The effect of treatment with dilute acid and alkali on the streaming birefringence of sodium deoxyribonucleate solution

When sodium deoxyribonucleate (DNA) is titrated from the neutral region to pH 2 or 12, and then back-titrated to neutrality, the backward titration curve differs from that obtained on forward titration. This has been explained[1] by supposing that the purine and pyrimidine bases take part in hydrogen-bonding which is irreversibly broken on titration. The structure proposed for DNA by CRICK AND WATSON[2] consists of two helical polynucleotide chains coiled round the same axis, the chains being held together by hydrogen bonds between pairs of bases. This structure is in agreement not only with the X-ray data, but also with the results of electrometric titration[3], and chemical analysis[4].

It seems reasonable to suppose that if the hydrogen bonds are irreversibly broken on titration, the molecule of DNA should undergo a large and irreversible change of shape on being brought to a pH where the amino groups of adenine and cytosine have been titrated. REICHMANN, BUNCE AND DOTY[5] have concluded from a study of the light-scattering of DNA in 0.2 *M* NaCl that the molecular weight and the molecular length both decrease with pH from pH 6.5 to 2.6, but on returning the solution to pH 6.5 the molecular weight and length are restored to their original value. This reversibility seems at first sight to conflict with the titration data, but in 0.2 *M* NaCl the p*K'* of the bases are decreased considerably[3], and it may be that at pH 2.6 in 0.2 *M* NaCl many of the chain-linking hydrogen bonds are still intact.

Changes in molecular shape are readily followed by measurements of streaming birefringence, and so the effect on the streaming birefringence of lowering the pH in steps to pH 3.5 and 2.0, and returning it to neutrality, was investigated. The sample of DNA employed, designated GI(1), was extracted from calf thymus tissue by the method of GULLAND, JORDAN AND THRELFALL[6], and it had a molecular weight of $7.9 \cdot 10^6$.[7] Initially, relatively concentrated DNA solutions were employed so that the maximum effect could be observed. The apparatus was similar to that described by EDSALL, RICH AND GOLDSTEIN[8], with a number of modifications and it allowed measurements of streaming birefringence to be made at gradients of 0–4300 G. Plots of extinction angle (χ) against G were extrapolated to zero G and the slope at the origin (tan α) was calculated. The results, plotted as pH versus log (tan α), are shown in Fig. 1a for aqueous solutions and in Fig. 1b for 0.1 *M* NaCl solutions. The same results were obtained whether the pH was changed by titration with 0.05 *N* HCl and NaOH, or by dialysis.

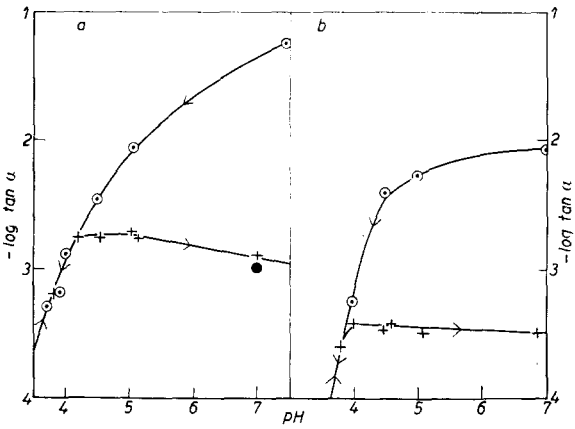

Fig. 1. Variation of log tan α with the pH of the solution. ⊙ lowering pH from neutrality; + raising pH from 3.5; ● raising pH from 2.0.

In both aqueous and 0.1 *M* NaCl solution, on lowering the pH tan α falls, slowly at first but with increasing rapidity until below pH 3.5 the birefringence becomes unmeasurable under the experimental conditions. On increasing the pH, from either pH 3.5 or 2.0, tan α at first increases again, but about pH 4 this increase ceases and thereafter tan α falls slightly. The more rapid fall above pH 5 for the aqueous solutions may be due to the gradual build-up of salt concentration during back titration. Further experiments were made to discover to what extent tan α is reversible when the pH is lowered only to pH 4 or higher, and the table shows the results. For solutions in 0.1 *M* NaCl, the birefringence is completely reversible down to pH 4.7; below this pH the degree of reversibility decreases rapidly to zero at pH 3.8.

