Protein-Independent Spontaneous Packaging of Long DNA Molecules into Highly Ordered Phases

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The necessity to confine large amounts of genetic material within the small cellular volume imposes extreme organizational demands upon the biological system. Whereas packaging processes in eukaryotes are mediated by various structural proteins, DNA organization in viruses, mitochondria, and prokaryotic or dinoflagellate chromosomes, which may result in local DNA concentrations of 800 mg/mL,[1] is characterized by low relative amounts of condensing proteins.[2] What are the factors responsible for the induction and regulation of packaging processes that take place in low-protein chromatin complexes? Here we report that DNA fragments which are significantly longer than the DNA persistence length, and hence are not expected to possess the ability to self-organize, are nevertheless capable of *spontaneously* forming highly-ordered packed phases at physiological salt conditions. The findings indicate that double-stranded DNA molecules exhibit a surprisingly large *intrinsic* tendency to undergo efficient packaging processes into ordered structures and, in addition, point toward a structural feature that might be involved in the induction of such processes.

DNA pellets were obtained by gentle centrifugation (Dynac II tabletop centrifuge, 1000 rpm, 1 × 30 min) of 50-mL solutions containing DNA fragments of 3000 ± 50 bp (5×10^{-5} M, in base pairs) in the presence of 6 mM $MgCl_2$ and 20% (v/v) ethanol, required to reduce the water activity to values corresponding to those found in the dense cellular environment.[1] Pellets immersed in supernatant solutions were transferred into 1.5-mm glass capillaries without any attempt to pull fibers or to flow-align the sample and were subjected to X-ray scattering measurements. In addition to prominent diffuse rings reflecting the secondary structural state of the molecule, 10 predominantly weak but sharp diffraction maxima, related to DNA–DNA lateral interactions, are observed (Figure 1). The high-order multiple reflections, extending up to a reciprocal spacing of 1/3.34 $Å^{-1}$, clearly indicate the presence of a remarkable degree of crystallinity within the sample. To our knowledge, such multiplicity of reflections has not been hitherto observed for specimens derived from unsheared, nonaligned DNA segments, including those exhibiting liquid-crystalline properties[3] or even a crystalline morphology.[4] The DNA phases obtained in the presence of $MgCl_2$ are hexagonally packed (a = 43.8 Å) with half a molecule in the asymmetric unit, an interhelical spacing of 27.5 Å, and a minimum domain size (calculated using the Sherrer equation[5]) of 152 Å. The spatial relationship of the molecules along the c axis could not be deduced from the data, yet a staggered arrangement, in which neighboring helices are translated or screwed vertically with respect to each other to enhance intermolecular interactions, seems probable.[3,6]

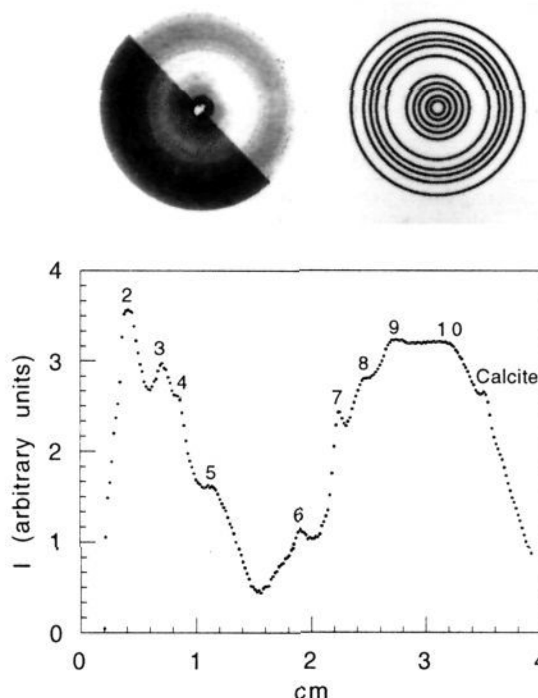

Figure 1. (a, top) Diffraction patterns of the condensed DNA phases[23] (induced at 6 mM $MgCl_2$ and 20% EtOH) recorded at over- and underexposure conditions. The sharp Bragg reflections indicating the presence of a long-range lateral order are schematically depicted, for clarity, in the right panel. These reflections correspond to reciprocal spacings of (1) 1/43.8, (2) 1/23.8, (3) 1/14.5, (4) 1/11.0, (5) 1/8.78, (6) 1/5.35, (7) 1/4.54, (8) 1/4.19, (9) 1/3.84, and (10) 1/3.37 $Å^{-1}$. The diffuse rings that are due to the secondary structure of the packed DNA molecules are listed in Table 1. Wide-angle X-ray scattering measurements were conducted using a Searle camera with Ni-filtered Cu Kα radiation (λ = 1.541 Å). The X-rays were generated by an Elliott G×6 rotating anode generator operating at 40 mA and 30 kV; the beam was further monochromated and collimated by two Frank mirrors and guard slits. Data were collected for 24 h at 19 °C. (b, bottom) Computer-generated plot of the averaged intensity of the overexposed film as a function of the radial distance from the pattern center. The film was digitized as 820 × 768 pixels using a UMAX scanner, followed by a circular-averaging analysis. Note that reflection 1 is only observable on the underexposed film and thus is not detected on this plot.

