# バイオ分析化学特論（６）

## 粘度滴定

竹中繁織
九州工業大学 物質工学研究系 応用化学部門

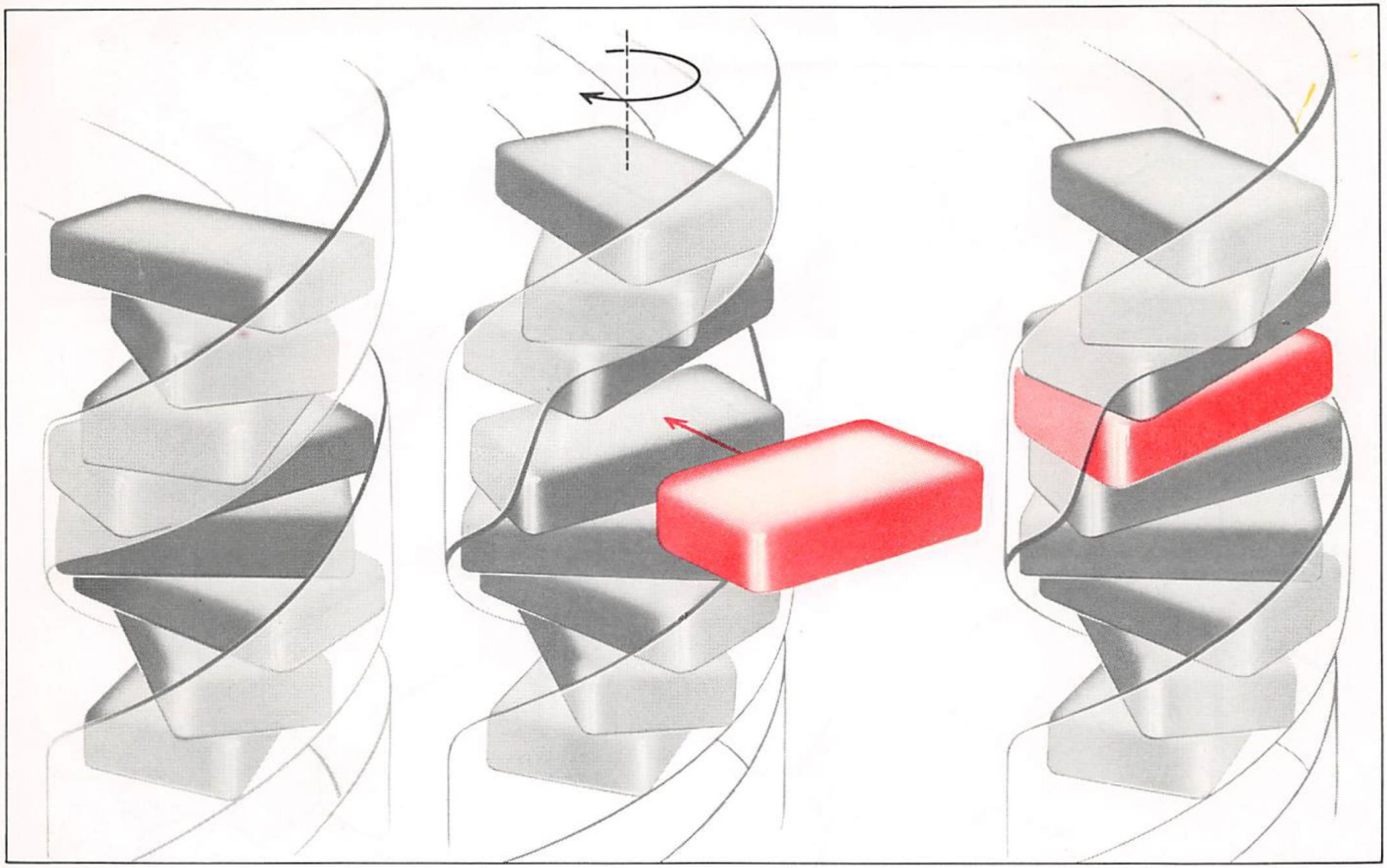
# 今回勉強すること。

1）DNA二重らせんは、約11塩基に360°巻いているが、長いDNAはさらに巻きすぎ（正の超らせん）や少し巻き戻されている（負の超らせん）状態で存在している。

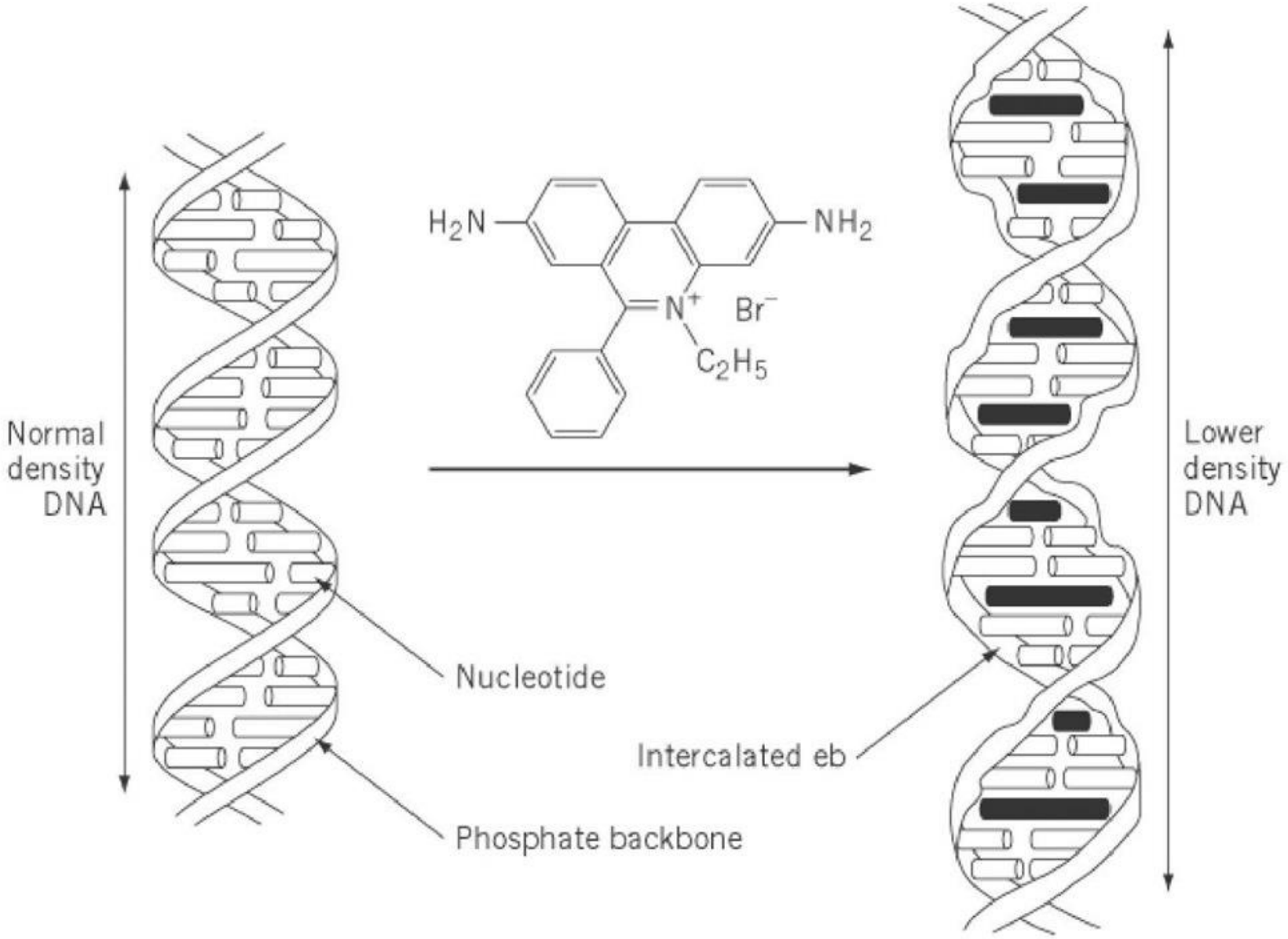
