines. At those conditions where DNA packaging processes are not induced (low NaCl concentrations), addition of cadaverine fails to alter the optical properties of the nucleic acids (Figure 8a). The shape of the CD spectra revealed by both the GC- and AT-rich DNA molecules indicates that the nucleic acid species remain in their fully extended, B-type conformation. In contrast, a salient modification of the DNA ellipicity, highly indicative of a B to A conformational transition, is observed when the DNA–NaCl–PEG mixture is supplemented with spermine. Notably, the extent of the spermine-induced conformational transition is composition-dependent; the optical properties that characterize the A-type secondary structure become considerably more conspicuous as a function of the GC percentage in the DNA samples (Figure 8b). A striking difference in the effect of the two polyamines on the optical, and hence structural, properties of the DNA molecules is observed at NaCl concentrations that are near to the critical value required for packaging. At a salt concentration that is just below this critical value (110 mM NaCl, see Figure 4), the addition of spermine to the DNA–NaCl–PEG system results in full-scale

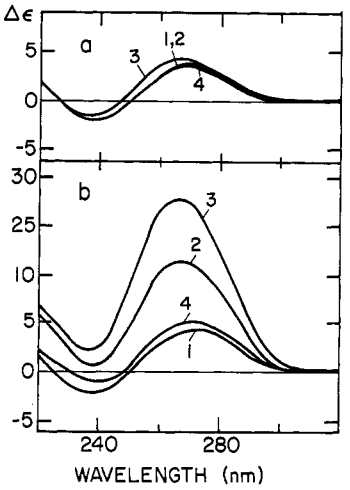

FIGURE 8: Effects of polyamines on the *secondary* conformation of DNA molecules. DNA concentration was 5×10^{-5} M in base pairs; NaCl and PEG concentrations were 10 mM and 150 mg/mL, respectively. (a) Cadaverine. (b) Spermine. (1) Calf thymus DNA; (2) calf thymus DNA + 25 μM polyamine; (3) *M. lysodeikticus* DNA + 25 μM polyamine; (4) *Cl. perfringens* DNA + 25 μM polyamine.

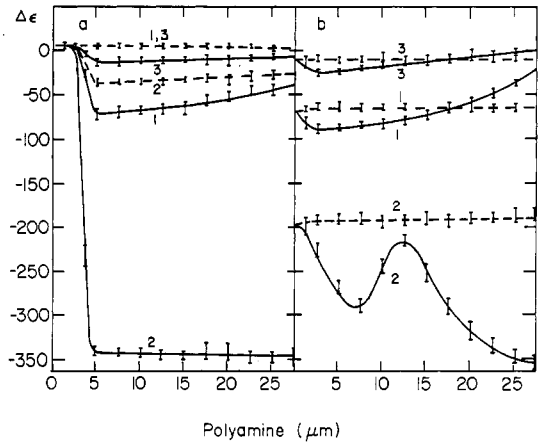

FIGURE 9: Effects of polyamines on the nonconservative ellipticities of DNA molecules condensed by NaCl and PEG. Solid and broken lines represent spermine and cadaverine, respectively. DNA concentration was 5×10^{-5} M in base pairs. (a) DNA + NaCl (110 mM) + PEG (150 mg/mL). (b) DNA + NaCl (120 mM) + PEG (150 mg/mL). (1) Calf thymus DNA; (2) *M. lysodeikticus* DNA; (3) *Cl. perfringens* DNA. It should be noted that each point on a given curve represents a CD maximum obtained from an independently prepared condensation mixture and not from a continuous polyamine titration.

DNA packaging, as inferred from the appearance of the large, nonconservative CD signals (Figure 9a). At a higher ionic strength (120 mM NaCl), where spermine is not required for DNA condensation processes, its presence in increasing amounts substantially alter the optical properties of the condensates (Figure 9b). Specifically, an initial increase followed by a sharp decrease of the CD intensities is observed for both the AT- and GC-rich DNA compact structures; a subsequent increase of the nonconservative CD magnitudes revealed by the GC-rich condensates—but not by the AT-rich species—upon a further increase of the spermine concentrations should be noted. In clear contrast, titration of the DNA–NaCl–PEG system with cadaverine does not affect the optical properties of the nucleic acids (Figure 9a,b). Thus, whereas spermine molecules exhibit a substantial effect upon DNA condensation processes, cadaverine molecules fail completely either to induce DNA packaging or to modify the structural features of the condensates.

