DNA condensation

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Recent progress in our understanding of DNA condensation includes the observation of the collapse of single DNA molecules, greater insights into the intermolecular forces driving condensation, the recognition of helix-structure perturbation in condensed DNA, and the increasing recognition of the likely biological consequences of condensation. DNA condensed with cationic liposomes is an efficient agent for the transfection of eukaryotic cells, with considerable potential interest for gene therapy.

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Abbreviations

bp	base pairs
CTAB	cetyltrimethylammonium bromide
DMPC	dimyristoylphosphatidylcholine
DSC	differential scanning calorimetry
EM	electron microscopy
FM	fluorescence microscopy
PEG	polyethylene glycol

Introduction

In dilute solution, the DNA of bacteriophage T4 has a radius of gyration of about 1000 nm, and a worm-like coil volume of $4\times10^9\,nm^3$. When packaged inside the T4 phage head, the DNA has an outer radius of only 50 nm and a volume of $5\times10^5\,nm^3$. Whereas phage have elaborate apparatuses for DNA packaging, a similar decrease in DNA volume to an orderly collapsed state can be produced *in vitro* simply by the addition of multivalent cations such as polyamines. It is this dramatic decrease in the volume occupied by a DNA molecule, provoked *in vitro* by chemical agents, that we define as condensation. For reviews of some of the biophysical aspects of DNA condensation, and references to earlier work, see [1–4] (polymer physicists often call this the 'coil-globule transition' [5]). In the condensed state, the helical segments are locally aligned, the volume fractions of solvent and DNA are comparable, and DNA helices may be separated by just one or two layers of water.

The condensation of single molecules has been observed in very dilute solutions of large DNAs. With plasmid-sized or smaller DNAs, it is more common that several molecules are incorporated into the condensed structure. Condensation is, therefore, difficult to distinguish clearly from aggregation or precipitation. Generally, the term 'condensation' is reserved for situations in which the aggregate is of finite size and orderly morphology.

DNA condensation bears some similarities to the much more intensely studied topic of protein folding. Both are characterized by the seeming improbability of the formation of compact, regular structures, their ready reversibility, the many types of non-covalent interactions that drive the process, and the requirement of the collapsed state for proper biological function. DNA condensation differs from protein folding in that no unique compact structure is formed, hydrophobic interactions do not drive collapse, and there is no ready functional assay for the condensed state.

This review focuses on recent developments in understanding structural and energetic aspects of condensation by multivalent cations, the implications of condensation for understanding some aspects of *in vivo* behavior, and potential applications of condensation by cationic amphiphiles for gene-transfer technology. We will not consider DNA packaging in phage, chromosomal or chromatin condensation, or polymeric coil–globule model systems.

Observation of DNA condensation

DNA condensation has been observed by a variety of techniques that detect changes in polymer size or chirality, including various forms of electron microscopy, total intensity and dynamic laser light scattering, sedimentation, viscometry, linear optical dichroism, and circular dichroism. A recent important addition to this list is fluorescence microscopy (FM), which was used to observe the condensation of single large T4 DNA molecules [6•,7••]. Fluorescence was produced by intercalated DAPI (4,6′-diamidino-2-phenylindole), and condensation provoked by the addition of high concentrations of polyethylene glycol (PEG). The radii of the uncondensed and condensed forms were ~3 μm and 0.7 μm respectively.

Agents that cause condensation *in vitro*

Chemical agents cause condensation by modifying electrostatic interactions between DNA segments, by modifying DNA–solvent interactions, by excluding volume to the worm-like coil, by causing localized bending or distortion of helical structure, or by some combination of these effects.