2）DNA二重らせんの塩基対間に平行挿入（インターカレート）する分子は塩基対間を広げるのでらせんは巻き戻される。

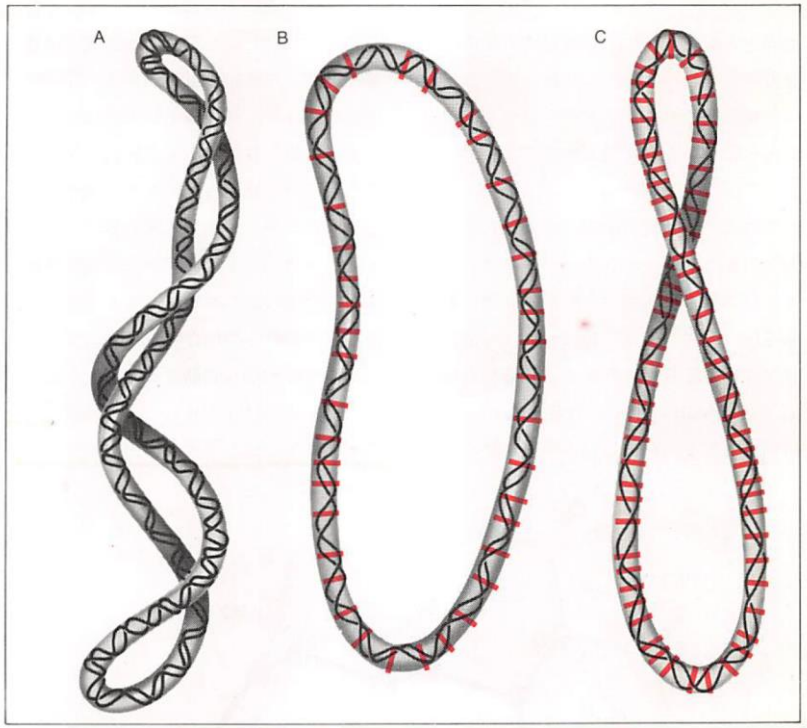
3）プラスミドDNAは二重らせんが環状になったウイルス分子である。通常は負の超らせんを有している。プラスミドは超らせん密度によって流体力学的体積が大きく異なる。これによって沈降係数やゲル電気泳動の移動度が大きく変化する。

