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INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
34100 TRIESTE (ITALY) - P.O.B. 586 - MIRAMARE - STRADA COSTIERA 11 - TELEPHONES: 224281/2/3/4/5/6
CABLE: CENTRATOM - TELEX 460392-1

SMR/111 - 13

SECOND SUMMER COLLEGE IN BIOPHYSICS

30 July - 7 September 1984

Lecture 1: Polyelectrolyte Properties of DNA

Lecture 2: DNA Packaging in Viruses and E. Coli

J. WIDOM
Laboratory of Molecular Biology
Medical Research Council Centre
University Medical School
Hills Road
Cambridge CB2 2QH
U.K.

Trieste Summer School for Biophysics Week 2, 1984 J. Widom

Lecture I: Polyelectrolyte Properties of DNA

DNA from bacteriophage λ is a linear, double-stranded molecule with a contour length of ~16μ

In aqueous solution, pH 7 with excess Na^+, behaves as a large random coil, with "Diameter of Gyration" = 2·Radius of Gyration of ~12,000 Å. Thus the DNA occupies a volume of solution greatly in excess of the volume of the hydrated DNA filament. This is due to two important properties of DNA:

a) stiffness for bending (and torsion); P = persistence length ~ 500 Å.
b) repulsion between DNA segments due to high negative charge density.

Backbone of DNA has one phosphate (negatively charged) for each base;

Projected onto DNA axis, charge density = -1 charge/base × 2 bases / 3.4 Å (double helical B-form DNA has one "base-pair" (2 bases) every 3.4 Å), so charge density = -1 charge / 1.7 Å

Because of this very high charge density, the molecule will have radically different physical properties

A) Qualitative Argument, from Onsager:

Consider positive (+1) mobile test-ions in field of infinitely long line-charge of charge-density $-1/b$ (b = distance in Å). Now consider subset of possible configurations where one ion is very near polyelectrolyte, at distance $\rho \le \rho_0$ where it feels unscreened Coulomb potential; let all other ions be at distances $\rho_j > \rho_0$. For test-ion at $\rho \le \rho_0$,

$$u(\rho) = \frac{2e^2}{b\varepsilon} \ln \rho$$

e = electronic charge; ε = bulk dielectric constant; $\frac{-1}{b}$ ~ poly-ion charge dens. ($= q/b$, $q=-1$)

Contribution of this set of configurations to phase-integral is:

$$A_i(\rho_0) = f(\rho_0) \int_0^{\rho_0} \exp\left(-u(\rho)/kT\right) 2\pi\rho \, d\rho$$

$f(\rho_0)$ = finite factor contributed by all ions j, where $\rho_j > \rho_0$
k = Boltzmann's constant; T = (Kelvin) Temperature

Define $\xi \equiv \frac{e^2}{b\varepsilon kT}$ dimensionless, proportional to poly-ion charge density

Then $A_i(\rho_0) = 2\pi f(\rho_0) \int_0^{\rho_0} \rho^{(1-2\xi)} d\rho$; if test-ions have valence $+N$, rather than $+1$, then

$$A_i(\rho_0) = 2\pi f(\rho_0) \int_0^{\rho_0} \rho^{(1-2N\xi)} d\rho$$

This integral diverges for all N, ξ such that $N\xi \ge 1$

Divergence of phase-integral implies physical instability: expect counterions to be adsorbed from infinitely dilute solution to reduce poly-ion effective charge density until $N\xi \le 1$.

For DNA in H_2O, 25°C, $\xi \sim 4.2 \gg 1$ even for $N=+1$.

∴ cations (+N) adsorbed until effective charge-fraction = $1/N\xi$

Residual charge-fraction (q_{eff}) depends only on N: DNA in H_2O, 25°C ...

