DNA COMPOSITION AND STABILITY TO ACID DENATURATION

Lyle G. Bunville and E. Peter Geiduschek*

Committee on Biophysics, The University of Chicago, Chicago, Illinois

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Of the many ways for destroying the secondary structure of DNA, acid denaturation was the first to be explored (Gulland et al., 1947). We, also, have been interested in this reaction as one means of investigating the factors that contribute to the stability of the native, helical DNA molecule. The present communication deals with the denaturation of DNA's of varying composition as studied in aqueous solution, using the techniques of differential spectrophotometry, viscometry, pH titration, and density gradient centrifugation on DNA samples isolated from bacterial and marine sources by detergent methods.** The differential spectrophotometric titration curves of the six DNA's listed in Fig. 2a all show an isosbestic point for protonation differential spectra of the ordered secondary structure at 272 $\pm$ 2mμ, at 25°C. and 0.10 ionic strength. Somewhat lower isosbestic points have been reported by Dove et al. (1959) and Lawley (1956) for calf thymus DNA. Denaturation occurs in a narrow pH region and is attended by a sharp rise in optical density at 250-270 mμ to a plateau at the low pH end of the transition (Fig. 1). The pH of half denaturation at 25°C., $pH_{1/2}$, may

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**Fuller details of isolation method, experimental procedure and results will be published elsewhere.

therefore be defined by these spectrophotometric titration curves.* Fig. 2a shows the variation of $pH_{1/2}$ with composition.

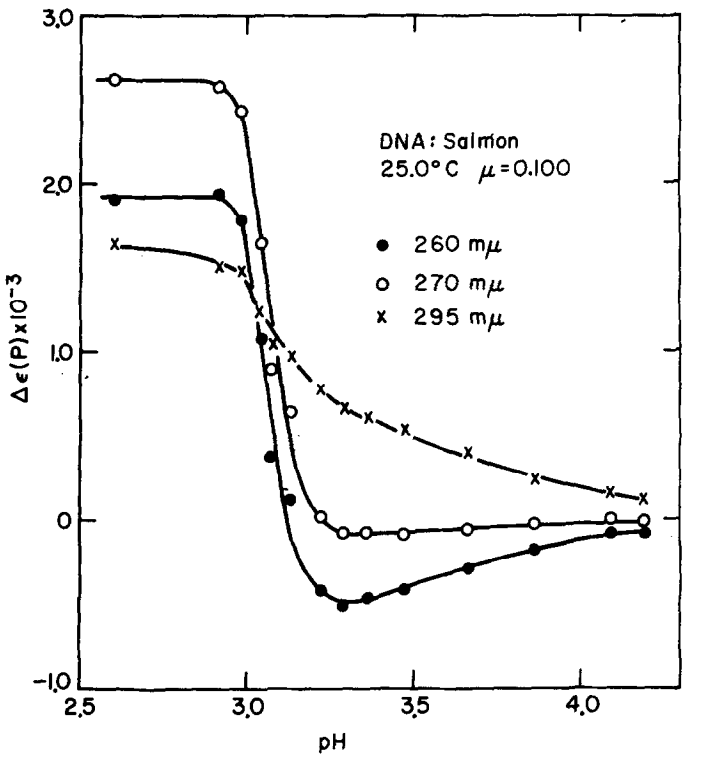

Fig. 1. Spectral difference curves as a function of pH at 25°C. and 0.100 ionic strength. DNA from salmon testis (adenine/guanine mole ratio 1.4). $\Delta\epsilon(P)$ is the change of phosphorus - molar extinction coefficient on going from pH 6.5 to the pH noted.

It is clear that, as the guanine-cytosine content of these DNA's is increased, the pH of half denaturation is lowered. Consequently, stability toward "thermal" and "acid" denaturation depend in the same manner on the composition of the DNA (Fig. 2b). The thermal stability of DNA's of varying composition was first reported by Marmur and Doty (1959), using spectrophotometric analysis. In our experiments we have used viscometric measurements

***The arbitrary choice of a given wavelength leads to systematic uncertainties of ± 0.02 pH units. However, for the subsequent discussion, pH differences will be the more important consideration, and for these the choice of wavelength is unimportant.

at 25°C. on dilute solutions heated to varying temperatures for 60 minutes and rapidly cooled to determine the maximum irreversible component of denaturation. $T_{1/2}$ is the temperature of the mid-point of the thermal transition at pH 7. Our results are similar to those of Marmur and Doty. However, the exact location of $T_{1/2}$ depends on the method of analysis. The viscometric and spectrophotometric assays differ in two respects: the reversibility factor in the viscometric analysis, and the presumed dependence of viscosity and optical density on long-range and short-range order respectively. The effects, on $T_{1/2}$, of these two factors compensate partially. Consequently for DNA from E. coli, the viscometric $T_{1/2}$ is only 2°C. higher than the spectrophotometric $T_{1/2}$.