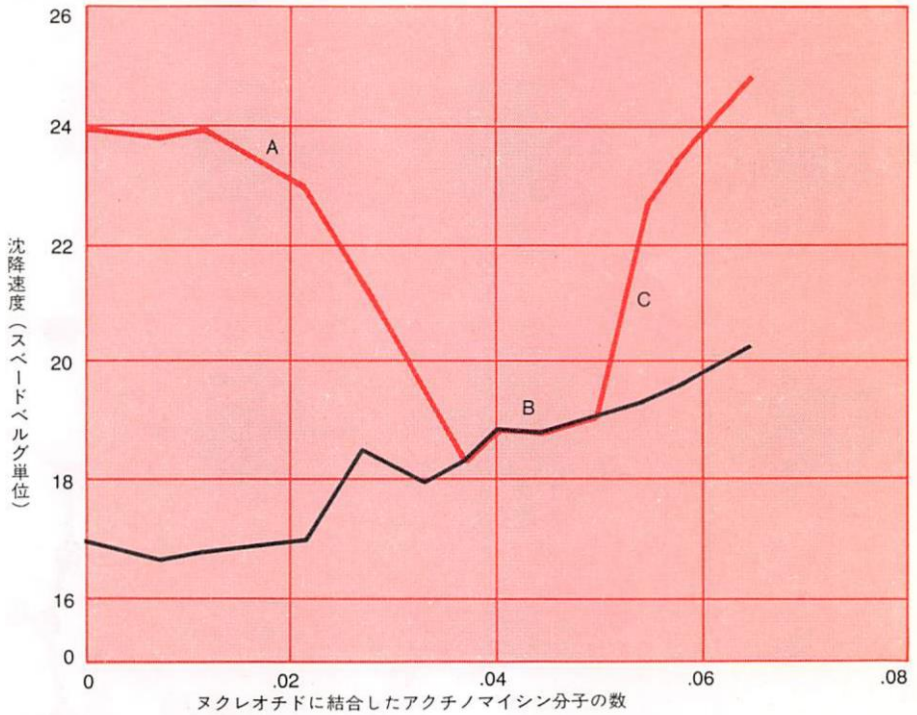
4）インターカレートする分子、インターカレータの濃度を変化させることによってインターカレータ分子が一分子結合することによる巻き戻し角を見積もることができる。



**挿入（intercalation）**　突然変異の原因となるアミノアクリジンと呼ばれる一群の化合物とDNAとの間の作用のしくみで，もっとも可能性の高いものとして，挿入の考えが最初に提案された。この一群の分子（色つきの平らな塊）は，隣り合った塩基対（灰色の平らな塊）の中にすべりこんでDNAの二重らせん（リボン状の構造）と結合すると考えられている。この過程でDNA分子は少し捩れがゆるんで（18度），隣り合った塩基対が，アミノアクリジン分子がぴったり入りこむのを許すのにちょうど十分な距離（3.4オングストローム）だけ，隣り合った塩基対が離れるようにする。挿入性の色素と結合すると，DNA分子が長くなり硬直して堅くなるので，特徴的に，DNA分子の粘度の増加と沈降係数のある程度の低下が観察される。切断されたDNAの溶液にアクチノマイシンを加えると，この2種類の効果が観察されるという事実から，アクチノマイシンもDNAと挿入によって結合すると考えられる。


Normal density DNA
$H_2N$
$NH_2$
$N^+$
$Br^-$
$C_2H_5$
Nucleotide
Intercalated eb
Phosphate backbone
Lower density DNA




**挿入の検査法**　色素や薬剤を加えていったときの切れ目のない“スーパーコイル型”(らせんがさらに捩れたもの）の環状DNAの水溶液の物理化学的性質を追求すると，挿入について感度よく検査できる。挿入性の色素はDNAの二重らせんを，挿入のすぐ側の部分で巻きもどす。この巻きもどしが起こる方向は，右巻きにスーパーコイルに巻いたDNAの構造（A）のひずみが，色素がますます結合するとともに，捩れの数が減少してゆるむような型のものである。巻きもどしが進むと，DNAは長く硬直してくるので，もとの凝縮した構造が失われる。したがって，DNAは超遠心で以前よりゆっくりと沈降する。切れ目のない環状DNAに十分な色素が結合すると，スーパーコイル構造がすべて失われる（B）。さらに色素が結合すると，左巻きのスーパーコイルが出現するようになり（C），ついには凝縮したスーパーコイルが再び現われる。このことはDNAが超遠心で沈降する速度の増加として検出される。アクチノマイシンDで実験すると，挿入の考えに一致するやり方で巻きもどす(赤い線)。対照の実験で，“切れ目の入った(nicked)”環状DNAでは，アクチノマイシンを加えても，沈降の性質はあまり変化しない（黒い線)。