Multivalent cations

Early studies indicated that in aqueous solutions at room temperature, a cation valence of +3 or greater is necessary to cause condensation (e.g. the naturally occurring polyamines spermidine^{3+} and spermine^{4+} and the inorganic cation $Co(NH_3)_6^{3+}$, polylysine, and basic histones.) However, recent results [8••] show that Mn^{2+} can produce toroidal condensates of supercoiled plasmid DNA, but not of linearized plasmid. Supercoiling appears

to aid Mn^{2+} in stabilizing helix distortions, and also provides a 'pressure' that enhances the side-by-side association of DNA segments, an effect also observed with Mg^{2+} [9,10]. High concentrations of divalent transition metals cause the aggregation of linear DNA, but not into ordered condensates [11,12]. In the diaminoalkane series $NH_3^+(CH_2)_nNH_3^+$ (n = 1–6), compounds with n = 3 and n = 5 cause compaction of single T4 DNA molecules, but those with n = 2, n = 4, and n = 6 do not [13], indicating that linker length and hydrophobicity, as well as charge, play a role.

Alcohol

80% ethanol is commonly used to precipitate DNA, but as little as 15–20% ethanol will cause condensation to toroids or rods if $Co(NH_3)_6^{3+}$ is also added to a solution at low ionic strength [14••]. Methanol and isopropanol behave similarly.

Basic proteins

A variety of basic proteins can produce toroidal or rod-like condensates. Four different proteins (sea urchin histone H1, sea cucumber histone f0, chicken erythrocyte histone H5, and clupeine) have little effect on the size or morphology of condensates with DNAs of various lengths [15]. Transition protein TP2, which is involved in chromatin condensation, shows GC-rich sequence preference and is zinc dependent [16].

Neutral crowding polymers

Even neutral polymers such as PEG, at high concentrations and in the presence of adequate concentrations of salt, can provoke DNA condensation through an excluded volume mechanism [17]. PEG and other crowding agents such as polyvinylpyrrolidone or albumin can enhance the effects of DNA-binding proteins such as the histone-like protein HU [18•,19•]. Low molecular weight PEG 200, on the other hand, disfavors the joining of λ-DNA ends (a model for condensation) apparently by reducing water activity [20]. Some biochemical and biological manifestations of crowding have been reviewed recently [21].

The condensation of single T4 DNA molecules by PEG has been observed by FM [6•,7••,22]. The critical concentration of PEG decreases with increasing degree of polymerization and salt concentration [22], demonstrating the importance of excluded volume and electrostatic repulsion effects. In concentrated PEG solutions, T4 DNA reverts to the coil state, an effect expected theoretically.

Cationic liposomes

When DNA is condensed with cationic liposomes composed of a mixture of cationic and fusogenic lipids, the complex becomes a very efficient agent for transfection of eukaryotic cells [23], with considerable potential interest for gene therapy [24•,25]. Although these complexes do not have the regular morphology that we have used to define condensation, great current interest in their structure–function properties warrants their consideration here. As summarized in [26•], optimal transfection depends on the choice of lipid composition, DNA:lipid ratio (there should be a slight excess of cation), and total concentration. A novel procedure has been developed to form hydrophobic complexes between cationic lipids and plasmid DNA, in which the DNA does not condense [27].

Part of the efficacy of liposome complexes is presumably due to the compact state of the DNA, which protects it from nucleases and allows it to pass more easily through small openings. The lipid coating on the DNA may also increase its permeability through cell membranes, although there is evidence [28] that membrane penetration occurs predominantly by endocytosis rather than fusion.

The structures of these complexes appear to depend on the type of microscopy used to view them, as well as their lipid composition and preparative details. Conventional electron microscopy (EM) studies suggest that cationic liposomes initially form clusters along the uncondensed DNA. At a critical density, these clusters coalesce by DNA-induced membrane fusion, and the DNA condenses to a form completely encapsulated by lipid [29]. Freeze-fracture EM shows liposome complexes and bilayer-covered DNA, with the DNA tubules connected to the liposome complexes as well as occurring free [30•]. Cryo-EM shows that in an excess of lipid charge, plasmids are trapped between lamellae in clusters of aggregated multilamellar structures [26•].

