DNA–DNA interactions

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The forces that govern DNA double helix organization are being finally systematically measured. The non-specific longer-range interactions – such as electrostatic interactions, hydration, and fluctuation forces – that treat DNA as a featureless rod are reasonably well recognized. Recently, specific interactions – such as those controlled by condensing agents or those consequent to helical structure – are beginning to be recognized, quantified and tested.

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Introduction

To ask about the physical forces and energies that involve DNA molecules is to ask whether there is more to DNA than its ability to carry the genetic code. During the initial excitement of cracking the code, it was tempting to set aside the idea that the packing of DNA in the cell relates to gene expression. Now that the controlled expression of genes is of primary interest, the work of packaging DNA is again an issue. The mechanical properties of DNA [1,2••] (persistence length, torsional rigidity), its polyelectrolyte character [3••] (charge density, counterion condensation), hydration [4] (counterion specificity, interactions with ligands) and liquid-crystalline packing properties [5] (mesophases and transitions between them) are all systematically investigated. DNA–DNA and protein–DNA interactions have become most pertinent.

So massive are these investigations that, in this opinion, we reluctantly omit material from several related topics. Thriving subjects — DNA condensation (expertly reviewed in this series in 1996 [6]), protein–DNA interactions, DNA supercoiling and the statistical mechanics of rod-like particles — appear only tangentially; we will refer to these subjects only when discussing the forces that underlie them. We examine the DNA liquid crystals upon which the force and energy measurements are being made. We review these measurements and then discuss them in terms of their component repulsive and attractive forces. During this review, we also suggest an experimenal strategy for gauging the strength and specificity of the physical forces between DNA molecules.

DNA liquid crystals – ordering in solution

As functions of salt species and concentration, of DNA stiffness and length, of temperature and osmotic stress, and of the activities of polycationic condensing agents [6], DNA, salt and water mixtures assemble into a taxonomy of beautiful liquid-crystalline phases of different symmetries and densities [5]. Over a wide range of DNA concentrations, entropic packing constraints are sufficient to align the molecules into orientational order but they are not strong enough to induce long-range positional order (crystallinity), hence 'liquid crystal'.

In gravimetric mixtures, two or more phases can co-exist. The activities of salt, water and DNA itself are equal in co-existing phases but are not yet known. In liquid-crystal samples prepared at known water (osmotic) and salt activities, the chemical potential of DNA at different concentrations can immediately be determined. Under these osmotic stressing conditions, it is possible to measure the repulsive forces between DNA molecules [7].

Condensing agents [3] that induce attractive interactions between DNA molecules can concentrate DNA enough to form liquid-crystalline phases. These agents are, typically, multivalent ions like manganese, cobalt hexammine, spermine, spermidine [8,9], protamine, polycations such as those used for nonviral gene therapy [10] and perhaps even polysaccharides such as levan [11]. Pelta *et al.* [12] showed that DNA forms both columnar and cholesteric phases when condensed by spermidine.

For reasons not stated, many gravimetric DNA, salt and agent preparations are diluted or concentrated by adding or evaporating water. If the DNA precipitates and if — as is probable — the agent stabilizes the condensate, then the agent : DNA ratio will be higher in the condensed phase. The consequence of this partitioning is that the active concentrations of agent or salt will change in the supernatant. The two phases will co-exist under conditions in which the activities of salt, agent and DNA are not known.

DNA condensation under osmotic stress has been combined with condensation by polycationic agents [13]. Different ratios of osmotic stress and polycation concentration have been used to measure the energies of condensation as well as the forces by which these agents hold the DNA molecules at finite but small (< 1 nm) separations. The chemical potentials are under control. With the Gibbs–Duhem equation, one can use the known

activities of each component to determine its contribution to the free energy of the unassembled DNA [14].

Modern biochemical and molecular biological techniques allow one to prepare monodisperse solutions of DNA whose length ranges from a few nanometers to several micrometres. This facility makes it possible to address fundamental questions. What determines the concentration at which a polymer solution expels a liquid-crystalline phase and how does this concentration depend upon DNA length [15••]?