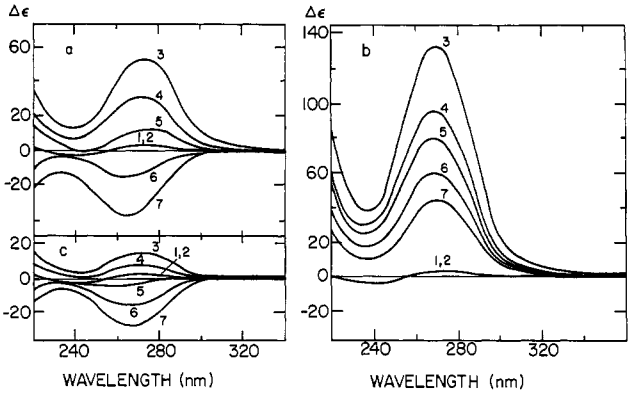

FIGURE 10: Effects of the ionic strength and base composition upon the CD signals exhibited by DNA molecules of three different GC base pair percentages, in NaCl–EtOH (35% v/v) solutions. (a) Calf thymus DNA. (b) *M. lysodeikticus* DNA. (c) *Cl. perfringens* DNA. NaCl concentrations were (1) 0, (2) 0.6, (3) 0.8, (4) 1.2, (5) 1.6, (6) 2.0, and (7) 2.4 M.

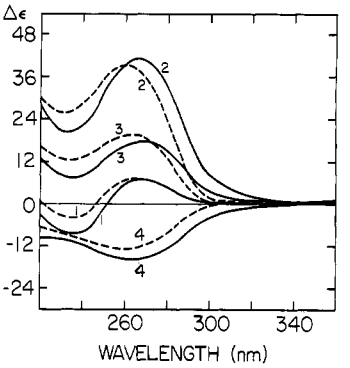

FIGURE 11: Contribution of scattered light to the CD of the condensed DNA particles. Solid lines represent CD measurements at a standard CD configuration, where the solid angle of light detection is 3° and at which most of the scattered light is not detected. Broken lines represent CD measurements obtained from placing the same samples near the detector, thus substantially increasing the acceptance angle. DNA (5×10^{-5} M in base pairs) condensation was performed in ethanol solutions (35% v/v) at NaCl concentrations of (1) 0, (2) 0.8, (3) 1.2, and (4) 1.8 M.

When DNA packaging is affected by means of NaCl and EtOH, both right- and left-handed long-range interhelical conformations are obtained. In 35% (v/v) ethanol and 0.8 M NaCl, the DNA molecules undergo a sharp transition into compact species that exhibit a positive nonconservative CD signal. The magnitude of the ellipticities is found to depend upon the nucleic acid composition, being significantly larger for the GC-rich condensates (Figure 10). The intensities are diminished as the salt concentrations are gradually increased; yet, whereas a sign reversal and large negative CD signals are finally obtained for the AT-rich condensed species, the GC-rich structures display reduced but still positive ellipticities even at the highest NaCl concentrations. As already mentioned, the packed particles do not reveal a significant tendency to form asymmetric quaternary aggregates. This is based on the observation that upon increasing the solid angle of CD detection the magnitudes of the ellipticities remain virtually unaltered, indicating minor, if any, contributions from differential light scattering (Figure 11).

Discussion

The in vivo packaging of DNA is characterized by the formation of structures in which the helices are ordered in a quasi-parallel organization and are separated from each other by less than 10 Å. X-ray studies conducted on T4 phage have indicated a separation of about 5 Å between the quasi-parallel

DNA helical segments (Earnshaw et al., 1978). In nucleosomal structures the dsDNA follows a helical path around the core with a radius of curvature of about 50 Å and a pitch of 28 Å, rendering the interhelical distance to be 8 Å (Finch et al., 1977). Such ubiquitous tight organization (Kellenberger, 1987) and the variety of chemical agents capable of inducing in vitro DNA condensation, as well as the salient morphological similarity exhibited by the resulting packed species, point toward a substantial intrinsic tendency of dsDNA molecules to undergo packaging processes. The extensive modulations of the extent of DNA compactness that occur during the formation of viruses, spores, sperm cells, and eukaryotic metaphase chromosomes, as well as during replication and transcription, indicate that nucleic acid packaging must be rigorously and continuously regulated. Such regulation is likely to be mediated through condensation–decondensation mechanisms whose effect is, in turn, triggered by minute, strictly controlled alterations of cellular conditions.