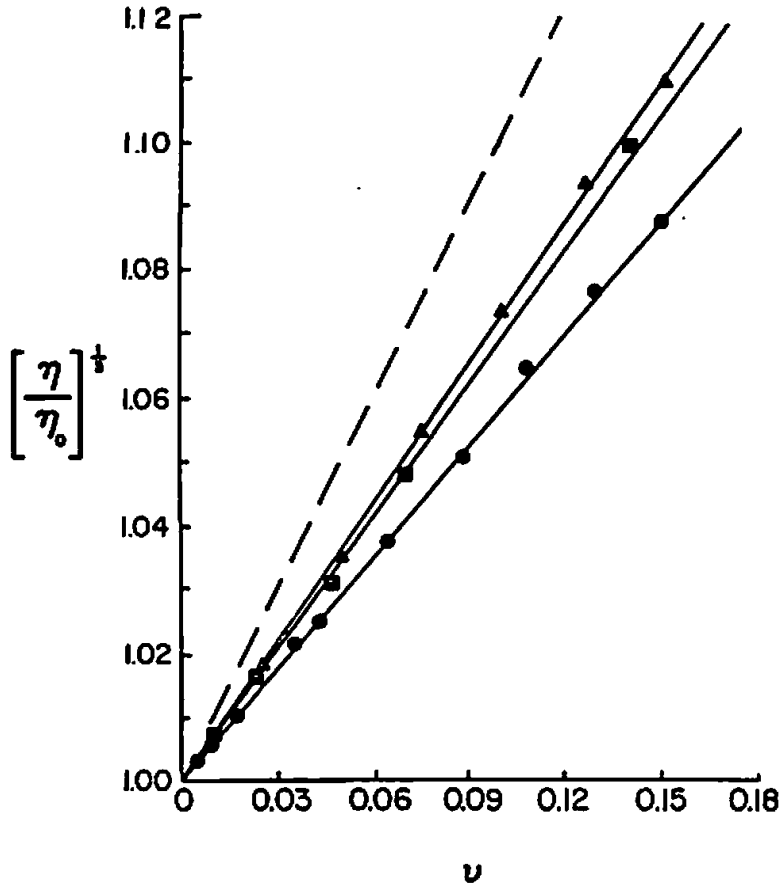



**Fig. 3.** Results from a viscometric titration of sonicated calf thymus DNA with quinacrine (▲), 9-aminoacridine (■), and ethidium (●), where $\eta$ is the reduced specific viscosity of the DNA–ligand solution and $\eta_0$ is the reduced specific viscosity of the DNA solution before the addition of ligand. The experiments were conducted at pH 7.0 and 25°C in PIPES buffer (0.01 *M* piperazinediethanesulfonic acid, $10^{-3}$ *M*, EDTA, pH 7.0). The dashed line is the theoretical slope predicted by Eq. (1) for a 3.4 Å length increase in the double helix for each intercalated ligand. The reduced specific viscosity ratio $\eta/\eta_0$ is plotted as a function of $\nu$, the moles of ligand bound per DNA base-pair equivalent. Note that many plots in the literature are drawn as a function of the moles of ligand bound per DNA nucleotide, $r$, and $\nu = 2r$.