A recent cryo-electron microscopy study shows how bacteriophage T7 DNA packs inside its virus capsid [16••]. DNA appears to spool axially around a connector core. This work addresses the old question as to how DNA packs into small spaces. The authors speak of quasi-crystalline packing, although liquid crystallinity cannot be ruled out.

Repulsive interactions

Liquid crystalline order enables direct measurement of intermolecular forces. In the osmotic stress method, DNA liquid crystals are equilibrated against neutral polymer solutions of known osmotic pressure, pH, temperature and ionic strength [17]. After equilibration, DNA–DNA separation is measured either by X-ray scattering, if the DNA subphase is sufficiently ordered, or by straightforward densitometry. The known DNA density and osmotic stress immediately provide an equation of state that can be codified in analytic form for the entire phase diagram. Then, with the local packing symmetry derived from X-ray scattering [17,18], if necessary corrected for DNA motion [19••], it is possible to extract the bare interaxial forces between molecules. *In vivo* observation of DNA liquid crystals [20] shows that the amount of stress needed for compaction and liquid-crystalline ordering is the same for DNA *in vitro* [21].

Direct force measurements, performed on DNA in univalent salt solutions, reveal two types of purely repulsive interactions between DNA double helices. At surface separations of less than ~1 nm (interaxial separation ~3 nm), an exponentially varying 'hydration' repulsion is thought to originate from partially ordered waters near the DNA surface. At surface separations greater than 1 nm, measured interactions reveal an electrostatic double-layer repulsion, arising from the negative phosphates along the DNA backbone. The measurements give no evidence for a significant DNA–DNA attraction. Charge fluctuation forces must certainly occur, although they appear to be negligible at least for liquid-crystal formation in monovalent ion solutions. At these larger separations, the double-layer repulsion often couples with configurational fluctuations and create exponentially decaying forces whose decay length is significantly larger than the expected Debye screening length [19••].

Short-range molecular interactions between DNA molecules appear to be insensitive to the amount of added salt. This has been taken to be evidence that they are not electrostatic in origin [22]. The term 'hydration force' associates these short-range forces with perturbations of the water structure around the DNA [18]. Alternatively, short-range repulsion has been viewed as a consequence of the electrostatic force that is specific to high DNA density and counterion concentration [23].

A recent measurement of the equation of state of DNA liquid crystals showed that, in addition to the bare hydration interactions at surface separations < 1 nm, there is also a strong configurational entropic contribution to the effective repulsion between DNA molecules [17,19••]. This fluctuation-enhanced repulsion comes from both coupling between bending fluctuations and screened electrostatic interactions between DNA molecules. These fluctuations mask the bare electrostatic force so that it appears only in its fluctuation-modified form. From these measurements, the form of the bare electrostatic repulsion, as well as the effective charge densities along the chain, can be extracted.

Measured DNA–DNA interactions between condensed molecules differ qualitatively from the predictions of the electrostatic double-layer theory. This is particularly true for DNA condensed by polycations. DNA that repelled with an exponential of characteristic length 3 Å when there was insufficient polycation for condensation was seen to repel with a characteristic distance of 1.5 Å after the DNA condensed [13]. As long as sufficient polycation is present to cause precipitation, the residual repulsion is independent of polycation and univalent salt concentrations. The halving of the decay constant has been rationalized in terms of a solvation picture in which there are regions of attraction and repulsion [22].

Attractive interactions

Unfortunately there is no means for direct measurement of DNA–DNA attractive interactions. Force measurements made without administration of condensing agents reveal only repulsive forces. With condensing agents, DNA will precipitate or, with inadequate amounts of agent, exhibit weakened repulsion.

Some ions (e.g. manganese) show a temperature-dependent attraction [24]. The attraction increases with increasing temperature. Condensed DNAs move closer together as the preparation is heated. Shrinkage of an assemblage held at constant osmotic stress is tantamount to an increase in entropy. Such an increase can be 'solvent entropy', caused by the release of water structured around the isolated DNA, or it can be due to the counterionic fluctuations that increase with molecular approach [25,26]. This latter concept goes all the way back to the work of Oosawa [27] and has recently been upgraded by numerical model simulations [28•] as well as analytical calculations [29,30•,31•]. It is not easy to distinguish between the two without paying overdue quantitative attention to the consequence of varied salt concentration and salt type.

