559. *Electrometric Titration of the Sodium Salts of Deoxyribonucleic Acids. Part VII.* The Reversible Dissociation at Low Temperatures.*

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Dissociation curves in 0·05M-sodium chloride of herring-sperm sodium deoxyribonucleate have been studied at 0·3°, 0°, −0·4° and −0·75° by continuous titration. A reversible dissociation curve was obtained for the undenatured double-helical structure at the lowest temperature. Titration at 25° of nucleate which had undergone a full titration cycle at −0·75° gave anomalous dissociation curves identical with those of the untreated material. By comparison of the relative positions of the reversible titration curves of the non-hydrogen-bonded nucleate and of a mixture of equal amounts of this and of the original form, the validity of the method used for calculating the extent of denaturation from the displacement of titration curves was confirmed.

Earlier papers in this series reported studies of denaturation that occurs when neutral solutions of sodium deoxyribonucleate in the double-helical form are titrated to and from low or high pH. At 25° this goes to completion, the nucleate is irreversibly and completely disordered, and any hydrogen bonds that exist after this are regarded as randomly disposed. However, when the acid titration cycle was carried out at 0·4°, the resulting neutral nucleate solution, on undergoing a further acid titration cycle at 25°, exhibited " hysteresis " in its dissociation curves to the extent calculated for a sample still containing 60% of the nucleate in the double-helical form.[1] From this it was inferred that at 0·4° all the originally hydrogen-bonded groups may be ionised but that on removal of the protons with rise in pH, 60% of these re-formed in the original double-helical structure. Thus, it appeared that if titrations could be carried out at sufficiently low temperatures, it might be possible to observe a completely reversible dissociation curve for the double-helical structure which would be in its original form after undergoing this titration cycle. If this were so, it would confirm previous ideas and might afford useful means for following denaturation processes by means of changes in the *reversible* dissociation curves characteristic of the deoxyribonucleate molecule in the helical and the denatured states. Such reversible curves should avoid some of the ambiguities in titrations at 25° which arise when denaturation is concurrent with ionisation. This reversible dissociation curve of the double-helical structure had already been inferred [1] from those of the denatured nucleate and of the 40% denatured nucleate obtained in titration at 0·4°. We now describe titrations made below 0·4°, whereby the approach to reversibility was examined more closely. Reversible dissociation curves of the double-helical nucleate were observed at −0·75° and are compared below with those for the denatured molecule and for mixtures containing equal amounts of these two forms.

Experimental

Herring-sperm sodium deoxyribonucleate [2] (1·4 mg./ml.) in 0·05M-sodium chloride was titrated electrometrically with acid to about pH 2·7, then back-titrated with alkali. Continuous titration was made in a cell with liquid junction and a glass electrode, with the procedure and precautions already described.[2] The solutions of the " denatured " nucleate were obtained either by heating the sample at 100° for 20 min. in 0·05M-sodium chloride or by subjecting it to a titration cycle at 25° in the same solution.

The titration vessel was immersed in a brine-bath kept at 0·3°, 0°, −0·4°, or −0·75° ± 0·05°. The contents of the titration vessel were allowed to attain the temperature of the bath during

* Part VI, *J.*, 1958, 4117.

[1] Cox and Peacocke, *J.*, 1957, 4724.
[2] Cox and Peacocke, *J.*, 1956, 2499.

30 min. before the titration. No freezing occurred even at the lowest temperature (but some of the solutions were supercooled).

0·05M-Sodium borate and 0·05M-potassium hydrogen phthalate were used to calibrate the

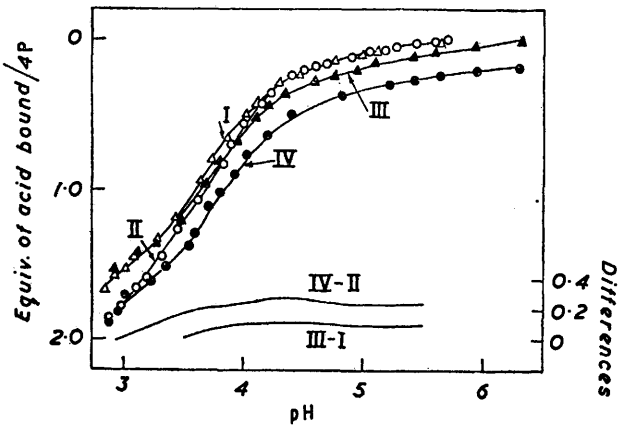

FIG. 1. *Titration curves of herring-sperm sodium deoxyribonucleate in 0·05M-sodium chloride at low temperatures.*

Upper figure: Left ordinate scale. I (△), *Forward-titration curve at* −0·4°. III (▲), *Back-titration curve at* −0·4°. II (○), *Forward-titration curve at* +0·3°. IV (●), *Back-titration curve at* +0·3°.