N	q_{eff}
1	0.24
2	.12
3	.08

B) Manning's Statistical-mechanical Analysis:

Define θ_N = # of N-valent counterions associated per DNA-phosphate
Then $q_{eff} = 1 - N\theta_N$
Assume linear, infinitely long; charges q_{eff} spaced at distance b (=1.7 Å)
Assume residual (q_{eff}) charges interact via screened coulomb potential, with screening contributed by bulk-solution salts.
Define $\bar{g}_x = G_x / n_p RT$: G = free energy; R = gas constant; n_p = #moles charged groups / poly-ion

For charges i, j $\quad u_{ij} = \frac{e^2 q_{eff}^2}{\varepsilon} \cdot \frac{1}{b|i-j|} \cdot \exp\left(-K|i-j|b\right)$ (K = Debye screening parameter)

work of charging polyion is $\bar{g}_{el} = -(1-N\theta_N)^2 \xi \ln(1-e^{-K\xi})$

Let associated counterions diffuse freely within volume V_p (cm³/mole) centered on poly-ion axis. No "chemical binding"

Let counterions have free concentration (not "associated") = C_N

associated counterions have local concentration $C_N^{loc} = \frac{10^3 \theta_N}{v_p}$ molar

$\therefore$ entropy of mixing term

$\bar{g}_{mix} = \theta_N \ln (10^3 \theta_N v_p^{-1} / c_N)$

Find θ_N for which $\bar{g} = \bar{g}_{el} + \bar{g}_{mix}$ is minimum : set $\frac{d\bar{g}}{d\theta_N} = 0$

$\therefore \quad 1 + \ln (10^3 \theta_N v_p^{-1} / c_N) = -2NS (1 - N\theta_N) \ln (1 - e^{-2\lambda})$

in lim $c_N \to 0$, [illegible] (note that $\lambda \sim \sqrt{c_N}$)
this equation is satisfied only for

$N\theta_N = 1 - (NS)^{-1} \Rightarrow 1/NS = 1 - N\theta_N = g_{eff}$ as found in Onsager's qualitative explanation, above (A).

in lim $c_N \to 0$ and for 1:1 salt (e.g. NaCl) this implies that

$v_p = 41.1 (S-1) b^3$; $\therefore \quad C_1^{loc} = 24.3 (Sb^3)^{-1}$

for DNA in H_2O, 25°C with 1:1 salt (Na+) $C_1^{loc} \sim 1.2$ molar !

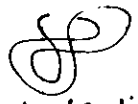
Thus the system is "potentiated" for N-valent cation binding, because were an N-valent cation to replace N monovalent ions, this would be accompanied by entropy increase of $nR \ln (C_1^{loc} / C_1)$

$\therefore$ expect binding constant of N-valent cation to go as:

$\ln K_N \sim n \ln C_1$

From v_p, find that the C_1^{loc} (= 1.2 molar) +1 counterions are contained within a volume of ~650 cm³/mole, which is equal to a ~7Å radius around the (20Å diameter) DNA surface.

Manning's theory is readily generalized for mixtures of counterions of differing valence : e.g. with +1, +N-valent ions ; set $g_{eff} = 1 - \theta_1 - N\theta_N$, and include extra terms in mixing entropy. Compute θ_1 and θ_N for $d\bar{g}/d\theta_1 = 0$ and $d\bar{g}/d\theta_2 = 0$.

DNA "Condensation" :  $\xrightleftharpoons{M^{3+}}$ ●

Diameter of Gyration ~ 12,000 Å

Diameter ~600-800 Å

Why is a trivalent (or higher-valent) cation required ?

a) Perhaps failure to neutralize sufficient charge
or b) Perhaps 3+ cations form "sandwich" between adjacent DNA segments

Calculations based on Manning's Theory suggest (a) is not the case.

X-ray results from J. Schellman support (b).

References:

Widom, J. & Baldwin, R.L. (1983) Biopolymers 22, 1595-1620

Widom, J. & Baldwin, R.L. (1980) J. Mol. Biol 144, 431-453

Schellman, J. & Parthasarathy, N. (1984) J. Mol. Biol. 175, 313-329

Manning, G. (1978) Q. Rev. Biophys. 11, p 179-246

Trieste Summer School for Biophysics 7 August 1984 J. Widom

Lecture 2: DNA Packaging in Viruses and E. Coli

A a) Double-strand DNA Viruses: phage λ

Assembly pathway: Construct independently empty capsids and tails

then, capsid + (DNA) —ATP→ + Tail → complete virus

volume of DNA ≈ volume of capsid.

