KINETICS OF THERMAL DEGRADATION OF DEOXYRIBONUCLEIC ACID (DNA) AND RIBONUCLEIC ACID (RNA)

Saul Kit *

The Section of Nucleoprotein Metabolism, Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas

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The data to be presented support the concept that the strands of the DNA duplex separate at higher temperatures. The properties of RNA and of thermally denatured DNA are in marked contrast to native DNA. The following are some of the properties characteristic of RNA and heat denatured DNA: a) The viscosity increases and the sedimentation constants decrease as the ionic strength of the medium is reduced (Cox and Littauer, 1959; Inman and Jordan, 1960; Kawade, 1959; Kit, in press; Rice and Doty, 1957). b) The optical density increases gradually when the nucleic acid is heated from room temperature to 95° (Doty, Marmur, Eigner, and Schildkraut, 1960). c) Hyperchromicity is observed in the presence of 6M urea (Kawade, 1959). d) The molecules react rapidly with formaldehyde (Fraenkel-Conrat, 1954; Kawade, 1959; Kit, in press). e)The potentiometric titration curves indicate rapid protonation between pH 7 and pH 4 (Dove, Wallace, and

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Davidson, 1959; Rice and Doty, 1957). The viscosity and sedimentation data are typical of single stranded polyelectrolytes which may vary in conformation between a random coil and a more extended thread-like form. The hyperchromicity induced by heating or by urea solutions is indicative of the disruption of secondary structure, while the potentiometric titration curves and other spectral changes show that the nucleic acid bases are relatively free to react with protons and formaldehyde.

There is, however, a property in which thermally denatured DNA has been reported to resemble native DNA rather than RNA. From measurements of the kinetics of enzyme degradation, it is inferred that native DNA (except for that from ϕ X174) is double stranded (Schumaker, Richards, and Schachman, 1956), whereas RNA is single stranded (Gierer, 1958). Cavalieri, Rosenberg, and Deutsch (1959) have shown that heat denatured calf thymus or E. coli DNA also manifest double strandedness when studied in this way. This observation and the fact that the molecular weight of E. coli DNA, unlike that of calf thymus DNA, is halved after heating (Cavalieri, Rosenberg, and Deutsch, 1959; Rice and Doty, 1957) led Cavalieri, Rosenberg and Deutsch (1959) to propose that the unit DNA molecule of E. coli is actually a dimer composed of two double helices, laterally bonded together, and that the bonds holding the dimer together are ruptured by heating.

In all the foregoing experiments, the heat denatured DNA was cooled to room temperature before additional measurements were made. As shown by Doty, Marmur, Eigner, and Schildkraut (1960), hydrogen bonds may reform on cooling. There is physicochemical

and biological evidence that renaturation takes place with bacterial DNA preparations, provided that cooling is gradual and the concentration of DNA exceeds a minimal value. Moreover, it is by no means certain that a single stranded DNA structure could not form sufficient intramolecular hydrogen bonds to result in the apparent two-stranded kinetics of degradation by deoxyribonuclease (Sinsheimer, 1960).

It seemed essential that strandedness studies on denatured DNA should be performed under conditions where one could exclude the reformation of hydrogen bonds between base pairs. This condition prevails with DNA at higher temperatures. Therefore, the kinetics of thermal degradation at 95° of heat denatured DNA and at 80° of RNA was investigated. Viscosity measurements were employed to follow the degradation of the nucleic acids.

The results, as plotted by the method of Schumaker, Richards, and Schachman (1956), are shown in Figures 1 and 2a. The slope of the curves are indicative of the strandedness of the molecules (see legend to Figure 1). For Lettre-Ehrlich tumor RNA, the slope was 0.86, in agreement with the concept that RNA is single stranded. Viscosity measurements of the DNA solution were begun immediately after temperature equilibration (6 minutes). At this time, the disruption of the native hydrogen bonded structure was essentially complete, as shown by spectrophotometric measurements (Figure 2b). The slope of the curve of Figure 2a is 1.10 for the first thirty minutes and thereafter decreases. The slope is clearly not 2.0, as predicted for double stranded DNA. These results show that DNA at 95° is either entirely single stranded or consists of duplexes held together by so few