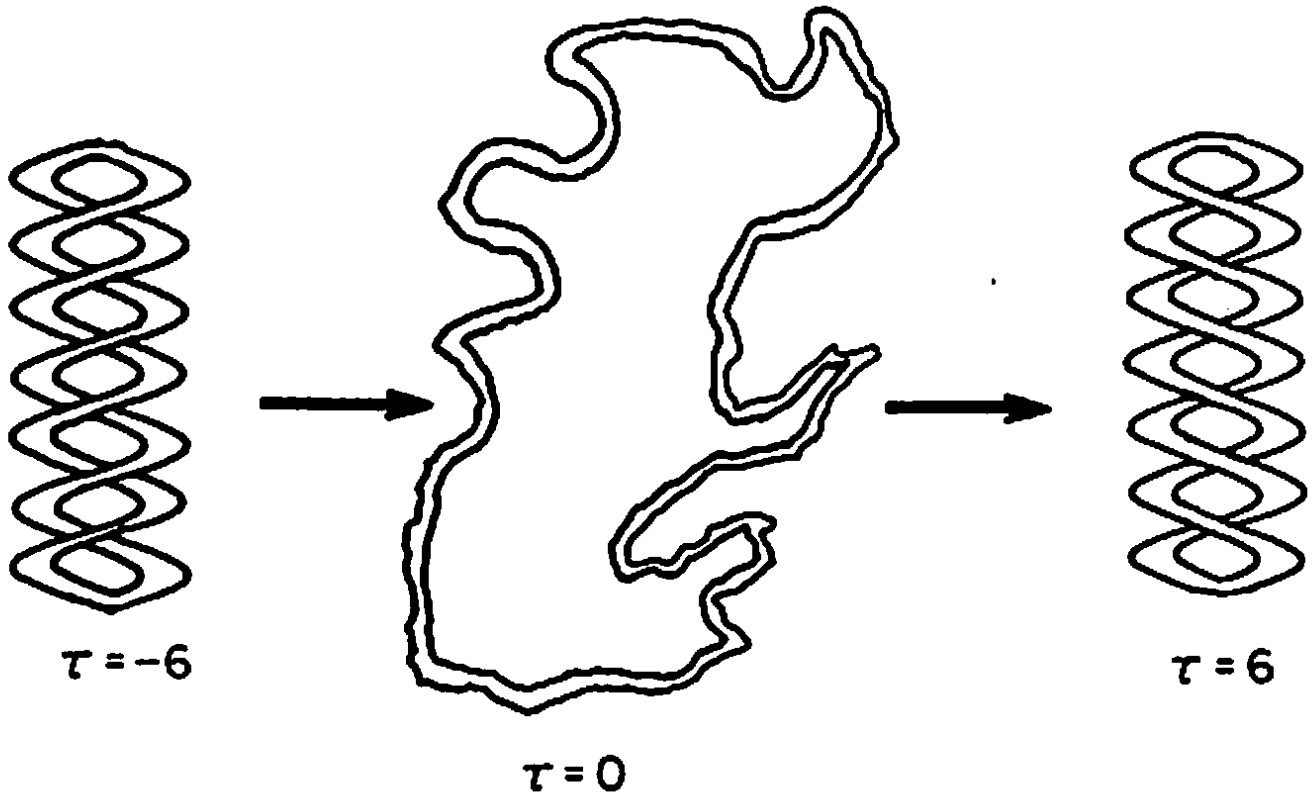



**Fig. 4.** Representation of the effects of the binding of an intercalating ligand to closed circular superhelical DNA. The diagram illustrates three stages of binding. On the left the DNA molecule has not bound enough ligand to remove the natural right-handed superhelical turns. In the center enough ligand has been added to remove the superhelical turns exactly (this is the maximum in a viscometric titration of superhelical DNA by an intercalating ligand). On the right more ligand has been added, and in this case unwinding of the double helix produces left-handed superhelical turns. The number of superhelical turns that the double helix makes about a superhelix axis is denoted by $\tau$.

