PLECTONEMIC STRUCTURE OF TOPOLOGICALLY CONSTRAINED, SUPERCOILED DNA

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Closed circular DNA usually exists in a supercoiled configuration, in which the duplex is wound around another part of the same molecule to form a higher order helix. Supercoiling is utilized in many cellular mechanisms. Here, we explore the extent to which supercoiling controls the compaction of pUC18 bacterial plasmid (2686 base pairs) in a liquid crystal [1,2]. For this purpose, the configuration of the superhelix is monitored with SANS through the phase transition.

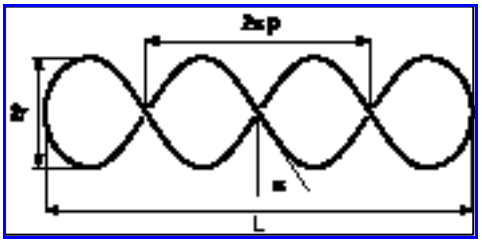

Figure 1. Plectonemic helix with length L, radius r, pitch p and opening angle α.

The topological constraint is characterized by the linking number deficit ΔLk, which is the number of turns the duplex is turned before closure to form a ring. ΔLk is conserved and is distributed among writhe Wr and excess twist ΔTw exerted on the duplex according to $\Delta Lk = Wr + \Delta Tw$. For a right-handed, regular supercoil without end loops, Wr is proportional to the number of crossings n when viewed perpendicular to the superhelical axis $Wr = -n\sin\alpha$, with the pitch angle α as in figure 1. It is convenient to define the normalized length $2L/l$, with l being the length of the DNA molecule. From integration along the contour follows

$$2L/l = p/\left(p^2 + r^2\right)^{1/2}, \qquad (1)$$

if the end loops are neglected. The pitch angle α is given by

$$\tan\alpha = p/r = \left(2L/l\right)/\left(1-\left(2L/l\right)^2\right)^{1/2} \qquad (2)$$

and the writhe reads

$$Wr = -lp/\left(2p\left(p^2 + r^2\right)\right). \qquad (3)$$

The *local* structure of the superhelix is fully characterized by p and r. These parameters determine α, Wr and $2L/l$.

In our experiments, the range of momentum transfer q exceeds L^{-1} by at least an order of magnitude. In this high q-range, the scattering is sensitive to interference over an extent on the order of r and p and effects of overall flexibility and/or branching of the supercoil are beyond observation. We can accordingly use the high-q approximate form of the form factor of a regular superhelix

$$P(q) = \frac{p}{qL}\left[J_0^2(qr) + 2\sum_{k=1}^{ap/2} J_{2k}^2\left(\left(q^2 - 4k^2/p^2\right)^{1/2} r\right)\right], qL >> 1 \qquad (4)$$

Note that $P(q)$ is sensitive to the DNA density per unit length projected on the superhelical axis L^{-1}. However, for $qr \gg 1$ and $qp \gg 1$ the scattering is essentially given by a single strand of the superhelix, which is proportional to the density per unit contour length l^{-1}. In both regimes, the form factor displays the characteristic q^{-1} scaling for rodlike particles, but the prefactor drops from L^{-1} to l^{-1}. Our data do however not comply with such idealized structure as depicted in figure 1. Accordingly, we have assumed a Gaussian distribution in r with standard deviation s_r. It is also assumed that α is constant, which implies that p is proportional to r with a proportionality factor given by equation (2). Interactions among supercoils are accounted for in the second virial approximation and the total scattering function takes hence the form

$$S(q) = NP(q)/\left(1 + 2A_2 r P(q)\right), \qquad (4)$$

with N the number of bases, A_2 the second virial coefficient and r the DNA density.

