DNA condensation

- extended DNA chains collapse into compact, orderly particles: only one or few molecules

- Characters of genomic DNA:

  Very long molecule, e.g. T4 phage = 160,000 bp ≙ 54 µm

  Virus capsid: ⌀ 100 nm !!

  → 540 fold compression

  E. coli = 'chromosome' length: 1.4 mm

  must fit into nucleolar region of 1 µm ⌀ : linear compression: ~1400

T4 radius of gyration ~ 950 nm : volume Compression ~6900!

- Energetic cost of compression:

  → loss of configurational entropy

  → tight bending of stiff helix

  → electrostatic repulsion

  organisms expend ~ $\frac{1}{2}$ ATP per base pair packaged!

DNA collapse or condensation can occur spontaneously in the test tube!

Overview

condensation: def. decrease in volume from
wormlike coil (diluted) →
compact state: fraction of water (solvent)
and DNA are comparable.

→ helices separated by 1 or 2 layers of
water

→ Commonly several molecules involved
in one structural unit (1 is possible, too)

Condensing agents:

- work by decreasing repulsions between
DNA segments: * neutralize phosphate charge
* reorientation of water dipoles
near DNA surfaces
* by multivalent ions (cationic)

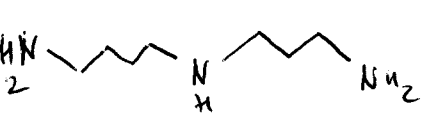
- by making solvent-DNA IA less favorable.
* adding Ethanol
* adding other polymer (PEG), excludes
volume to ten DNA

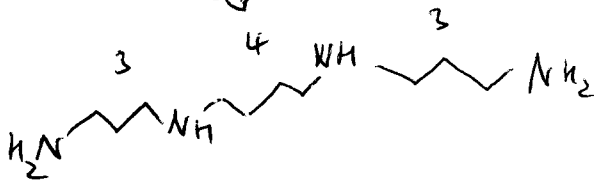
- by causing localized bending / distortion (→ facilitates condensation
* multivalent cations

— Cations: charge +3 and greater required,

Commonly used

* Spermidine^{3+} and Spermine^{4+}





* $[Co(NH_3)_6]^{3+}$

* Histones H1, H5 (basic proteins)

* divalent metal cations: - only under special conditions
in aqueous solutions
in - water - alcohol solutions

— alcohols and neutral polymers - ethanol

- PEG

⇒ Ψ - DNA:
polymer - salt - DNA
(condensed)

— cationic liposomes

— addition of polycations of $[Co(NH_3)_6]^{3+}$ to very dilute DNA solutions: toroids and rods

toroids: - size distribution regardless of DNA length
- helical fibers can sometimes be seen around ~~the toroid~~ wrapped around the toroid
additional small amount of rods

water-alcohol mixtures: greater proportion of rods, due to higher non-polarity of the solvent

→ more increasing alcohol concentration: rods + toroids replaced by more exclusively aggregated structures

at $\varepsilon < 65$ (solvent) + $[Co(NH_3)]^{3+}$: DNA collapses to a network of multistranded fibers

also: B-> A transitions undergone!

A-DNA: strongly self-adhesive, aggregates to fibrous networks, not allowing time for more compact/orderly condensates to form!

Kinetics / Reversibility

- dilute DNA: 30 - 120 min for complete condensation (10^{-10} M DNA molecules)

decondensation: very rapid, few seconds needed

on dilution of condensation agent

readily reversible process!! of condensation of DNA

- minimum DNA size: ~ 400 bp needed to form stable particles

Toroids

- well defined role in the middle, circumferentially wound double helical strands

- theoretical reasoning:

free energy of DNA as sum of compressive, repulsive, elastic contributions

compressive: - external osmotic pressure
- poor solvent quality

repulsive: - excluded volume of the DNA

elastic: - bending → phase diagrams, also dependent on DNA length, Φ
shorter: toroids
longer: spherical globules

Experimental Estimates of Intermolecular free energy

- λ DNA + optical tweezers:
  - * low concentrations of salt (spermidine)
  - * DNA molecule held at 80% extension (looping can still occur)
  - * Force rises abruptly to 4 pN ($[Co(NH_3)_6]^{3+}$) or 0.5 pN with spermidine!

$\Delta G_1 = F\Delta x$ ↓ free energy change in moving 1 base pair, 3.4 Å, $\sim -13.6 \cdot 10^{-22}$ J $\hat{=} \frac{1}{3}$ kT / bp

Spermidine : $\sim 2 \cdot 10^{-22}$ J

- osmotic stress technique:

Change of Bragg spacing on change of osmotic pressure

20 mM $[Co(NH_3)_6]^{3+}$: $-0.17\ k_BT$ per base pair
+ 0.25 M NaCl

Ionic Interactions. Manning's Counterion condensation theory

- ~ 90% of DNA charge must be neutralized for condensation

Equations for competitive counterion condensation of two cations with different valences:

(Theory = tough 4) – continuum model $\varepsilon_{solvent}$ = const
– Ions ≙ point charges
– polyion = line of indefinite length, characterized by ξ

(1) $1 + \ln \frac{1000\,\theta_1}{c_1 V_{P1}} = -2 z_1 \xi (1 - z_1\theta_1 - z_2\theta_2) \ln (1 - e^{-\varkappa b})$

(2) $\ln \frac{\theta_2}{c_2} = \ln \frac{V_{P2}}{1000\,e} + \frac{z_2}{z_1} \ln \frac{1000\,\theta_1 e}{c_1 V_{P1}}$

(3) $V_P(z) = 4\pi e N_A (1+z)\left(\xi - \frac{1}{2}\right) b^3$

$z_1, c_1, V_{P1}, \theta_1$
- z_1 ↓ Valence
- c_1 ↓ molar Concentration
- V_{P1} (territorial) binding Volume
- θ_1 fraction of DNA phosphate occupied

for cation of type 1 (= lower valent ion)

$\varkappa = \lambda_D^{-1}$ screening length

ξ = Counterion condensation parameter

$= \frac{L_{Bjerrum}}{b} = \frac{q^2/\varepsilon k_B T}{b}$

$b = \frac{\text{charge spacing}}{(e/N)}$

Ions for one can push together like charges having KT

Iterative numerical solutions:

$\tau = z_1\theta_1 + z_2\theta_2$ (total fraction of DNA charges neutralized)

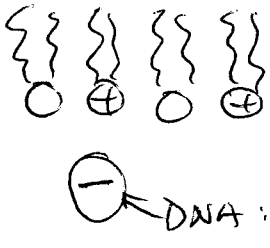
– collapse occurs when $\tau \approx 0.89 - 0.90$

– Condensation is determined by total charge, rather than by the binding of the multivalent cation

$\theta_2 = 0.1$ for Mg^{2+} - Spermine^{4+} - solutions

Lipid - DNA complexes

- cationic lipids needed (not occuring in nature)
- additionally : helper lipid (neutral, naturally occuring)



- 1 : 1 : 1 mixtures spontaneously formed from liposome (vesicle) and DNA solutions

* DOPC : DOTAP : DNA → L_α^c - Phase

DOPE : DOTAP : DNA → H_{II}^c - Phase

- release of counterion drives formation of complex!

* $T\Delta S / \Delta F \approx 0.97$!! at the isoelectric point, when amount of cationic lipid is varied.

possible

→ Application: gene transfer → gene therapy ?
(successfully used for transfer of GFP!)