DEFORMATION OF DNA, III.* THE EFFECT OF GLYCOL AND GLYCEROL ON THE ULTRAVIOLET ABSORBANCE OF DNA. RENATURATION BY DILUTION**

Edward L. Duggan

with the assistance of S. L. Bunch, S. L. Louie and A. N. Brown

Department of Biochemistry
University of California School of Medicine
San Francisco 22, California

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The demonstration (Marmur and Lane, 1960; Doty, Marmur, Eigner, and Schildkraut, 1960) that transformant DNA may be inactivated and reactivated by heating has emphasized the need for mild treatments leading to denaturation and, possibly, renaturation.

Our finding that 96% glycol (Duggan and Bunch, 1961) will increase the relative absorbance at 260 mμ to a value of 1.50-1.54 may provide one mild treatment for use by researchers in studies of the active microbial DNA's. Comparative studies of methanol, glycol and glycerol have demonstrated that glycol is unique in the ability to open the hydrogen-bonded structure of DNA at room temperature. For methanol, our results confirm Geiduschek (1956) that there is no change in A_{260} as methanol increases to 90% concentration. Glycerol, while less effective than glycol, as measured by relative absorbance, may be preferred on the basis of less interference with the bacteria during the test of activity.

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* Previous paper in series: V. L. Stevens and E. L. Duggan, J. Am. Chem. Soc., 79, 5703 (1957).

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Methods and Materials

Chicken erythrocyte DNA and calf thymus DNA were used in this investigation. The chicken erythrocyte DNA was prepared by the use of Dupanol and Versene with a five minute blending at 60°, otherwise as described by Stevens and Duggan (1957). The preparation was never subjected to acid treatment (below pH 5.8), to drying nor to chloroform-octanol treatment. After repeated precipitations with ethanol from 0.3 M NaCl solution, the final solution of DNA (0.2%, in 0.3 M NaCl) was stored frozen. This material had an intrinsic viscosity at zero shear of 2.5 ml./gm. in 0.15 M NaCl; the value did not change during storage. In contrast to the results of Cavalieri, Deutsch, and Rosenberg (1961), the viscosity value did not change as a result of chloroform-octanol extraction. On occasion, dry calf thymus DNA (Worthington) was dissolved by homogenization and used in parallel studies to obviate the criticism that changes in the chicken DNA are unique to this type of DNA. The heat treatments were done in a thermostatted bath. The absorbance values were taken from full spectral curves determined at room temperature in the DK-2 spectrophotometer.

Results

Figure 1 presents the results of identical experiments on both types of DNA, chicken erythrocyte and calf thymus. Both samples increase in A_{260} as the glycol concentration is increased to 96%. The curves show a marked similarity in shape to the melting curves of Marmur and Lane (1960). Position of the curve on the absiccsa has been shown to be highly dependent upon

cation concentration, shifting the mid-point of the S shaped curve to 89% glycol in 6 mM NaCl.

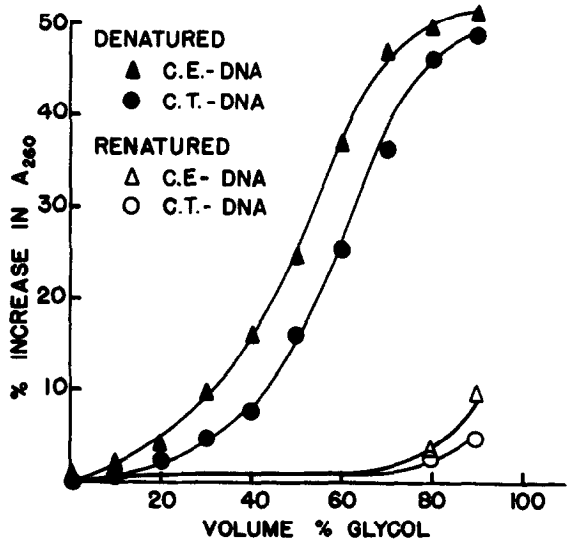

Fig. 1. The effect of glycol concentration on the relative absorbance of chicken and calf DNA. The two upper curves were obtained and read in glycol containing 0.2 mM phosphate buffer, pH 7.0, after 2 hours at room temperature. The "renatured" DNA samples (lower curves) were five-fold more concentrated in DNA at the various glycol levels. Then they were diluted with aqueous M NaCl until the final molarity of 0.8 M NaCl was reached.

Using 9 mm. spacers in the cuvets, the same relative absorbance was obtained by treatment of more concentrated DNA (to 0.02% concentration) in 90% glycol. Therefore, the samples yielding the lower curves exhibited the same absorbance increase before dilution as the other samples. The low absorbances of these samples after dilution indicate that the DNA's have reformed a hydrogen-bonded structure, in the low glycol, high salt medium.

Relative viscosity measurements on chicken DNA in 96% glycol (6 mM NaCl) indicate a decrease to 40% of the initial value in saline; after removal of the glycol by dialysis, the relative viscosity became 77% of its initial value. The values from

absorbance and viscosity measurements indicate strand separation and reformation, without definitive location of single-strand or reformed double-strand limits. The incremental dilution method for absorbance-return holds a certain analogy to the strand reformation by slow cooling of a heat-denatured sample (Doty, et al., 1960).

