

Circular DNA – How can we describe it?

General:

Has been known since 1962, that circular DNAs exist, they were isolated from several organisms: bacterial viruses, tumor and other animal viruses, bacterial plasmids and sex factors, bacteria, mitochondria, chloroplasts, kinoplasts, and sperm. Essentially all of the DNA of *organelle genomes* is **circular**).

Observation of circular DNA

The physical experimental techniques that have been used to show circular DNA are

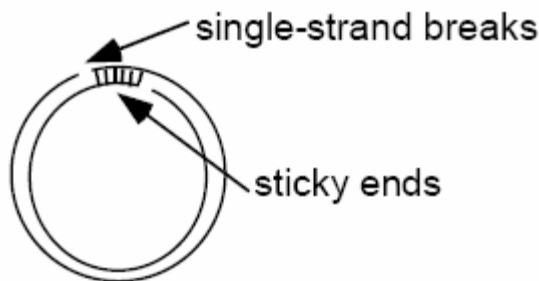
- Electron microscopy
- Autoradiography
- Sedimentation velocity
- Buoyant density titrations with dye molecules
- Light scattering
- Viscosity measurements
- In addition it was also shown by the genetic *linkage maps* (this relates to how far genes are separated from each other on the physical DNA molecule – the farther apart two gene sequences are, the less correlated are the two genetic traits).

The circular DNA can exist in many different forms, such as,

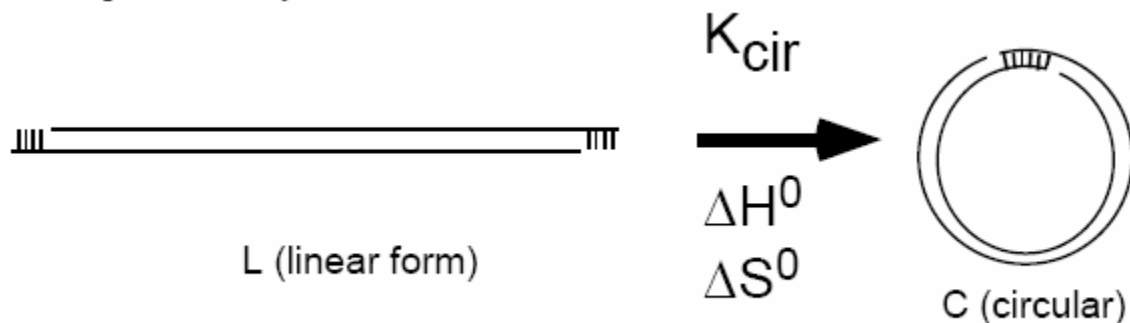
- Single-stranded c-DNA
- Intact double-stranded c-DNA (closed circles with both strands covalently linked)
- Nicked ds-c-DNA (only one strand covalently linked)
- Concatenated circles

Many ways were found to shift between these forms, involving enzymes, chemical means, exposure to x-rays, heating, and even physical agitation. Nicked ds circular DNA can be resealed (ligated) by enzymes (ligases).

Let's take the example of a mutant of λ DNA, $\lambda b_2 b_5 c$ of the λ phage (MW= 25.8×10^6 - this is about 39000 base pairs). We will see that we can account nicely for the circularization reaction using a very simple theory of chemical reactions and the polymer statistics we just developed. We will deal with the case where the circle is closed, but the strands are not covalently closed; this is a molecule with "sticky ends". There are single-strand breaks on both strands, but they are in different places, and the DNA can be held in a circular form through the complementary sequences of the "sticky ends".



It is possible to use sedimentation velocity experiments to study the equilibrium between the linear form (L) and the circular monomer (C) form. The two forms have different frictional coefficients and sediment in a centrifuge with different speeds. We represent this equilibrium by:



L \leftrightarrow C with an equilibrium constant “K_{cir}”

This reaction will behave as any other monomolecular reaction. Assume that this is a true equilibrium constant between two real species L and C, and vary T (temperature), measuring [C] and [L]. Then the van't Hoff equation yields:

$$\frac{d \ln K_{cir}}{d(1/T)} = -\frac{\Delta H^0}{R} \quad ; K_{cir} = \frac{C}{L}$$