Any mechanism proposed to explain DNA packaging must address the free energy factors that oppose this process. Those include the loss of conformational entropy by the dsDNA on going from extended, random-coiled to ordered, condensed phases, the energy required to affect bending and local kinking, and the electrostatic repulsions that dominate the unfavorable contributions (Reimer & Bloomfield, 1978; Wilson & Bloomfield, 1979). According to Manning (1980, 1981), DNA condensation would occur spontaneously once a critical charge neutralization is reached and thus no interhelical attractive energy contributions are required. The requirement for such attractive factors in conjunction with charge neutralization has, however, been indicated by Bloomfield et al. (1980), who suggested that favorable interactions for dsDNA tight packaging are provided by London dispersion forces. On the basis of the results and considerations presented in this study, we suggest that conformational changes within the secondary structure of the DNA molecules are crucially involved in nucleic acid long-range packaging processes by mitigating the unfavorable free energy contributions related to DNA bending and kinking as well as by acting as a packaging regulation factor.

The issue of a potential link between DNA secondary conformation and its higher-order structural features remains controversial, although intensively studied. X-ray scattering of DNA condensed by either neutral and charged polymers, polyamines, or salts has been interpreted in terms of a secondary conformation closely related to the B-form, thus precluding any significant contributions from other polymorphs (Suwalsky et al., 1969; Maniatis et al., 1974; Evdokimov et al., 1976; Damaschun et al., 1978; Skuridin et al., 1986). Notably, these studies were conducted on condensed states that exhibit both positive and negative nonconservative CD spectra, indicating that the B-form prevails for both right- and left-handed long-range conformations of the ordered DNA condensates. In sharp contrast, all known nucleic acid polymorphs have been implicated in DNA packaging when the packed particles were studied by means of CD or Raman spectroscopy. The nonconservative ellipticities revealed by condensed DNA species obtained in the presence of random lysine–leucine copolymers were suggested to reflect ordered tertiary organization and not modifications within the secondary conformation, which was assumed to remain in the B-form (Ong et al. 1976; Reich et al., 1980). It has been argued, however, that the long-range right-handed tertiary structures adopted by DNA–polylysine condensates supplemented with heavy metals are associated with the A-type secondary conformation (Shin & Eichhorn, 1977). A distorted structure, described as an intermediate between the B and C forms, was proposed to be displayed by DNA–polylysine condensed species (Weiskopf & Hsueh, 1977), wheras a C-type structure was suggested to characterize ethanol-induced condensed DNA species (Eickbush & Moudrianakis, 1978). Laser Raman studies (Zacharias et al., 1983) as well as CD measurements combined with electron microscopy (Thomas & Bloomfield, 1985; Castleman et al., 1984) of ordered aggregates obtained from poly(dG-dC) and its methylated derivative indicated that the condensed species adopt a perturbed secondary conformation, being an intermediate between the B and Z forms. Treatment of poly(dG-dC) with $[Co(NH_3)_6]Cl_3$ was shown to elicit packaging through a modified yet ill-defined conformation, being either A or a variant of Z forms (Eichhorn et al., 1982). Moreover, dsRNA condensation processes were found to be concomitant with an A to A′ secondary transition (Steely et al., 1986). On the basis of these conflicting observations, it has been suggested that, although not specifically required for nucleic acid packaging processes, secondary conformational transitions are not incompatible with their occurrence (Huey & Mohr, 1981; Granados & Bello, 1981).

It is well established that the secondary structural transitions of nucleic acids are crucially affected by their state of hydration, a property that depends upon the water activity of the DNA solutions as well as upon the DNA base composition and sequence (Saenger, 1983; Wolf & Hanlon, 1975; Dickerson et al., 1982). Thus, whereas the B conformation prevails at high water activity, a B to C transition is observed as the ionic strength is increased; a B to A conformational change is obtained in the presence of dehydrating agents. Sequences such as poly(dG-dC) undergo a B to Z transition at high salt concentrations, a process facilitated by the presence of dehydrating agents or polyamines. AT-rich sequences reveal a lower tendency to undergo conformational changes due to the particularly stable spine of hydration that runs along their minor groove and stabilizes the B form (Drew & Dickerson, 1981; Kopka et al., 1982).