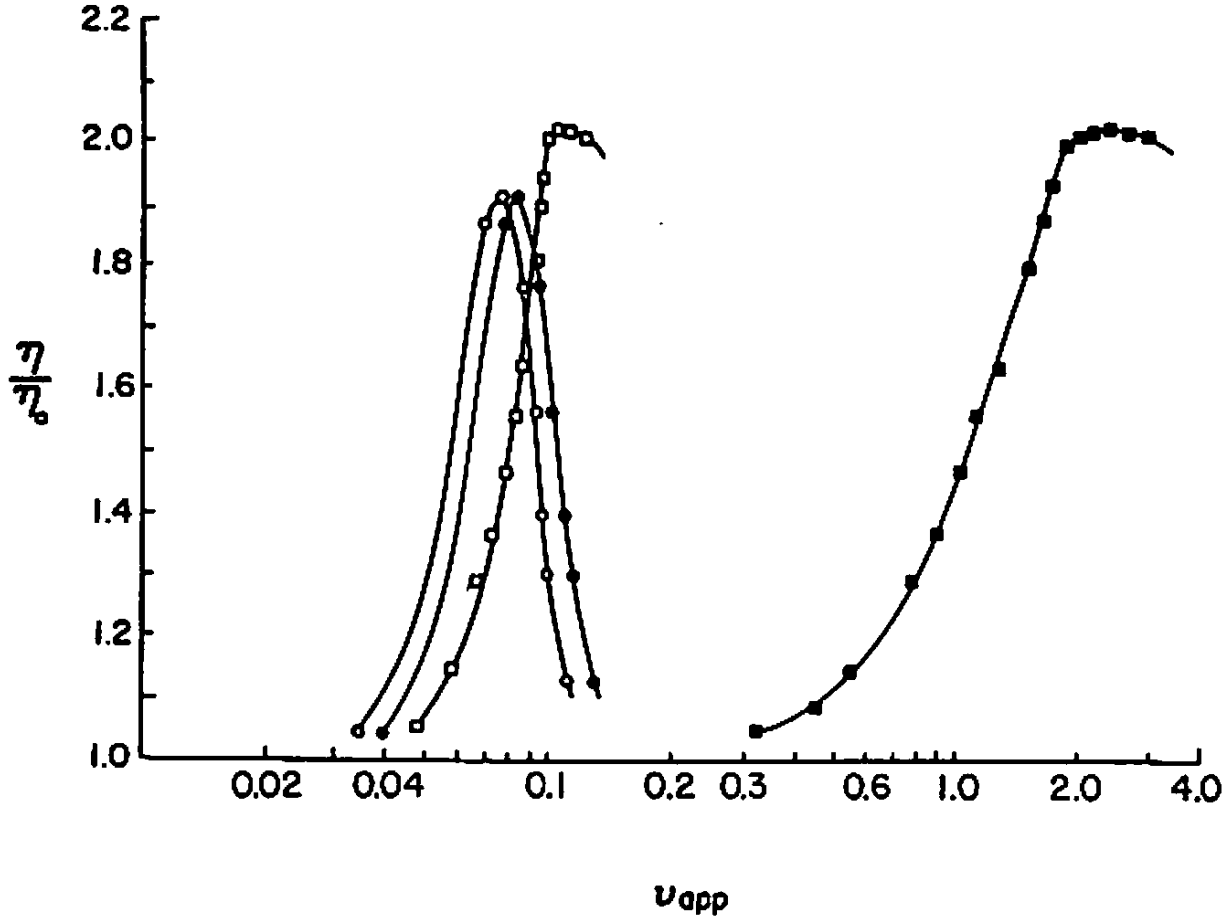



**Fig. 5.** A viscometric titration of closed circular superhelical Col $E_1$ DNA plotted as a function of $\nu_{app}$, the total moles of ligand added per base pair equivalent of DNA (●, ethidium; ■, chloroquine; $\eta/\eta_0$ is defined in Fig. 3). The titrations are also shown corrected for the amount of ligand bound, $\nu$ (○, ethidium; □, chloroquine). Since ethidium and chloroquine have quite different binding constants under these experimental conditions, the corrected results are much closer than the direct experimental results. This illustrates the necessity of quantitating binding as in Eq. (2) in order to evaluate unwinding angles accurately. The experiment was conducted at 25°C in PIPES buffer (see Fig. 3) with 0.1 *M* NaCl.

turns in all of the experiments, the amount of ligand bound per base pair to remove the superhelical turns completely, $\nu'$, should be constant. This quantity is related to other experimentally measurable quantities through the expression

$$C'_t = \nu' N'_t + C' \tag{2}$$

where $C'_t$ is the total amount of ligand and $N'_t$ is the total nucleic acid concentration at the maximum in a viscometric titration. A plot of $C'_t$ as a function of $N'_t$ allows the determination of $\nu'$ and $C'$, the free ligand concentration. All primed quantities refer to those characteristic of the closed circular DNA with all superhelical turns removed, the maximum in a viscometric titration. If a standard of known unwinding angle is available, the unwinding angle of an unknown ligand can be determined using

$$\phi_k \nu'_k = \phi \nu' \tag{3}$$

where $\phi_k$ and $\phi$ are unwinding angles for a known and an unknown ligand, respectively, and $\nu'_k$ refers to the known compound. Ethidium bromide has evolved as a standard for use in determinations of unwinding angles by this method. Several different types of experiments have indicated that this