$$\Delta S^0 = R \ln K_{cir} + \frac{\Delta H^0}{T} = \frac{\Delta H^0}{T_m}$$

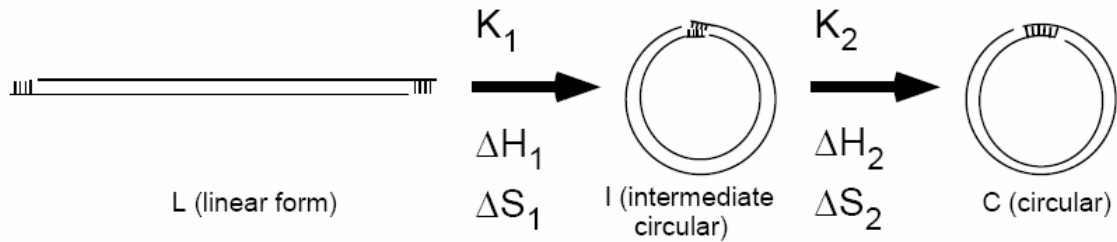
When $T = T_m$, then $K_{cir} = 1$, and $\ln K_{cir} = 0$; that is, T_m is the temperature where $\frac{1}{2}$ of the molecules are in the circular form, and $\frac{1}{2}$ are in the open linear form.

Some experimental results:

$\Delta H^0 = -91$ kcal/mol, $\Delta S^0 = -280$ cal/mol-deg, in 0.13 M Na⁺,
and $T_m = 50.6$ °C;

$\Delta H^0 = -85$ kcal/mol, $\Delta S^0 = -250$ cal/mol-deg, in 1.0 M Na⁺,
and $T_m = 63.6$ °C.

The cyclization reaction has been hypothesized to take place in two steps:



It is assumed that the first step closes the circle so that the ends are both within a small volume, δV , but there is no electrostatic repulsion between the two strands. Therefore one assumes that $\Delta H_1=0$, and the first free energy change comes only from a change in entropy, ΔS_1 . We can calculate this by the Boltzmann equation for the entropy:

$$S = k \ln \Omega$$

where Ω is the number of configurations of equal energy accessible to the molecule. $\Omega(\delta V)$ is the number of configurations where the ends are simultaneously within the small volume element δV . So in the first step, the entropy change is:

$$\Delta S_1 = S(\delta V) - S(V) = k \ln \frac{\Omega(\delta V)}{\Omega(V)}$$

For a random polymer chain of length L and segment length b (this is the Kuhn statistical length, and is the apparent length of the segments so that the polymer behaves as a random polymer, with no excluded volume, it is about 500-

700 Å) is within a small distance l of the beginning of the chain, we have:

$$\frac{\Omega(\delta V)}{\Omega(V)} = \left(\frac{3}{2\pi Lb} \right) \int_0^l \exp\left[-3l'^2 / 2Lb \right] 4\pi l'^2 dl'$$

This is a normalized function, so $\Omega(v)=l$ (the ends have to be somewhere). If $l^2 \ll Lb$, then we have

$$\exp\left[-3l'^2 / 2Lb \right] \approx 1, \text{ and we have } \int_0^l \frac{4\pi l'^2 dl'}{3} = \delta V$$

So that

$$\Delta S_1 = R \ln \left[\left(\frac{3}{2\pi Lb} \right)^{3/2} \delta V \right] = R \ln(w_{js} \delta V);$$

where w_{js} is the “**Jacobson-Stockmayer**” factor. This factor, which has units of inverse volume, represents the concentration of one end of the molecule in the neighborhood of the other.

Now we have to estimate some lengths; assume that $b = 717 \text{ \AA}$ (this is one estimate for DNA, and is almost independent of the ionic strength), and the $L = 13.2 \text{ microns}$ for this DNA. Assume also that $dV = 500 \text{ \AA}^3$; this is about a sphere with a radius of 8 \AA . Using these values, we calculate $\Delta S_1 = -43 \text{ cal/mol-deg}$. Using the thermodynamic values we found above, and remembering that $\Delta H = \Delta H_2 = -91 \text{ kcal/mol}$, we can say for 0.13 M Na^+ that $\Delta S_2 = -237 \text{ cal/mol-deg}$.

Now we can use this value of ΔS_2 and the value of $\Delta H_2 = \Delta H$ to calculate the **number of base pairs** “ n ” that form the “sticky ends” (see the figure). We use canonical values of Δh and Δs , which are the **enthalpy** and **entropy** changes per mole of **base pair formation** found from other experiments (we will see how these are determined later when we cover helix-coil transitions of oligomers and polymers of DNA). **$\Delta h = -7.7 \text{ kcal/mol}$, and $\Delta s = -23 \text{ cal/mol-deg}$** . These are useful values to stick in your memory. The two following equations should agree.

$$n = \frac{\Delta S_2}{\Delta s}; \text{ and } n = \frac{\Delta H_2 + 7000}{\Delta h}$$

The additional term 7000 kcal is because of the additional stacking interactions at the junction points (see the figure).

These estimates give:

$n \approx 10$ base pairs at the “sticky ends of the λ DNA molecules.

This estimate agrees with other ways of estimating the number of base pairs at the end of this DNA molecule. This derivation and the results support the use of the above model where DNA is assumed to act as a worm-like polymer chain we derived earlier.