Electron micrographs and x-ray solution scattering suggest that the DNA is organized as a "spool", with DNA segments quasi-parallel and closely spaced

Lambda DNA is linear, and so has 2 ends, which are genetically and physically distinct. The left end is known to be packaged first; the right end (packaged last) is inserted into the tail, and exits first on injection of DNA back out of capsid.

The persistence length of DNA is ~500Å, ≈600Å, the diameter of the phage capsid; also, the inside of the capsid is thought to be "lined" with DNA-binding sites.

Therefore, expect that the first DNA packaged (left end) will be on the outside of the spool, in contact with the capsid, and that the right-end will be near the middle, as the DNA fills the capsid.

Two related models of this sort were tested experimentally:

1. grow phage on thy⁻ E. Coli in a synthetic medium containing no thymine, but an excess of BrdU (Bromodeoxyuridine, a light-sensitive thymine analogue which is efficiently incorporated into DNA).
2. purify phage
3. irradiate with long-wave UV light (low-dose: only BrdU reacts); this produces 0 or 1 crosslinks between capsid proteins and some point on the DNA.
4. Lyse phage with EDTA; this "cracks" open the capsid and allows the DNA to come out.

(a): look in microscope (EM): find broken-capsid+tail complexes attached to DNA; measure distance to nearest end divided by total (contour) length

or (b) cleave molecules with a restriction enzyme: cuts DNA into many pieces, but cleaves at exactly the same sites on every molecule.

Label ends with radioactive ^{32}P

now we have both naked DNA, and DNA-capsid complexes. In CsCl gradients of mean density ~1.6, $\rho_{max} = 1.65$ and $\rho_{min} = 1.55$, naked DNA has buoyant density >1.5 and will sink. The DNA-capsid complexes have $\rho \leq 1.5$ and float.

Collect the set of all molecules that float; digest away proteins with protease, and run on agarose gel: identify which fragments were able to be crosslinked.

Result: in (a) find points of contact everywhere between the ends and the middle

in (b) find that all restriction fragments tested (Eco RI, Hind III, some of which are very small) could be crosslinked.

Therefore, we propose that not all phage are identical: in each, the DNA is organized in a spool-like fashion, with the bulk of the DNA quasi-parallel, but the mode of packaging allows the DNA to follow a different path in each virus particle.

B) Single-strand (RNA) viruses

Tobacco Mosaic Virus (TMV): helical structure, may be solved directly at high resolution by x-ray fiber diffraction using many heavy-metal derivatives.

symmetry of RNA conforms to symmetry of capsid; each group of three bases of RNA make equivalent interaction with one capsid protein monomer. High resolution details of this interaction will soon be available

TBSV (Tomato Bushy Stunt Virus) Icosahedral structure; the RNA has lower symmetry than the capsid: consequently, random orientation of the virus particles in the crystal causes the RNA to be invisible. The capsid protein has 3 domains; two are responsible for maintaining the capsid structure. The third domain is in the interior of the virus and is also invisible. This suggests that it may connect to the capsid surface through a flexible hinge to facilitate interaction with RNA in more than one non-equivalent conformation.

E Coli: genome organized in 40-50 supercoiled domains (in one large double-stranded circular DNA molecule).

There are 2 "histone-like" proteins; yet there is no proof that the chromosome is built (or packaged) out of fundamental repeating units such as the nucleosomes of eucaryotic cells.

References:

Widom, J. & Baldwin, R.L. (1983) 171, 415-437

Earnshaw, W.C. & Casjens, S.R. (1980) Cell 21, 319-331

Widom, J. (1984) Nature (News and Views) 309, 312-313

Harrison, S.C. (1983) J. Mol. Biol. 171, 577-580