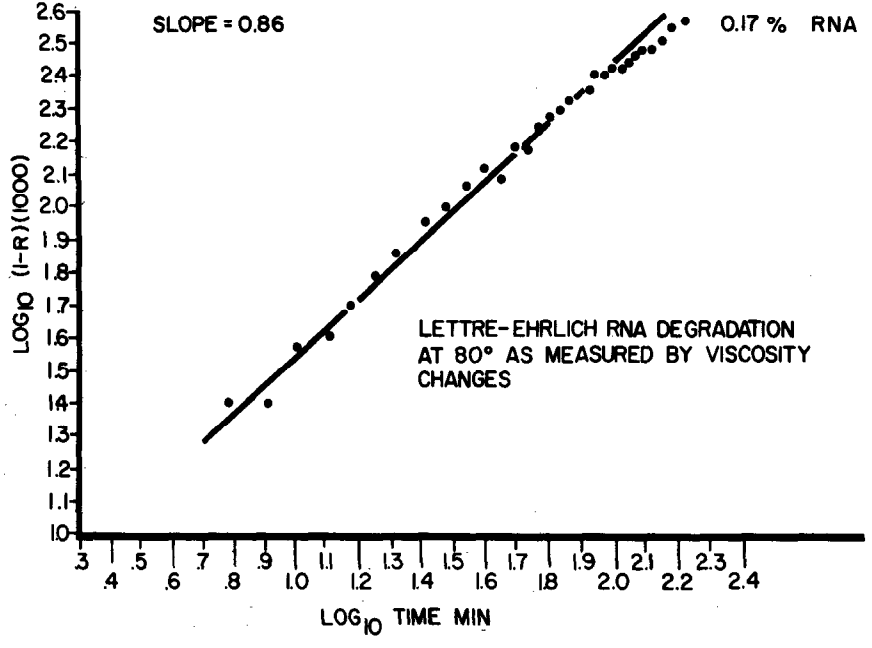

Figure 1

Thermal degradation of Lettre-Ehrlich carcinoma RNA at 80°. The strandedness of RNA was estimated by the method of Schumaker, Richards, and Schachman (1956) from the equation:

$$\log_{10} (1-R^{obs}) = n \log_{10} t + \text{constant}$$

R is defined as the ratio of the observed specific viscosity at any time to the initial specific viscosity; n equals the number of strands possessed by the RNA molecule; and t equals the time in minutes. RNA was dissolved in 0.05M NaCl, 10^{-3}M versenate, 10^{-3}M

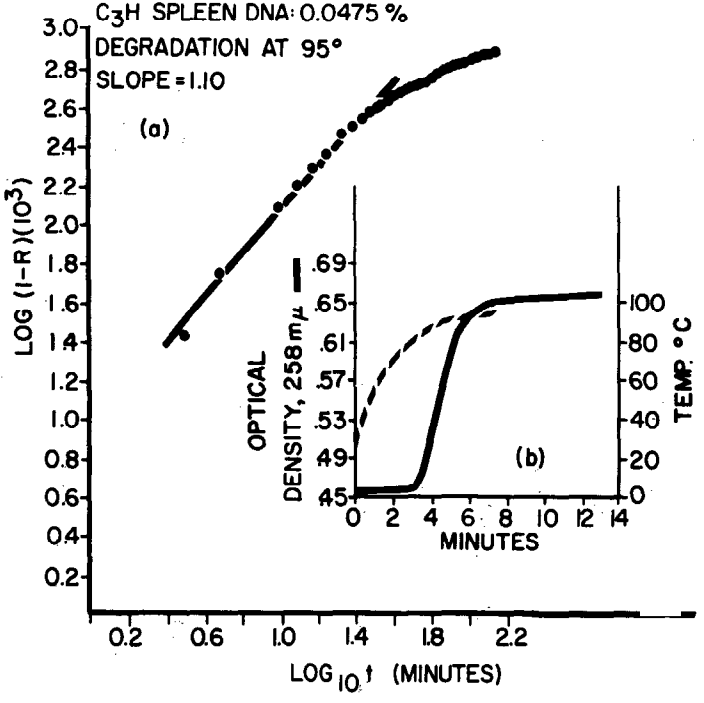

Figure 2

a) Thermal degradation of mouse spleen DNA at 95° as measured by viscosity changes. DNA was dissolved in 0.15M NaCl, 10^{-3}M versenate, 10^{-3}M phosphate.

b) Properties of DNA solution after exposure to thermostatically controlled temperature of 95°. The increase with time of the ultraviolet absorption and the tempera-

hydrogen bonds that single strand kinetics is manifested. The biological and biophysical experiments of Doty, Marmur, Eigner, and Schildkraut (1960) lend plausibility to the first interpretation, but molecular weight determinations are required for a decisive answer. Molecular weight measurements must be performed, however, under conditions where the reformation of hydrogen bonds between nucleic acid base pairs is prevented.

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