Differential scanning calorimetry (DSC) has been used to characterize the binding of DNA and RNA to sphingosine-containing dimyristoylphosphatidylcholine (DMPC) liposomes [31]. The DSC melting profiles of the mixed liposomes, but not those of neat DMPC, are significantly altered by the nucleic acid, demonstrating binding to the cationic component. Binding to sphingosine derivatives in which the amino group is N-acetylated, and thus has no positive charge, is much weaker or absent [32], indicating that binding is electrostatic.

The condensation of single molecules of T4 DNA in the presence of the cationic surfactant cetyltrimethylammonium bromide (CTAB) has been observed by FM [33]. The coil-globule transition is a sharp function of surfactant concentration, changing from all-coil to all-globule within 9.4–20 μM CTAB, with both forms (but not intermediate states) co-existing between these concentrations. The condensed form swells somewhat at CTAB concentrations >1 mM, due to the penetration of the surfactant into the globule. The binding isotherm of CTAB to DNA has been measured by potentiometric titration [34], and binding increases sharply in the coil–globule transition region. Apparent cooperative binding reflects the bimodality of the coil–globule distribution. The condensation induced by CTAB is reversible, and polyacrylic acid will induce the globule→coil transition.

Morphology of condensed particles

Toroids

Much interest has been drawn to DNA condensation because the condensed particles often assume a striking toroidal shape. One turn of DNA circumferentially wrapped around a toroid of outer radius 50 nm will contain about 930 base pairs (bp). Atomic force and electron microscopy [35] show that sperm DNA packaged by protamines adopts a toroidal structure with inner and outer radii of 7.5 and 45 nm respectively and containing up to 60 kbp of DNA.

Non-toroidal shapes

Although toroids are the most common morphology in DNA condensation from aqueous solution, the addition of alcohol (methanol, ethanol, or isopropanol) tends to produce more rod-like structures when condensation is provoked by $Co(NH_3)_6^{3+}$ [14••]. The alcoholic solvent and condensing ligand may act synergistically to locally destabilize the double helix, permitting DNA foldbacks that lead to rod-like condensates. Condensation with permethylated spermidine also produces a large proportion of rod-like particles [36], perhaps due to the more hydrophobic character of this ligand.

The addition of larger volumes of alcohol produces ramified fibrous aggregates, accompanied by a B→A conformational transition of the plasmid DNA [14••]. The transition occurs at a lower concentration of either ethanol or $Co(NH_3)_6^{3+}$ than would be required with either alone, indicating that they act cooperatively. It is speculated that the A-DNA strongly self-adheres and rapidly aggregates into fibrous networks, disallowing the annealing required to form more compact and orderly condensates.

Different manipulation methods can also produce different morphologies of highly aggregated complexes, which can be visualized in fluorescence and bright-field microscopy [37]. Vigorous mixing yields globular complexes with different degrees of compaction when poly-L-lysine or histone H1 is the condensing agent. Molecular networks or single cable-like structures can be obtained if hydrodynamic shear is minimized.

Liquid crystals

Short DNA molecules do not normally form toroidal aggregates: their length is insufficient to nucleate such structures [1]. At very high concentrations near 200 mg/ml, mononucleosomal DNA of about one persistence length spontaneously forms a liquid crystalline phase [38]. This transition takes place in the presence of monovalent salt due to repulsive excluded volume interactions between the rod-like molecules. A liquid crystalline phase can also be formed by adding spermidine to a dilute solution of mononucleosomal DNA [39•]. In this case, formation of the ordered phase is due to attractive interactions between the parallel rods.

Long DNA, 8 kbp or ~54 persistence lengths, has been found to undergo a transition from the isotropic to the anisotropic phase in 0.1 M NaCl [40]. The anisotropic phase appears at 13 mg/ml and the isotropic phase disappears at 67 mg/ml. The first of these concentrations is much lower than expected for a polymer with the stiffness of DNA; this is speculated to reflect local, sequence-dependent variations in DNA flexibility.