Lower figure: Right ordinate scale. Difference curves.

glass-electrode system. The pH value [3] assigned to the phthalate solution over the temperature range used was 4·008 and the interpolated value [4] given to the borate solution was 9·39 ± 0·01 for the range +1° to −1°.

Results.—The titration behaviour at low temperatures is shown in Fig. 1 where curves I and II are the irreversible forward-titration curves at −0·4° and 0·3°, respectively, and III and IV the corresponding reversible back-titration curves. Fig. 1 also gives a plot of the difference in acid bound between the forward- and back-titration curves for each of these temperatures and it can be seen that the difference was much less at the lower temperature. An intermediate pair of curves was also obtained at 0° but is omitted from Fig. 1 for clarity.

FIG. 2.

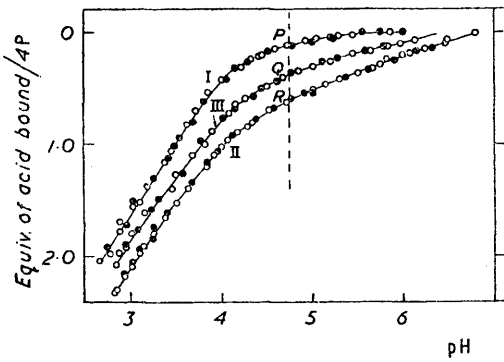

FIG. 3.

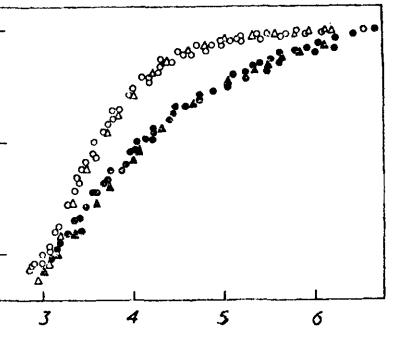

FIG. 2. *Reversible dissociation curves of sodium deoxyribonucleate in 0·05M-sodium chloride at* −0·75°. I, *Original untreated nucleate.* II, *Denatured nucleate.* III, *Mixture of* I *and* II *in equimolar proportion (on P basis).*

○ *Forward-titrations with acid.* ● *Back-titrations with alkali. P, Q, R, see text.*

FIG. 3. *Titration curve of sodium deoxyribonucleate at* 25° *in 0·05M-sodium chloride after a low-temperature titration cycle.*

○● *Forward- and back-titration curves of nucleate that has previously undergone a complete titration cycle at* −0·7° *in 0·05M-sodium chloride (several experiments).*

△▲ *Forward- and back-titration curves of the original untreated nucleate.*

The reversible dissociation curve of the double-helical structure was observed when the temperature was reduced to −0·75° and is shown as curve I in Fig. 2. This reversibility suggested that at this temperature the nucleate had not become denatured and that the hydrogen

[3] Steinhardt and Harris, *J. Res. Nat. Bur. Stand.*, 1940, **24**, 335.
[4] Steinhardt, Fugitt, and Harris, *ibid.*, 1940, **25**, 519.

bonds had re-formed on back-titration. This was confirmed by submitting the neutral nucleate solution obtained by a complete titration cycle at −0·75°, to a further titration cycle at 25°; the points shown in Fig. 3 were then observed. Points on the forward- and back-titration curves of a previously untreated nucleate are also shown in Fig. 3 and it is apparent that the two sets of observations are coincident within experimental error. Thus the complete titration cycle at −0·75° had not permanently modified the dissociation behaviour of the various groups in the nucleate and it is reasonable to conclude that the double-helical structure remained intact since the curves of Fig. 3 are characteristic of this structure.

The reversible titration curve of the non-hydrogen-bonded nucleate at −0·75° was obtained by titrating, at this temperature, a neutral solution of nucleate which had been previously denatured either by heat or by acid-treatment. This curve, which is markedly different from the reversible curve of the hydrogen-bonded structure, is shown as II in Fig. 2.