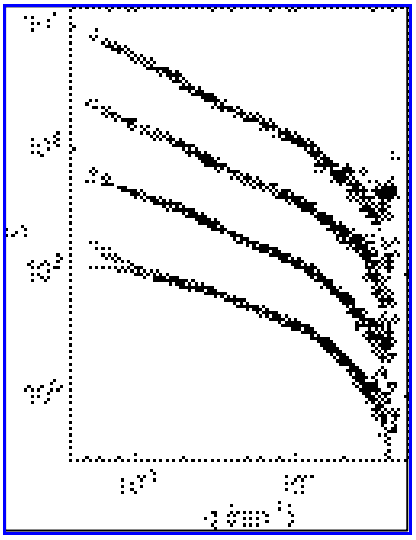

Figure 2. Structure factor S vs momentum transfer q for 3 (Δ), 6 (◊), 11 (□) and 27 (o) g DNA/dm^3 in 0.05 M NaCl.

Due to the presence of a significant distribution in r and p, the structure factors do not exhibit strong oscillatory behaviour. They do show however, the anticipated q^{-1} scaling and the drop in prefactor from L^{-1} to l^{-1} with increasing q. The plectonemic structure is most clearly demonstrated in figure 3, where the structure factors are normalized in a way that they go to unity at high q. The normalized structure factor extrapolates to $2l/L$ for $q \to 0$ and in the absence of interactions. In the fit procedure, we have optimized r, its distribution width s_r, a and A_2. The other parameters are derived through equation (2) and standard variance propagation. Note that the margins are not related to error, but rather to variation in molecular shape.

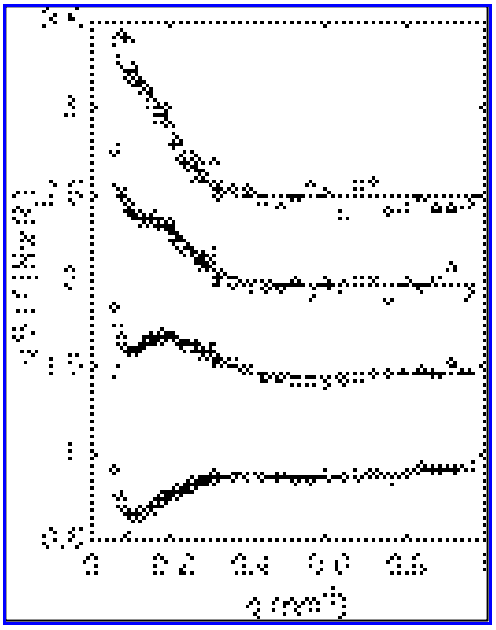

Figure 3. As in Figure 2, but for the normalized structure factor. Lines represent a fit with parameters in table 1. To avoid overlap the data are shifted along the y-axis.

With increasing concentration through the phase transition, r and p are seen to decrease significantly. Because of the (near) constancy of a, Wr decreases and the number of superhelical turns increases ($Wr = -n \sin a$). According to the fact that the sum of the excess twist and the writhe are conserved, this decrease in Wr should be compensated by a positive twist exerted on the duplex if ΔLk is conserved. Apart from the change in physical size of the supercoil, the associated increase in molecular free energy is of great importance in controlling the phase boundaries.

Table 1. Parameters resulting from the fit of the structure factor to the scattering data. Note that the margins are related to a variation in molecular shape.

c (g/dm^3)	r (nm)	p (nm)	α(°)	$2L/l$	Wr	A_2 (10^6 nm^3)
3	10±4	21±9	65	0.91	-6±3	-
6	9±5	14±7	57	0.84	-7±4	0.55
11	8±4	13±7	59	0.86	-9±5	1.22
27	5±3	6±4	52	0.79	-14±10	1.92

References

[1] Zakharova S S, Jesse W, Backendorf C, Egelhaaf S U, Lapp A and van der Maarel J R C, Biophys. J. **83** (2002) 1106

[2] Zakharova S S, Jesse W, Backendorf C and van der Maarel J R C, Biophys. J. **83** (2002) 1119