Liquid crystalline domains of DNA are seen in electron micrographs of dinoflagellate chromosomes [41], and in X-ray scattering from bacteria with high copy number plasmids [42••]. *In vitro* studies of supercoiled, nicked, and linear plasmids shows that the structure of the liquid crystalline phase is determined by the supercoiling density and handedness of the plasmids, rather than by environmental factors, as is the case for linear DNA molecules. This leads to the suggestion that "supercoiling-regulated liquid crystallinity represents an effective packaging mode of nucleosome-free, topologically-constrained DNA molecules in living systems" [43••].

Mechanism of condensation

A statistical thermodynamic model has been proposed [44] for the formation of toroids from DNA condensed with crowding polymer in high salt. It is based on a theory of the undulation enhancement of the electrostatic interaction in hexagonal arrays of semi-flexible polyions. The chemical potential of a toroid is split into bulk, surface, and curvature contributions, and minimized to obtain optimal toroid dimensions as a function of the concentrations of polymer and salt. The range of toroid stability is also predicted.

The size and morphology of condensed DNA particles appears to be determined at least as much by kinetics as by thermodynamics [1]. A single molecule of T4 DNA has been shown to undergo a first-order transition, a discrete change between expanded coil and condensed globule [7••]. FM is interpreted as showing an initial slow nucleation, in which the contour length of the coil decreases with constant speed, followed by faster growth of the globular structure. It is speculated that T4 DNA is too large to form a single orderly toroid; the initial nucleus may be toroidal, whereas the subsequent more rapidly formed globule is disordered though compact. Although the resolution of the microscope, 0.3 μm, is too coarse to be certain where nucleation occurs, a theoretical model predicts that it proceeds from one end [45].

A kinetic mechanism that seems consistent with the FM observations has been proposed and is based on an ingenious structural model for toroids [46••]. In this model, DNA is coiled with a constant radius of curvature into a series of equally sized contiguous loops which process about the toroid axis. This model is consistent with most observations in the literature. Kinetically, it is proposed that a DNA molecule in solution spontaneously

forms a loop with two sequence-separated sections in close contact. A condensing agent binds to this contact and stabilizes the loop. Successive condensing agents bind both looped and extended DNA to continue toroid formation. Quantitative analysis of this kinetic model leads to a distribution of toroid sizes in good agreement with measurements.

Structure of condensed DNA

It is generally assumed that condensed DNA is in the B-conformation, an assumption often supported by circular dichroism spectroscopy. Nevertheless, there is considerable evidence that the solvent conditions that lead to condensed DNA can also distort DNA secondary structure [47]. Recent work shows that the coupling between secondary structure and condensation depends to some extent on base sequence and supercoiling.

Raman spectroscopy of protamine–calf thymus DNA complexes shows a modified B-form with appreciable unstacking of the bases [48]. Divalent transition metals cause substantial deviations from normal B-form vibrational spectra in the base and backbone regions [49,50]; changes are also seen with trivalent cations such as La, Eu, and Tb ions [51] and cobalt hexaammine and cobalt pentaammine [52]. Mn^{2+} can produce toroidal condensates of supercoiled plasmid DNA, but not of linearized plasmid [8••]. Mg^{2+} does not cause condensation, and neither $MgCl_2$ nor NaCl reverses the effect of $MnCl_2$, indicating that the Mn-induced condensation mechanism is not primarily electrostatic. It is hypothesized that supercoiling cooperates with Mn^{2+} to stabilize helix distortions, and also provides a 'pressure' that enhances lateral association.

Specific DNA sequences affect both secondary structure and condensation. Some sequences may be converted from B- to Z-conformation by ligands that also induce condensation. The condensation of pUC18 plasmids by hexaammine cobalt is enhanced by the insertion of $(dC–dG)_n$ (n = 12 and 20) sequences which were demonstrated to have been converted to Z-form [53•]. Blocks of $(dA–dC)_n·(dG–dT)_n$ (n = 23 and 60) inserted into plasmids pDHf2 and pDHf14 were converted to Z-DNA by the naturally occurring polyamines spermidine^{3+} and spermine^{4+} (but not by putrescene^{2+} or inorganic trivalent cations) at concentrations that would normally induce condensation [54]. Runs of adenines (A-tracts) have been extensively investigated as sources of sequence-induced DNA bending. Condensates of different morphologies are formed with DNAs of identical base composition depending on whether the DNA sequence is random or tandemly iterated A-tracts are short (<3) or long (>3) [55]. Longer in-phase A-tracts, which cause sequence-directed bending, form unusually small toroids.