The data obtained from the three condensation systems described under Reslts clearly indicate that all the factors which are capable to elicit and modulate packaging processes bear an intimate relation with the DNA hydration states. High concentrations of salt are required to affect packaging in the NaCl–PEG and NaCl–EtOH systems. The low ionic strength that is needed to induce DNA condersation in the $MgCl_2$–EtOH system is interpreted in terms of the substantial effect of Mg^{2+} ions upon nucleic acid hydration states (Wolf & Hanlon 1975). Also, the presence of relatively large amounts of alcohols or PEG, acting as dehydrating agents, is required for packaging. Notably, no condensation processes are induced by the weakly dehydrating MeOH, whereas the extent of packaging efficiency revealed by the other alcohols correlates with their dehydrating capacity (Figure 1b). The effect exerted by the pH of the nucleic acid solutions upon the long-range organization of the DNA molecules further supports the correlation. As indicated in Figure 1d, low pH values prevent packaging while neutral and slightly basic pH values promote such processes. We suggest that this observation correlates with the specific organization of the water molecules along the DNA species. At neutral pH, the first layer of water that runs along the grooves is bound to a second, less-defined outer layer that serves to complete the tetrahedral environment. As the pH is reduced, some of the water molecules that form the second shell are replaced by protons that are bound through hydrogen bonds to the primary water layer. Such a tetrahedral

organization will conceivably strengthen the hydrogen bonds between the first layer and the nucleotides, stabilizing consequently the primary hydration shell. Thus, environmental modulations that destabilize the first hydration layer of the nucleic acid molecules and, as such, facilitate secondary conformational transitions, i.e., an increase of the ionic strength, a higher dehydrating capacity, and higher pH values, are shown here to be closely associated with DNA packaging processes.

The correlation between secondary transitions and packaging is further supported by additional observations. The B to A or B to Z conformational changes are characteristically cooperative, a phenomenon that is casually related to the cooperative nature of the modulations of the DNA hydration states. Indeed, all the factors shown to be involved in DNA packaging elicit such processes through highly cooperative modes. Specifically, a complete transformation of fully extended DNA molecules into fully condensed structures is shown to follow from a minute alteration of the $MgCl_2$ and alcohol concentrations or of the pH values in the first packaging system (Figure 1) and minute modifications of the NaCl and PEG or NaCl and EtOH concentrations in the second and third condensation mixtures, respectively (Figures 4 and 10). The second observation that supports the notion of a direct correlation between the secondary and the higher order conformational changes of nucleic acids is concerned with the conspicuous effects of the base composition upon these two structural processes. As already mentioned, AT-rich sequences reveal a substantially lesser tendency toward secondary conformational transitions due to the spine of hydration that stabilizes the B form; GC-rich DNA species are, in contrast, characterized by relatively facile B to A and, under certain conditions, B to Z transitions. Indeed, in all the packaging systems a clear correlation between the percentage of GC base pairs and the magnitudes of the nonconservative CD signals is observed (Figures 2, 5, and 10; the extremely large ellipticities revealed by the GC-rich DNA species upon their condensation by NaCl and PEG, shown in Figure 5, should be noted). As the optical properties of the condensates discussed in this study are not significantly affected by contributions from differential scattering (Figure 11), the magnitudes of the nonconservative CD spectra are assumed to reflect the extent of order and compactness within the particles (Kim et al., 1986). Consequently, the observations indicate that the tendency of DNA molecules to undergo secondary conformational changes correlates with their tendency to pack into ordered, condensed structures. Moreover, the particular effects of base composition on the modes and extent of condensation clearly indicate that factors other than mere charge neutralization must be involved in the packaging processes. Electron microscopy studies of the various condensed species support these notions; whereas the GC-rich DNA structures appear, under all packaging conditions, as ordered toroids, the AT-rich species are observed as irregular particles (Figures 3 and 7).