TABLE I

REVERSIBILITY OF TAN α ABOVE pH 4

Initial and Final pH	6.01	6.90	7.45
Lowest pH	4.69	4.30	3.83
Fraction of reversibility	0.80	0.17	0.00

Below pH 3.8, tan α is again reversible but to a limited extent only.

Since tan α is a function of the molecular length and of the intermolecular interaction these results are consistent with a picture of DNA in which the two polynucleotide chains forming the double helix separate when all the hydrogen bonds involving cytosine and most of those involving adenine are broken by titration. On back titration to neutrality the double helix is not re-formed. The single chains, below pH 3.8, show much greater flexibility and smaller asymmetry than the unchanged DNA molecule.

This work is being extended to lower concentrations of DNA where the value of tan α (*i.e.* $(\tan \alpha)_0$) is independent of concentration, to obtain estimates of the molecular dimensions, and to the alkaline side of neutrality. At pH 6.0, $\log (\tan \alpha)_0 = -2.54$.

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[1] J. M. GULLAND, D. O. JORDAN AND H. F. W. TAYLOR, *J. Chem. Soc.*, 1131 (1947).
[2] F. H. C. CRICK AND J. D. WATSON, *Proc. Roy. Soc.*, A223 (1954) 80.
[3] D. O. JORDAN, A. R. MATHIESON AND S. MATTY (in the press).
[4] E. CHARGAFF, S. ZAMENHOF AND C. GREEN, *Nature*, 165 (1950) 756.
[5] M. E. REICHMANN, B. H. BUNCE AND P. DOTY, *J. Polymer Sci.*, 10 (1953) 109.
[6] J. M. GULLAND, D. O. JORDAN AND C. J. THRELFALL, *J. Chem. Soc.*, 1129 (1947).
[7] G. J. HOWARD, *Thesis*, Nottingham (1953).
[8] J. T. EDSALL, A. RICH AND M. GOLDSTEIN, *Rev. Sci. Instr.*, 23 (1952) 695.

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L-Azaserine as an inducing agent for the development of phage in the lysogenic *Escherichia coli*, K-12*

The ability of an agent to induce the development of active bacteriophage from the prophage state in lysogenic bacteria can be correlated with mutagenic and carcinogenic activities[1]. These and other radiomimetic agents bring about cytological changes in bacteria characterized by chromosomal aberrations and filamentation[2].

L-Azaserine (O-diazoacetyl serine) is a new antibiotic which is receiving special attention because of its anti-neoplastic activity[3]. As a mutagenic agent in bacterial systems, azaserine was more active than nitrogen mustard[4]. Induction of filament formation in *Escherichia coli* by azaserine has also been observed[5]. Because of these radiomimetic properties, we predicted that azaserine could induce the formation of active phage (*lambda*) in the lysogenic *Escherichia coli*, strain K-12. This was indeed true.

TABLE I

EFFECT OF L-AZASERINE ON BACTERIA AND *lambda* PHAGE IN CULTURES OF *Escherichia coli*, K-12

Medium	L-*Azaserine* μg/ml	*Bacteria per ml*		*Phage per ml*	
		0 h	*5 h*	*0 h*	*5 h*
S–G*	0	$2.14 \cdot 10^6$	$1.25 \cdot 10^8$	0	$5.8 \cdot 10^4$
	0.1	$2.14 \cdot 10^6$	$1.00 \cdot 10^6$	0	$2.3 \cdot 10^7$
Nutrient	0	$8.43 \cdot 10^6$	$1.03 \cdot 10^9$	0	$1.6 \cdot 10^5$
Broth	200	$1.44 \cdot 10^6$	$< 10^3$	0	$> 5.0 \cdot 10^7$

* S–G = Salts-Glucose medium.

Cells growing in the respective media were harvested at the logarithmic phase of growth, washed twice with media and inoculated into fresh media to give the initial colony counts indicated for time zero. Samples were taken at intervals and plated for colony formers and for free phage. Free phage was measured as plaque formers on a streptomycin resistant mutant (C-Sr) of the indicator, strain C, in the presence of streptomycin (method of BERTANI[6]).