Crystal structure analysis of oligonucleotides in the R3 space group shows self-fitting of B-DNA molecules through groove–backbone interactions [56•]. These interactions can, in turn, trigger sequence-dependent changes of secondary structure involving rearranged hydrogen bonding [57••]. Such interactions might well occur in condensed DNA as well as in crystals.

Intermolecular forces in condensation

DNA condensation arises from a complex interplay of interactions [2,3]. These include entropy loss upon collapse of the expanded worm-like coil, stiffness which sets limits on tight curvature, electrostatic repulsions which must be overcome by high salt concentrations or by the correlated fluctuations of territorially bound multivalent cations, hydration which must be adjusted to allow mutual accommodation of the water structure surrounding surface groups on the DNA helices as they approach, and repulsive excluded volume interactions with other polymers in the solution. Attractive free energy may also come by bridging through condensing ligands, and by interhelical binding between bases whose normal intraduplex pairing and stacking have been disrupted by interactions with solvent or ligand. Recent attention has focused largely on counterion fluctuations and hydration forces. Because at close range both hydration and ionic forces appear to depend on the effect of apposing surface lattices on the fluctuating correlations of ions and water molecules between them, the two types of force may prove difficult to disentangle.

Correlated counterion fluctuations

DNA condenses in the presence of multivalent cations when 89–90% of its charge is neutralized [58]. This is true not only in water, but also in aqueous solutions in which the dielectric constant is lowered by alcohols [14••] or raised by osmolytes [59•]. The counter-ions not only screen coulombic repulsions between the DNA phosphates, they also produce attraction through correlated fluctuations of the ion atmosphere. This idea was first put forward by Oosawa in 1970 [60], and was recently applied to DNA [2]. A similar but more detailed model based on the Poisson–Boltzmann theory has been developed and applied to synthetic polyelectrolytes [61•]. It correctly predicts that aggregates will redissolve as salt is added due to the screening of short range electrostatic attractions. Detailed Monte Carlo calculations on hexagonally packed DNA predicts that divalent cations will lead to a net attraction at 5–15 Å between surfaces, depending on ion size and salt concentration [62•]. Experimentally, a charge of +3 or greater is required for condensation in aqueous solution at room temperature, but theory and experiment agree that divalent cations will be effective if conditions are only slightly different.

Hydration force

Forces between DNA double helices in hexagonal array have been measured directly as a function of distance, pushing the molecules by osmotic stress and measuring spacing by X-ray diffraction [63•]. Exponentially decaying forces are observed that are insensitive to counterion

valence, structure or concentration, arguing against forces based on electrostatic double layer interactions or ligand bridging [64]. Instead, Rau and Parsegian proposed that polyvalent ligands bound to DNA double helices appear to act by reconfiguring the water between macromolecular surfaces to create attractive long-range hydration forces. Enthalpy and entropy are measured by varying temperature [65,66]; the entropy ΔS of the Mn^{2+}-induced DNA-assembly transition is positive, attributed to the release of water from the surfaces. ΔS is increased by changing the Mn anion from the chloride ion to the chaotropic perchlorate ion. Although the insensitivity of DNA–DNA forces to counter-ions may be explicable by the extreme non-linearity of electrostatic interaction near the highly charged DNA surface, it is hard to explain the positive ΔS by an electrostatic mechanism.

Directly measured forces between DNA helices in ordered arrays in univalent salt have been parameterized to allow use in molecular modeling calculations [67••]. Long-range electrostatic interactions take configurational fluctuations of the DNA into account.