The notion that secondary conformational transitions affect and modulate nucleic acid packaging must, however, be reconciled with the ambiguity concerned with the nature of the secondary structures within the condensed states and, in particular, with the X-ray diffraction studies, which indicate that the B-form prevails in such states. We suggest that the process of nucleic acid packaging is induced and its mode, extent, and modulations regulated by partial, segmental transitions of the dsDNA secondary conformation. Specifically, it is suggested that, under these concentrations of salts, dehydrating agents, or polyamines and at those pH values that induce cooperative DNA condensation processes, the nucleic acid's secondary conformation is no longer represented by a single structural motif. Instead, due to subtle alterations of the DNA hydration state, the B-type double helix is interspersed with short segments that adopt other conformations. Models which imply that local segments of either A or Z DNA may exist in an otherwise B helix have been pointed out; moreover, it has been shown that the junctions between the various polymorphs might induce a substantial local bending at the site of the junction as well as long-range structural fluctuations, which propagate along the macromolecule (Selsing & Wells, 1978; Wartell et al., 1982; Arnott et al., 1982; Lavery, 1988; Manning, 1988; Sheardy & Winkle, 1989). Thus, we propose that the increased overall flexibility required for tight packaging is provided by the bending and the enhanced conformational fluctuations that characterize the B-DNA segment when flanked at both ends with a different structural motif. Moreover, as the A and B forms are energetically similar, and since strand separation is not required for a B to Z transformation (Wang et al., 1979), it might be suggested that such non-B-DNA clusters travel along the B-type helix like a bubble, thus propagating the junction-induced structural flexibility.

The effects exhibited by the various condensation systems upon titration with polyamines further indicate the involvement of secondary structural transitions in DNA packaging processes. The addition of spermine to the packaging mixtures performed at an ionic strength that is high enough to elicit condensation even in the absence of the polyamine results in substantial changes of the CD signals. At those salt concentrations that are slightly below the threshold required for DNA packaging, the addition of spermine induces full-scale condensation processes. In sharp contrast, the optical properties revealed by the various packaging systems are found to be completely insensitive to the presence of a different polyamine, namely, cadaverine (Figures 1c and 9). An interpretation for the polyamine effect is provided by the modification of the *conservative* CD signals observed upon the addition of spermine and spermidine to the DNA–NaCl–PEG system at low ionic strength, at which no DNA packaging occurs (Figure 8). Under such conditions, a clear B to A secondary transition is affected specifically by the spermine molecules but not by the cadaverine species, which leave the B-form unaffected. Indeed, detailed studies of DNA in water–ethanol solutions have indicated that spermine and spermidine promote a conformational change into the A form, whereas other polyamines, such as cadaverine or putrescine, stabilize the B polymorph (Minyat et al., 1978). Such different effects are conceivably associated with the difference in the overall electrostatic charge of the spermine and cadaverine, being +4 and +2, respectively, as well as with the different N^+–N^+ distances, which lead to a DNA-conformation-dependent mode of binding between the polyamines and the nucleic acids. A prominent correlation between the effects of polyamines upon DNA secondary structures on one hand and upon ordered packaging on the other is pointed out: cadaverine fails to affect either process, while spermine induces both. Moreover, the extent of the secondary and higher order conformational modulations elicited by the spermine is found to correlate with the percentage of GC base pairs: its effect on the B to A or B to Z transitions as well as on the condensation processes are significantly more pronounced in GC-rich DNA molecules.

The proposed hypothesis that causally relates local structural perturbations within the B-DNA helix with packaging pro-

cesses provides a straightforward interpretation to a large body of experimental results. As only a limited number of short segments are suggested to reveal non-B-DNA structures and, in particular, as those clusters are assumed to travel along the B-type helix, they cannot be detected by X-ray scattering studies, which provide an average conformation. A rationale for the ambiguity concerned with the secondary conformation of the nucleic acid condensed states is, consequently, pointed out. Moreover, the significantly higher tendency of GC-rich sequences to undergo secondary structural transitions than that characterizing the AT-rich segments accounts for the larger extent of compactness and order revealed by the GC-rich condensed DNA particles. Finally, we have recently been able to show that the presence of DNA-binding drugs severely interferes with nucleic acid packaging processes (Reich et al., 1990b). In terms of the proposed model, it is suggested that the tight complexation of either intercalating agents or groove binders, which usually stabilize the B form, strongly reduces the extent of DNA bending as well as prevents the propagation of the dynamic effects caused by the junction sites. Indeed, it has been shown that the curvature which characterizes kinetoplasts is dramatically reduced upon complexation of the DNA with distamycin (Griffith et al., 1986).