Chaotropic (water structure breaking) anions, added to DNA solutions, qualitatively encourage DNA precipitation at low levels of condensing agent. It has been argued that this sensitivity to the entropy of water in the bath, as a result of the chaotropic ion, indicates an important contribution due to the release of water. All condensing agents, from manganese, cobalt hexammine and the polyamines to the cationic protein protamine that wraps into the DNA grooves, show the same exponential repulsion, with 1.5 Å characteristic length [13].

Different agents condense DNA into different DNA–DNA separations; however, these separations are independent of salt and agent concentration 13]. It would be startling if the attractions that condense DNA in all these cases were driven by ionic fluctuations.

An alternative mechanism is the presumed attraction between polycations and the counterions present in the space between them [32,33]. These attractive forces bear some similarity to the forces in wetting or to forces between two macroscopic hydrophobic moieties. These ideas are still quite controversial, however, and are not generally accepted.

Intimations of repulsive interactions

Under physiological conditions, DNA is highly charged (two negative charges per base pair or 3.4 Å of its length). In an electrolyte solution, the DNA's net negative charge creates an accumulation of counterions close to its surface. These counterions that screen part of the bare charge and lead to an 'effective' charge density that is felt at long distances. In principle, this effect can be captured by a nonlinear Poisson–Boltzmann theory [34].

In electrophoretic mobility measurements, it is the 'effective' charge density that is responsible for the force dragging the molecule through solutions or gels when an electric field is applied. The problems with interpreting electrophoretic mobility measurements are discussed in detail in [35]. Although extensive measurements were performed on DNA [36–38], the resulting 'effective' charge densities come out at between 10% and 60% [38,39] of the bare charge of DNA. This discrepancy exists not because of experimental uncertainty, but because of the different theoretical treatments of the measured mobilities.

Intimations of attractive interactions

A few experimental results exist that suggest the presence of attractive interactions under noncondensing conditions. Some light scattering experiments [40,41] in a 1 : 1 electrolyte solution at high concentration (1M NaCl) indicate aggregation. The onset of the double-twisting blue phase [5] is an alternative possibility. Similarly, electron micrographs of the tightening of supercoils [42], as a function of increasing salt concentration, suggest strong side by side associations. Other light scattering and fluoresence results contradict these findings [43].

Under salt-free conditions, isotropic solutions of short fragment DNA solutions show a pronounced peak for the structure factor measured by small angle X-ray scattering [44]. Such peaks are well known from other studies in polyelectrolyes [45] and have sometimes been interpreted as indicating attractive interactions between polymers.

Helix-specific interactions

Kornyshev and Leikin [46••], recently solved the formidable problem of a helix–helix interaction that takes into account the helical pitch, the number of helical strands, the spacing between charges or other chemical groups along the helix, and the number of base pairs per turn. Their formulation applies to hydration forces and electrostatic double-layer forces. From simple symmetry considerations, the theory shows how repulsion can weaken and even turn to attraction depending upon small changes in the helical charge pattern caused, for example, by counterion adsorption. For example, there is a slight difference between the helical pitch of DNA in solution [47] compared to DNA in any condensed array [48]. Their theory connects the short decay constant of hydration repulsion between guanosine four-stranded helices [49,50••] and between collagen triple helices [51] with the helical pitch of these structures.

Helix interaction is so specific that there is an optimal orientation angle between two interacting helices; the tendency is essentially to lock the two molecules into a separation-dependent angle [46••]. This tight orientational preference might be the essential element in analyses of the transition from hexagonal arrays to cholesteric packing [52•].

Conclusions

Measured forces and free energies and entropies compel more critical testing of molecular assembly theories. Use of this information will be especially valuable in the examination of supercoiling, condensation and even protein–DNA interaction. Speculation about causative forces can not be validated without directly measuring these forces. Experiments without well defined thermodynamic variables will produce many effects but not enable careful thought about cause and effect.

Liquid-crystalline phases are best routinely prepared under osmotic stress rather than under stoichiometric conditions. Otherwise there is no way of knowing the ionic and water activities that create the ordered structures under examination. The typical neglect of the chemical potentials of water, salt and DNA are as self-defeating as it would be to ignore pH and temperature.

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