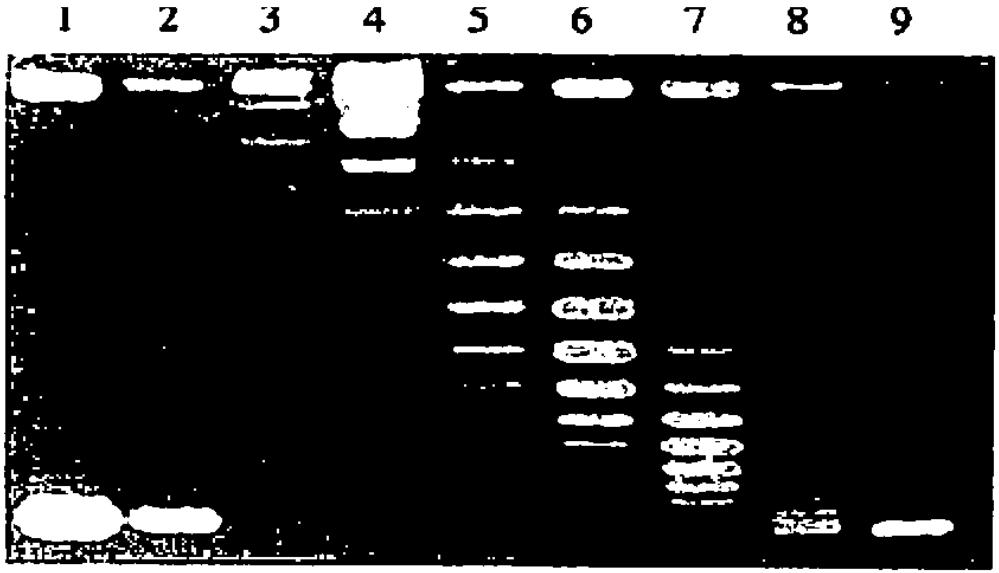



**FIGURE 2.35.** Electrophoresis analysis of topoisomers of pBR322 DNA, produced by ethidium bromide binding and the topoisomerase I relaxation, followed by the removal of the drub and enzyme. (1). Native intact pBR322 DNA; (2) native pBR322 DNA treated with incubation, phenol extract, and ethanol precipitation in buffer T (35 mM Tris-HCl, pH = 8.0, 73 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 5 mM spermidine, and 0.01% bovine albumine) containing no topoisomerase I and no ethidium bromide. (3–9). Ethidium bromide was added prior to topoisomerase. DNA concentration = 10 μg/mL. Ethidium concentrations (μg/mL): (3) 0; (4) 0.25; (5) 0.50; (6) 0.75; (7) 1.00; (8) 1.25; (9) 1.50 [71].

**TABLE 2.5. Angle of Unwinding of DNA Double Helix Caused by Ethidium Binding as Derived from Writhing Number (τ) and Equilibrium Constant**

| Sample Number | Total Drug Concentration | | Bound Drug Concentration ($\times 10^{-5}$ M) | $m$ | $\Delta\beta$ | $\phi$ |
|---|---|---|---|---|---|---|
| | μg/mL | $\times 10^{-5}$ M | | | | |
| 4 | 0.25 | =0.0635 | 0.0252 | 66° | 2° | 11° |
| 5 | 0.50 | =0.1269 | 0.0498 | 130° | 5° | 14° |
| 6 | 0.75 | =0.1904 | 0.0741 | 194° | 7° | 137° |
| 7 | 1.00 | =0.2538 | 0.0979 | 256° | 10° | 14° |
| 8 | 1.25 | =0.3173 | 0.1212 | 317° | 15° | 17° |
| 9 | 1.50 | =0.3807 | 0.1441 | 377° | 20° | 19° |

from Fig. 2.35 that an ethidium bromide concentration of 0.25 μg/mL causes an average linking number change of $\Delta\alpha = 2$ for the plasmid, while 0.50 μg/mL causes a change of $\Delta\alpha = 5$, 0.75 μg/mL causes a change of $\Delta\alpha = 7$, and 1.0 μg/mL causes a change of $\Delta\alpha = 10$. These observed $\Delta\alpha$ values are considered to be entirely attributable to the amount of unwinding ($\Delta\beta$) caused by the drug binding, where $\beta$ is given by

$$\beta = \frac{N}{h} \tag{2.4}$$

Here, $N$ is the total number of base pairs involved in the closed circular duplex and $h$ is the number of base pairs involved in one pitch of the DNA helix. It is possible to define an angle $\theta$ that measures the clockwise rotation (viewed along the helical duplex axis) needed to reach a base pair from the adjacent base pair located closer to the viewer:

$$\theta = \frac{360^\circ}{h} = \frac{360^\circ\beta}{N} \tag{2.5}$$

The change in $\theta$ on drug binding is the difference of the angles between the unbound ($\theta_0$) and bound ($\theta$):

$$\Delta\theta = \theta - \theta_0 = \frac{360^\circ\Delta\beta}{N} \tag{2.6}$$

If the number drug molecules that bind to one closed circular duplex molecule is given by $m$, then the value of the unwinding angle ($\phi$) due to one drug molecule should be