Alterations of environmental parameters, which include the ionic strength, pH, hydrophobicity, and polyamine concentrations, are shown to possess the ability to induce and modulate nucleic acid packaging processes through their effect upon the DNA secondary conformations. Similar parameters are operative in vivo; a high local charge density as well as a hydrophobic, dehydrating environment is provided by DNA-binding proteins such as histones and protamines. Alterations of these parameters might be brought about by reversible acetylation and phosphorylation processes, which occur within such proteins during the cell cycle (Alberts et al., 1983; Hohmann et al., 1976). Likewise, slight modulations of the pH values are reported to occur between interphase and mitosis and, as such, to accompany the aggregation of chromatin into chromosomes (Guo & Cole, 1989). These changes, as well as small alterations of the cellular concentrations of salts and polyamines (Prasad et al., 1987; Feuerstein et al., 1986), are all known to occur in living systems in a strictly regulated mode. It may be suggested, consequently, that such slight, controlled fluctuations of cellular parameters result in the formation of short segments characterized by a non-B-DNA conformation; the perturbations induced by the conformational discontinuities at the junctions between the modified segments and the native B-form are proposed to be involved in the regulation of DNA condensation processes.

Conclusions

Nucleic acid condensation processes are suggested to be causally related to the occurrence of short non-B-DNA segments within the B-type double helix. The formation of these structurally modified clusters is, in turn, affected by slight alterations of the ionic strength, pH values, hydrophobicity, and polyamine concentrations. The substantial local and long-range structural fluctuations of the dsDNA molecules that result from the conformational discontinuities at the junction sites between the non-B-DNA segments and the B-type helix enhance the overall elastic response of the nucleic acids and hence enable extensive condensation processes. The extent of packaging is shown to correlate with the type of the modified segments and with the frequency of their occurrence. These parameters depend upon environmental factors as well as upon the DNA composition. Sequences characterized by a larger tendency to undergo secondary conformational changes are found to attain a higher extent of compactness and order. DNA-binding drugs that were shown to interfere with or prevent long-range DNA condensation processes are suggested to exert this effect by stabilizing the B conformation and preventing secondary structural modulations.

The factors shown to induce and modulate nucleic acid packaging are biologically significant. The ionic strength and the hydrophobic environment in the DNA vicinity are determined and regulated in vivo by histones and protamines. Similarly, the pH values and polyamine concentrations are strictly and continuously regulated in biological systems. Thus, the DNA condensation model presented in this study points toward a hitherto unconsidered mechanism by means of which secondary structural motifs might display a regulatory role, i.e., by acting as a functional link between cellular parameters on one hand and the mode, extent, and timing of nucleic acid packaging on the other.

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Calcium Retards NH_2OH Inhibition of O_2 Evolution Activity by Stabilization of Mn^{2+} Binding to Photosystem II†

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ABSTRACT: Calcium is required for oxidation of water to molecular oxygen by photosystem II; the Ca^{2+} demand of the reaction increases upon removal of 23- and 17-kDa extrinsic polypeptides from detergent-derived preparations of the photosystem. Employing the manganese reductant NH_2OH as a probe to examine the function of Ca^{2+} in photosystem II reveals that (1) Ca^{2+} slows the rate of NH_2OH inhibition of O_2 evolution activity, but only in photosystem II membranes depleted of extrinsic proteins, (2) other divalent cations (Sr^{2+}, Cd^{2+}) that compete for the Ca^{2+} site also slow NH_2OH inhibition, (3) Ca^{2+} is noncompetitive with respect to NH_2OH, (4) in order to slow inhibition, Ca^{2+} must be present prior to the initiation of NH_2OH reduction of manganese, and (5) Ca^{2+} appears not to interfere with NH_2OH reduction of manganese. We conclude that the ability of Ca^{2+} to slow the rate of NH_2OH inhibition arises from the site in photosystem II where Ca^{2+} normally stimulates O_2 evolution and that the mechanism of this phenomenon arises from the ability of Ca^{2+} or certain surrogate metals to stabilize the ligation environment of the manganese complex.

The formation of molecular oxygen from water by photosystem II (PSII)[1] is proposed to occur by means of a linear, four-electron oxidation process involving five so-called S-state intermediates [$S_0 \rightarrow S_4$ (Joliot & Kok, 1975)]. In dark-adapted material, S_1 predominates, and evidence exists to indicate that a concerted oxidation of water occurs after formation of the S_4 state (Radmer & Ollinger, 1986), sug-

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[1] Abbreviations: Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; EDTA, ethylenediaminetetraacetate; EPR, electron paramagnetic resonance; MES, 2-(*N*-morpholino)ethanesulfonic acid; Mn, manganese ligated to photosystem II in oxidation states higher than +2; PS, photosystem; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

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