The dependence of secondary structure stability upon pH, temperature and ionic strength may be conveniently shown in terms of phase diagrams (Geiduschek and Holtzer, 1958). At constant ionic strength, the dependence of $pH_{1/2}$ on temperature would be represented by a family of curves called denaturation isotones, since they apply to processes occurring at constant ionic strength. The data of Fig. 2b provide two points on each of these isotones and suggest that for DNA's of varying composition, isotones do not intersect.

In order to understand the significance of the above results in terms of the stability of the secondary structure, we also need to know the charge state at the pH of denaturation. Comparison of pH and spectrophotometric titrations yields the required information: those DNA's richest in guanine and cytosine must be more strongly protonated in order for denaturation to occur. At 25°C. and 0.10 ionic strength, the number of protons bound per nucleotide pair at the pH of half denaturation varies from 0.72 to 0.87 for the range of compositions studied. At

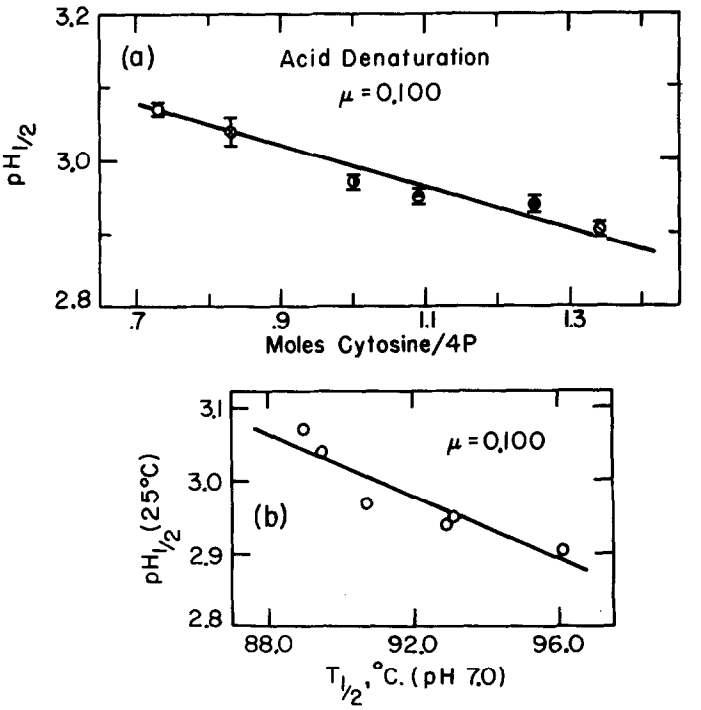

Fig. 2. (a) $pH_{1/2}$ as a function of base composition at 25°C. and 0.10 ionic strength. $pH_{1/2}$ is defined as the pH at which one-half the maximum extinction coefficient change at 270 mμ is obtained. Sources of DNA: O Strongylocentrotus drobachiensis, ⊗ salmon, ◑ E. coli, ◓ A. aerogenes, ● Serratia marcescens, ⊘ pseudomonas fluorescens.

(b) $T_{1/2}$ at 25°C. and pH 7, 0.10 ionic strength, versene buffer as a function of $pH_{1/2}$. $T_{1/2}$ is the temperature at which 60 minutes heating will reduce the intrinsic viscosity, measured at 25°C. after rapid cooling, to one-half its original value.

lower temperatures, considerably higher degrees of protonation are required.

Finally, the above data permit us a closer look at the relationship between the breadth of the acid denaturation transition, and the heterogeneity of the DNA samples. We find that in each of the nucleic acids investigated, half of the denaturation (from 25 to 75% of the observable change, using spectrophotometric

criteria) occurs within 0.08-0.12 pH units. Only a fraction of this breadth is due to heterogeneity in guanine-cytosine content. The following example is drawn from data on Strongylocentrotus DNA. If the width of the DNA band in CsCl gradient centrifugation is entirely assigned to density heterogeneity (a considerable over-estimate), the half width of the base composition distribution is 4.6 mole per cent guanine plus cytosine (Rolfe and Meselson, 1959; Sueoka et al., 1959). Using the data of Fig. 2a, we may estimate an upper limit of 0.027 pH units for the contribution of the compositional heterogeneity to the half width of the acid denaturation. The observed half-width is 0.09 pH. The difference, or approximately 0.06 pH units, is to be assigned as the average breadth of the transition in each molecular species. This suggests that the acid denaturation at high ionic strength, though sharp, is not an all-or-none process (Sturtevant et al., 1958; see, however, Oth, 1959). However, the extent to which intramolecular heterogeneity broadens the transition, is not, at present, clear. While short-range deviations from random distributions of nucleotide sequences are well-known (Jones et al., 1957; Shapiro and Chargaff, 1957), attempts to demonstrate substantial long-range heterogeneity have proved unsuccessful (Sueoka et al., 1959; Guild et al., 1960).

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