$$\phi = \frac{\Delta\theta}{m} = \frac{360^\circ\Delta\beta}{m} \tag{2.7}$$

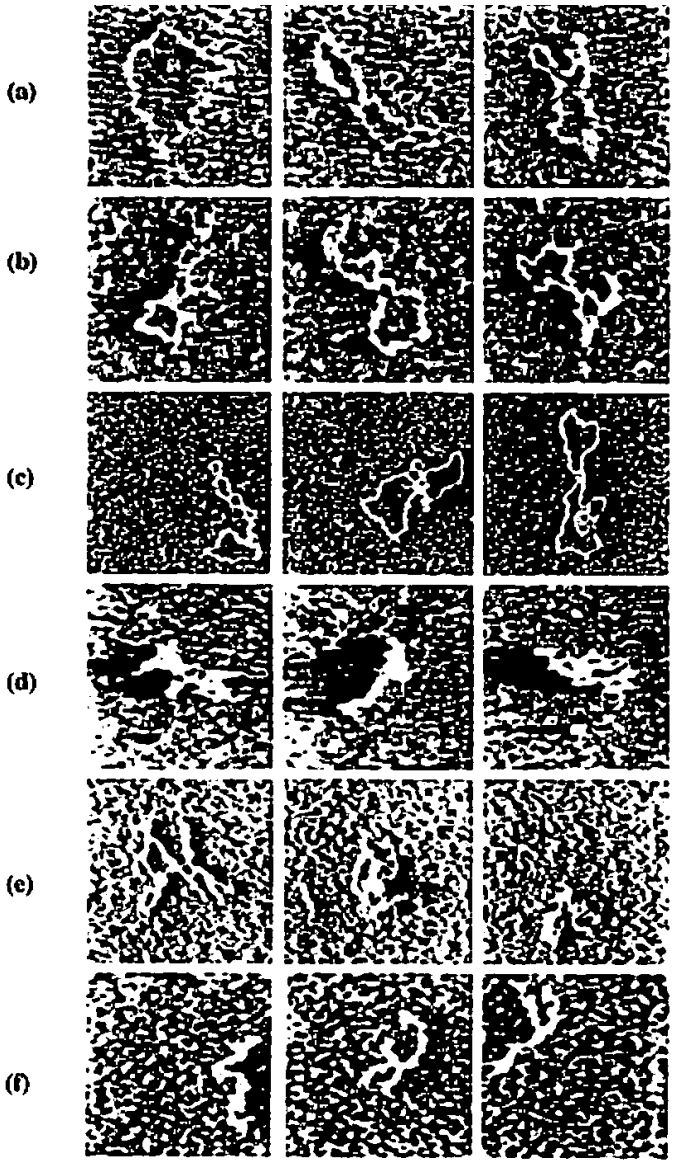



**FIGURE 2.36.** Atomic Force Microscopic images of plasmid pBR322 DNA in aqueous solutions, containing ethidium bromide. DNA concentration: 0.05 μg/mL. Ethidium bromide concentrations (μg/mL): (a) 0, (b) 0.75, (c) 0.80, (d) 1.0, (e) 2.0, (f) 10. Magnification—every square here has an area dimension of 1 × 1 μm [71].

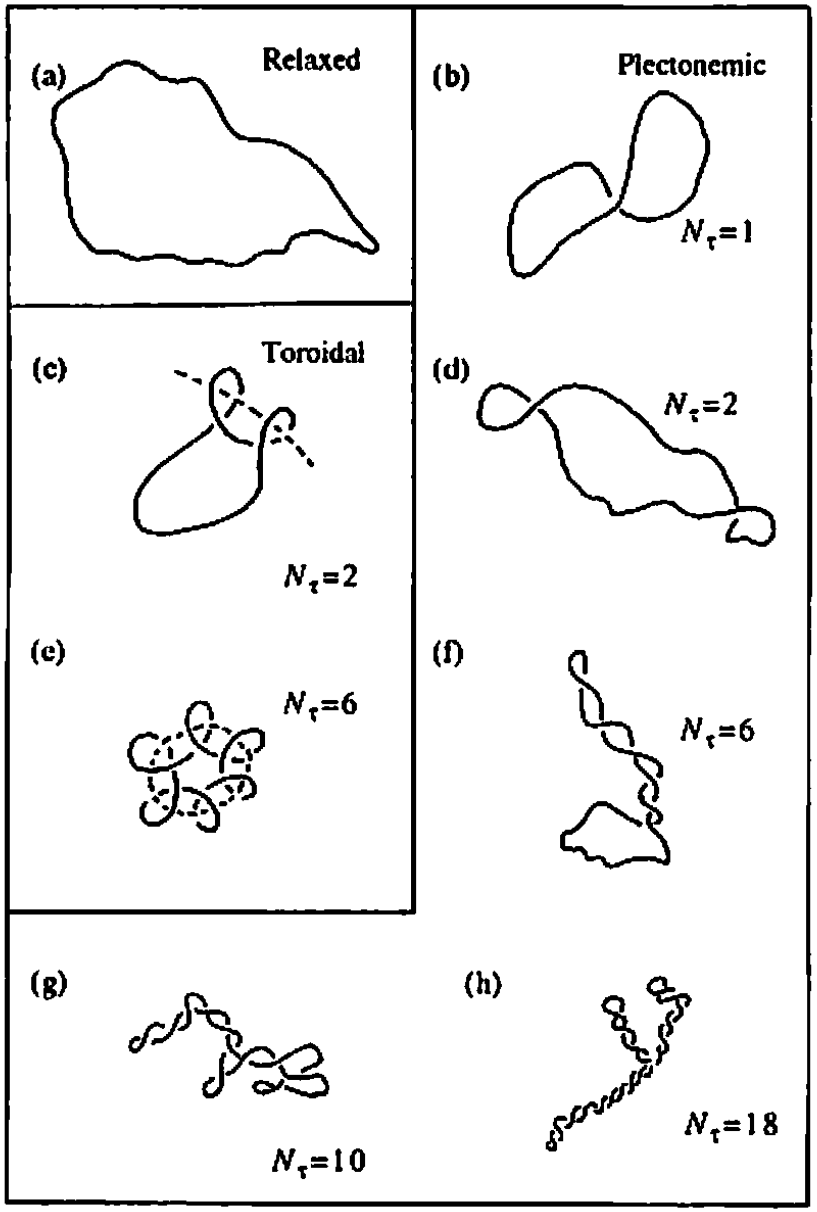



**FIGURE 2.37.** Diagramatic illustrations of possible tertiary structures of plasmid pBR322 molecules [71].