Notably, packed DNA structures obtained in the presence of a broad range of NaCl concentrations reveal only one reflection, consistent with a substantially lower order (data not shown). This observation indicates that highly ordered DNA condensation processes cannot be considered as a simple consequence of charge neutralization and screening of electrostatic repulsions. Since multivalent cations such as Mg^{2+}, spermine, or spermidine are known to occupy fixed positions along the DNA[7] and are capable of cross bridging between adjacent molecules,[8] we suggest that the reported long-range order might be enhanced and stabilized by the site-specific binding of these cations. This interpretation is supported by the observation that condensed phases of long DNA molecules have been found to exhibit multiple reflections,

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Table 1. DNA Secondary Conformation by Wide Angle X-ray Scattering[a]

DNA	peak/Å					
	1	2	3	4	5	6
sample	12.9 ± 0.2[d]	8.31 ± 0.07	5.39 ± 0.02	4.20 ± 0.04	e	3.34 ± 0.01
A-DNA[b]	12.6	7.44	5.32	4.11	3.65	3.27
B-DNA[b]	12.4	8.69	5.54	4.38	3.87	3.34
C-DNA[c]	11.4	8.42	5.53	4.13	3.53	3.30

[a] Scattering curves were obtained with a Joyce-Loebl densitometer. [b] The spherically averaged scattering curves were calculated using the Debye equation. Atomic coordinates for the A- and B-DNA canonical forms were taken from ref 21, using 25 base pairs for each configuration.[22] Water-weighted scattering factors were taken from ref 14. [c] Taken from ref 22. [d] The shift of this peak toward smaller scattering angles relative to the corresponding peak of the canonical forms is commonly observed in condensed DNA phases.[22] [e] Experimentally undetectable due to strong water background scattering.

and hence a high-order arrangement, in the presence of NaCl, but only under extreme dehydrating conditions (i.e., 80% EtOH).[9] Conceivably, at such a low water activity a complete ion delocalization, implied by the counterion condensation theory,[10] is no longer applicable even for monovalent ions. Under these conditions monovalent ions may occupy specific positions along the DNA and consequently stabilize an ordered organization.

On the basis of circular dichroism (CD), electron microscopy, and X-ray diffraction studies, a packaging model has been proposed, according to which the DNA molecules fold into quasi-parallel, tightly packed arrays that are gently twisted relative to each other.[11] Such a twist is predicted to result in large nonconservative CD spectra, provided that a minimum chromophore density of 1 chromophore/nm^3 is attained.[12] The chromophore density of the DNA samples used in this study was derived from the observed distances and lattice geometry, considering each nitrogen base as a unit chromophore, and found to be 0.9 chromophore/nm^3. Since the DNA–$MgCl_2$–EtOH system indeed exhibits large nonconservative CD signals,[13] the DNA packaging model seems to be applicable to the current system as well.

In order to identify the secondary conformation of the DNA molecules within the condensed phases, we have compared our data to the scattering curves of randomly oriented A- and B-DNA canonical forms, calculated by using the Debye equation with water-weighted scattering factors reported by Langridge.[14] Comparison of the experimental results and the theoretical values (Table 1) indicates that DNA packaging is accompanied by secondary conformational changes of the DNA. Specifically, the observed scattering pattern corresponds to a superposition of contributions from both the A- and B-DNA forms, as pointed out by the positions of the diffuse maxima. In a previous study we proposed that alterations in cellular parameters, such as the ionic strength, presence of specific polyamines, and pH, effect the formation of short non-B-DNA segments along the B-DNA double helix, and that the resulting conformational discontinuities act to increase the DNA elastic response, thus facilitating cooperative condensation processes.[13b] Although an unambiguous distinction between the presence of an intermediate secondary DNA conformation or the coexistence of two distinct forms along the same DNA molecule within the packed phases cannot be made, the reported observations support the notion that DNA secondary structural polymorphism might be involved in the induction of protein-independent DNA packaging processes.

The results presented in this study underscore and generalize the notion that relatively long DNA segments can undergo efficient and spontaneous packaging into highly ordered phases under very mild ionic strength and dehydrating conditions, without the assistance of DNA-condensing proteins such as histones. The packaging processes apparently involve DNA secondary conformational modulation, and the resulting ordered structures can be stabilized either by multivalent cations (metal cations and polyamines[15]) or by extensive dehydration.[9] Significantly, recent crystal structure analyses have indicated that DNA decamers form well-ordered quasi-infinite rods which are "glued" together by hydrated magnesium or calcium ions.[16] Since the factors that promote crystallization of short oligonucleotides and those involved in the formation of paracrystalline phases from long DNA species are likely to be similar, these studies further underline the intrinsic and general tendency of DNA molecules to pack into ordered conformations, as well as the crucial role displayed by multivalent bridging cations upon the packaging processes.

The results and considerations presented above, combined with the numerous indications for the presence of efficiently packed protein-independent liquid-crystalline DNA organizations in biological systems,[17] point toward a new perspective to the fundamental issue concerned with the role of histone and histone-like proteins. It is well-known that initiation of transcription is inhibited by histone complexes bound to promoters;[18] recent studies suggest that transcription is regulated by competitive binding of histones and transcriptional activators to promoters.[19] We propose that an additional and important function of the ubiquitous structural nucleoproteins, is to actually *prevent* a spontaneous collapse of the DNA molecules into regular, highly-ordered organizational forms (such as those present within the viral capsids[20]) which are incompatible with the selective unfolding processes required for all DNA transactions.

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