Figure 2 summarized the effects of combined heat and glycol treatments. It was expected that a lower concentration of glycol would be effective in maintaining separation of the base pairs, once their hydrogen bonds were broken by thermal energy. This was found to be true to a limited extent. The sample without glycol increases to 25% absorbance increment, then forms a

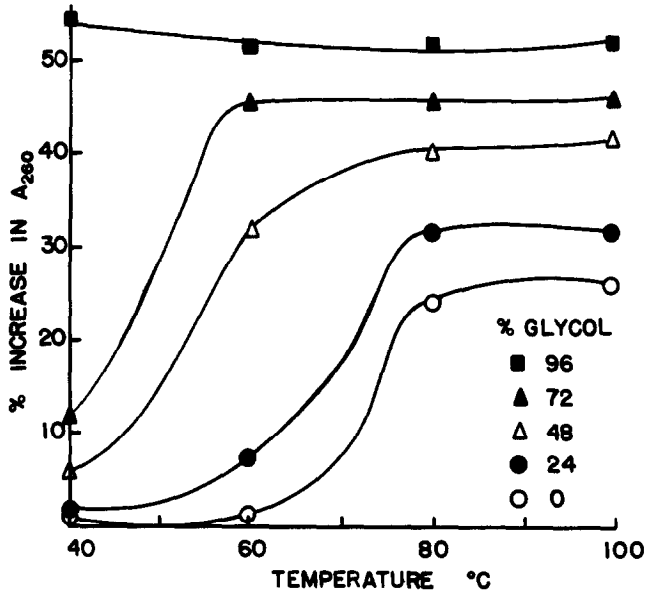

Fig. 2. The effect of heating at various glycol levels on the relative absorbance of chicken DNA at 260 mμ. The stock DNA initially contained 10 mM phosphate buffer, pH 7.0, and 300 mM sodium chloride. The dilution with glycol and water reduced total cation level to 6.2 mM concentration. The individual points are A_{260} values after 10 minutes at temperature and cooling to room temperature.

plateau, even though the DNA absorbance is still capable of an additional 25% increase. Such a plateau does not occur in the absorbance values at treatment temperature. This must be the result of reformation of hydrogen bonds occurring as the sample is cooled. As the glycol concentration is increased, two effects take place: a) The plateau level increases, indicating that hydrogen bonds are not able to reform as effectively. b) The temperature at which the plateau occurs is lowered so that in 96% glycol it is below room temperature. The highest absorbance increase of 53% is maintained for all temperatures, even below room temperature. We are inclined to view this as the "absorbance limit" for the chicken DNA (from an A_p in 0.3 M NaCl of 7,100 to an A_p in glycol of 10,800).

Figure 3 presents the data obtained from a study of the effect of ionic strength on the absorbance-return step for various calf thymus DNA samples heated at high glycol levels. The techniques are a combination of those producing Figure 2 and the lower curves of Figure 1. It is apparent that the return to lower relative absorbance values from the 1.50 limit is a common function of sodium ion level for all curves in Figure 3. Return to lower absorbance is also possible after heating the glycol-DNA mixtures.

Electron microscope pictures of sprayed, palladium-shadowed DNA samples in water and in glycol showed that the large DNA aggregates (100 A° diameter) in the aqueous DNA samples were completely missing in glycol-treated samples. The field of the glycol-treated specimens showed patches of amorphous material without observable structure or regularity.

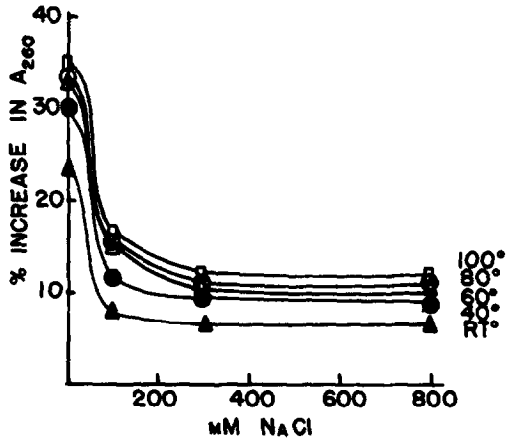

Fig. 3. The influence of ionic strength on the renaturation of DNA after heating in 90 volume per cent glycol. Aliquots of the same solution of calf thymus DNA in 90% glycol were heated 10 minutes at the indicated temperatures. Aliquots of these heated and cooled solutions were then quickly diluted 1:5 with sodium chloride solutions to give the indicated molarities of Na^+. The DNA stock solution initially contained 0.01 M phosphate buffer, pH 7. The starting absorbance level in 90% glycol is shown by the "limit" marking.

The effect of high glycerol concentration and heating have been studied, with results comparable to those shown in Figure 2, with the exception that 96% glycerol (13.1 M) yields a curve similar in location to that of 72% glycol (13 M). The equal effect of the two compounds on the molar basis implies that these denaturants form hydrogen bonds using only two hydroxyl groups of the glycerol.

To our knowledge this is the first study of the effect of glycol and glycerol as denaturants of DNA. It is interesting that glycol serves to expose the tyrosyl residues of ribonuclease to spectral titration (Sage and Singer, 1958). All activity could be recovered if the titration and exposure to glycol were rapidly done. The spectral changes of several proteins and viruses were also investigated after glycol treatment. No change was found on the treatment of chymotrypsin, serum albumin or tobacco mosaic

virus. A canary pox virus specimen showed appreciable absorbance increase at 260 mμ, beyond the change due to decreased turbidity. Yeast RNA showed an increase of 27% in 95% glycol.

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