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Superhelix Densities of Circular DNA's: A Generalized Equation for Their Determination by the Buoyant Method

Abstract. *Equations for the measurement of the supertwisting of circular DNA's from banding positions in buoyant density gradients containing intercalating dyes have required the use of SV40 DNA isolated from virions as a reference DNA. These equations are modified to allow the use of any closed circular DNA of known superhelix density as a reference DNA.*

Covalently closed duplex circular DNA (duplex circular DNA containing no single strand scissions) was first characterized in 1963 by Weil and Vinograd (*1*). Since that time a large number of DNA's with similar properties have been found, including the *Escherichia coli* chromosome, various prokaryotic and eukaryotic viral and plasmid DNA's, and organelle DNA's. Alteration of the duplex winding of closed circular DNA's causes tertiary conformation changes (supertwists or superhelical turns) as a result of the topological constraint present in such DNA's. Recently, both Griffith and Germond *et al.* have shown a relation between chromatin structure and the number of superhelical turns present in closed circular DNA (*2*). Others have suggested that the degree of supertwisting may be important in replication (*3*), transcription (*4*), and recombination (*5*) of DNA. The topological properties giving rise to superhelical turns are not limited to circular DNA's but are also relevant for linear DNA's lacking single-strand discontinuities and whose ends are fixed so that they cannot rotate about each other.

Several methods for the determination of the superhelix density (σ) of closed circular DNA's have been developed (*6*). One of these, the measurement of separations between bands of closed and open circular DNA's in buoyant gradients containing high concentrations of the intercalating dyes ethidium bromide or propidium diiodide (*7, 8*) has been widely used since it is possible to determine σ by this technique in a single buoyant density separation in a preparative ultracentrifuge. This procedure is also ideal for determinations in which radioactively labeled DNA is used (*9*).

A little known limitation of the buoyant separation method (*7, 8*) is that only SV40 DNA or a DNA with an equal superhelix density may be used as a reference DNA. The σ_0 for SV40 (the subscript zero refers to standard measurement conditions of 2.8*M* CsCl, 20°C, and neutral *p*H) was used in the derivation of the equations used in this method (*7*, appendix, equations I-1, I-8, and I-9) and SV40 DNA was the reference DNA for the experimental verification of this procedure (*8*).

The published equations can be easily generalized for use with any closed circular DNA of known σ_0 as a reference DNA. The previously published equation (*8*) is

$$\Delta\sigma_0^{\text{unk-SV40}} = \sigma_0^{\text{unk}} - \sigma_0 = a\left(\frac{\Delta_r^{\text{unk}}}{\Delta_r^{\text{SV40}}} - 1\right) \quad (1)$$

where $\Delta\sigma_0^{\text{unk-SV40}}$ is the difference in superhelix density between the unknown DNA (σ_0^{unk}) and SV40 (σ_0^{SV40}), Δ_r^{unk} and Δ_r^{SV40} are the distances between bands of closed and open circular DNA's for unknown and SV40 DNA's, and a is a constant. [Correction factors for differences in base composition between unknown and reference DNA's and for differences in banding positions in individual tubes are omitted here, but they are available in (*6–8*)]. If σ_0^{ref} and Δ_r^{ref} are substituted in Eq. 1 for σ_0^{unk} and Δ_r^{unk} to generate a second equation, Δ_r^{SV40} may be eliminated to give the general form of the equation for use with any reference DNA

$$\Delta\sigma_0^{\text{unk-ref}} = \sigma_0^{\text{unk}} - \sigma_0^{\text{ref}} = (b + \sigma_0^{\text{ref}})\left(\frac{\Delta_r^{\text{unk}}}{\Delta_r^{\text{ref}}} - 1\right) \quad (2)$$

where $\Delta\sigma_0^{\text{unk-ref}}$ refers to the difference in superhelix density between the unknown DNA and the reference DNA. The factor a in Eq. 1 is replaced by the sum of two independent factors, $b + \sigma_0^{\text{ref}}$, where b is dependent on the intercalating agent and σ_0^{ref} is the superhelix density of the reference DNA.

The value of a in the original equation was computed on the assumption that the helix unwinding angle per molecule of intercalated ethidium bromide was 12° (*10*). More recent data (*11*) indicate that 26° is a better value for this unwinding angle, and thus values of b for separations measured in CsCl gradients containing ethidium bromide or propidium diiodide are, respectively, 0.33 and 0.29. The corrected value of σ_0 for SV40 is −0.084. The PM2 DNA (corrected value of $\sigma_0 = 0.11$) is a good alternative reference DNA as it is easily prepared and its σ_0 has been well characterized (*8, 12*). Intracellular forms of SV40 or PM2 DNA's should not be used as they are more heterogeneous in σ_0 compared with DNA isolated from virions (*9, 13*). Specific details regarding superhelix determinations by this method have been described (*6, 8*). In calculating the number of potential superhelical turns in any DNA, differences between in vivo and measurement environmental conditions (such as salt and temperature) must also be considered (*14*).

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