Long-range attractive force

Although DNA condensation and aggregation usually require multivalent cations, mononucleosomal DNA has been found to aggregate at a critical concentration that increases with added NaCl [68,69•]. This unexpected result may provide evidence for a poorly understood long-range attractive force that is manifested only in the third virial coefficient, when second virial coefficient repulsions are reduced by added salt [70•]. Attraction has been predicted in univalent salt solution between parallel polyelectrolyte rods, modeled as infinite linear arrays of uniformly spaced monovalent charge sites [71••]. The force is repulsive for distances either much less than, or of the order of, the Debye length, but can be attractive at intermediate distances. Attraction results from the enhanced translation entropy of the condensed counter-ions as they share the space between the polyions. The relationship between this theoretical prediction and the experimental results [68,69•] remains to be clarified.

Biological significance

Increased attention is being devoted to the biological mechanisms and consequences of DNA condensation, particularly in prokaryotes where the extent and significance of DNA compaction is less obvious than in eukaryotic chromatin. Early work has shown that DNA condensation is required for the efficient catenation and recombination of plasmids by topoisomerases [72]. The rate of DNA renaturation, and of strand exchange with double-stranded DNA without proteins, is greatly accelerated by DNA condensation [73]. In these cases, the concentration of reactants in a confined search space is enhanced.

Condensation induced by DNA-binding proteins such as HU probably underlies the stability of compacted bacterial nucleoid or kinetoplast DNA. Condensing proteins can function at lower concentrations when their effect is enhanced by crowding, for example, by other intracellular proteins [18•,19•]. The formation of liquid crystalline arrays may be required for the efficient intracellular packaging of high copy number supercoiled plasmids [43••].

More specific effects probably come into play in condensed DNA. The close and potentially geometrically specific interaction of DNA helices can aid the construction of molecular recognition motifs for the formation of nucleoprotein complexes [56•]. This may be abetted by the modification of secondary structure by groove–backbone interactions [57••]. Many of the solution conditions that cause condensation also modulate the secondary structure of DNA, and topological linkages require coupling between writhe and twist in supercoiled DNAs. It has therefore been proposed that "the secondary structural polymorphism which characterizes ... DNA molecules might [play] a regulatory role by acting as a functional link between cellular parameters and the extent, mode, and timing of nucleic acid packaging processes" [47]. Likewise, "the close and specific approach of DNA segments occurring in genome packaging, DNA looping, synapsis formation or supercoiling can contribute directly to the secondary structure changes needed for DNA processing" [57••].

Conclusions

Our insights into DNA condensation have increased due to the application of new techniques, the investigation of a wider range of condensing agents and solvent conditions, the deeper understanding of intermolecular forces, and the application of recombinant DNA technology to produce supercoiled plasmids with defined sequences. Activity in the area has increased because cationic liposome–DNA complexes may potentially be useful in therapeutic gene transfer, and condensation and DNA secondary structure modification may be linked in biologically significant ways.

Although recent progress has been significant, much remains to be done. One continuing important direction for future research is the more extensive experimental and theoretical study of non-covalent interactions that stabilize the condensed state, and that lead to the adoption of toroidal or alternate morphology. The kinetic pathways leading to condensation are only sketchily understood, and it is likely that there are important differences between the monomolecular condensation of large DNAs and the multimolecular condensation of smaller ones. Another important theme is the systematic study of the effects of specific DNA sequences on condensation, which will have two aims: firstly, to understand the effects of sequence on the binding of condensing ligands and on the surface lattice that modulates ion and water structure in the solvent layer between apposing helices, and secondly, to elucidate the influence of local bending and twisting on the close approach of specific regions within global

supercoiled structures. Research on cationic lipids, on condensing proteins, and on other condensing agents both naturally occurring and designed, will surely continue. The similarities between some aspects of DNA condensation and protein folding are striking. The analogy suggests that research on DNA condensation will continue to be intellectually rich and practically significant, and also that the problems are not likely to be quickly solved.

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