Curve III in Fig. 2 is the reversible titration curve at −0·75° of a nucleate sample consisting of a mixture of equal amounts, with respect to nucleotide phosphorus, of untreated nucleate and of the denatured form. The ratio PQ/PR (Fig. 2), 0·54 ± 0·03, should represent [5] the proportion of the hydrogen bonds broken in the mixture, with no systematic variation according to the position of the point Q along III. Comparison of this value of 0·54 with the true value for this mixture of 0·50 indicates the limits of reliability of this type of computation from the dissociation curves of mixtures. This result supports the validity of the method used earlier in this series [5] for calculating the extent of denaturation from the displacement of titration curves.

Discussion

It has been shown that curve I (Fig. 2) obtained at −0·75° represents the reversible dissociation curve of the nucleate. As has been suggested earlier,[1] these observations can be interpreted in terms of the double-helical structure. It appears that at this low temperature the nucleate molecule retains its double-helical configuration in spite of the ionisation of the amino-groups and consequent disappearance of the hydrogen bonds. The highly specific structure of the molecule seems to be " frozen " into stability. This observation implies that, even for titrations carried out at 25°, ionisation of the amino-groups is a necessary but not sufficient condition for the loss of the double-helical structure. This accords with the mechanism of reversible dissociation which has been given previously [6] and which may be represented as:

$$\{\text{Helix}\}\text{–}NH_3^+ + (\text{–NH·CO–})\{\text{Helix}\} \rightleftharpoons \{\text{Helix}\}\text{–}NH_2 \ldots (\text{–NH·CO–})\{\text{Helix}\} + H^+$$

where $-NH_3^+$ and $-NH_2$ represent the charged and the uncharged form of the 1 : 6-amino-system capable of being hydrogen-bonded, although not necessarily defining the location of the proton; (–NH·CO–) is the system to which these groups are hydrogen-bonded in the double helix; the terms in the braces represent the configuration of the polynucleotide chains, and the dotted line represents the hydrogen bonds which link these systems.

The foregoing observations indicate that for permanent loss of the double-helical structure to occur (i) the hydrogen bonds must disappear as a result of ionisation and (ii) there must also be present enough thermal energy to cause rotation of the groups originally involved in hydrogen bonds. This rotation must attain the stage where there is a negligible chance of the double-helical form being recovered.

The results support an explanation, previously offered by one of us,[7] of some earlier observations on the effect of acid on sodium deoxyribonucleate. Reichmann, Bunce, and Doty [8] originally reported that light-scattering studies showed that the nucleate molecule collapsed on addition of acid to pH 2·6 in 0·2M-sodium chloride but that re-neutralisation of the solution to pH 6·5 restored the molecular size to its original value. Mathieson and

[5] Cox and Peacocke, *J.*, 1956, 2646.
[6] Cox and Peacocke, *J.*, 1958, 4117.
[7] Peacocke, *Chem. Soc. Special Publ.*, No. 8, 1957, p. 163; see also Shooter, *Progr. Biophysics Biophys. Chem.*, 1957, **8**, 327.
[8] Reichmann, Bunce, and Doty, *J. Polymer Sci.*, 1953, **10**, 109.

Matty [9] did not observe this restoration of the macromolecule to its original shape after re-neutralisation.

This apparent discrepancy can now be clearly attributed to the fact that in the work of Reichmann *et al.*[8] the acidification and re-neutralisation of the deoxyribonucleate were carried out in a cold room. The increased stability of the double-helical structure at these low temperatures and its reversible protonic dissociation under these conditions now explain the reversal in molecular shape and size which they observed. Mathieson and Matty's studies [9] were at 25° where the dissociation of the double-helical structure is irreversible, and thus no restoration of molecular shape is to be expected. Geiduschek [10] recently has reported light-scattering and viscosity measurements which support this explanation: whereas nucleate after acid-treatment at 25° has irreversibly collapsed, titration at 0° left the size and shape of the molecule unchanged.

The reversible dissociation curve of the fully denatured nucleate (II, Fig. 2) has been observed, and it is to be expected that partly denatured samples would give curves between I and II (Fig. 2). Curve III of Fig. 2 represents such a sample by being the reversible dissociation curve of a mixture of denatured and untreated nucleate. The position of such curves relative to I and II should allow determination of the extent and character of denaturation, regardless of the presence of such interfering effects as breaks in the main chain. This technique has been applied in a study of the denaturation of sodium deoxyribonucleate caused by γ-rays,[11] work on which will be more fully reported elsewhere.

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Department of Microbiology,
The University, Birmingham, 15. [*Received, February 18th,* 1959.]

[9] Mathieson and Matty, *J. Polymer Sci.*, 1957, **23**, 747.
[10] Geiduschek, *ibid.*, 1958, **31**, 67.
[11] Peacocke and Preston, *J. Polymer Sci.*, 1958, **31**, 1.