the extensive degradation of host DNA by these enzymes is consistent with the previous evidence that the ϕX174 DNA is a ring structure, resistent to exonuclease attack (*8*). That the RF also resists degradation under these conditions suggests that it, too, is a ring. In confirmation of these results, recent electron micrographs (*9*) of RF preparations show the presence of ring structures of the expected contour length (*10*).

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References and Notes

1. R. L. Sinsheimer, *J. Mol. Biol.* **1**, 43 (1959).
2. R. L. Sinsheimer, B. Starman, C. Nagler, S. Guthrie, *ibid.* **4**, 142 (1962).
3. G. D. Guthrie and R. L. Sinsheimer, *ibid.* **2**, 297 (1960).
4. M. Hayashi, M. N. Hayashi, S. Spiegelman, *Science* **140**, 1313 (1963).
5. C. C. Richardson and A. Kornberg, *J. Biol. Chem.*, in press.
6. I. R. Lehman, *ibid.* **235**, 1479 (1960).
7. J. D. Mandell and A. D. Hershey, *Anal. Biochem.* **1**, 66 (1960).
8. W. Fiers and R. L. Sinsheimer, *J. Mol. Biol.* **5**, 408 (1962).
9. A. K. Kleinschmidt, A. Burton, R. L. Sinsheimer, *Science*, this issue.
10. We thank Dr. C. C. Richardson and Dr. I. R. Lehman for generous gifts of the *E. coli* exonuclease III and the *E. coli* exonuclease I. One of us (A.B.) has been a postdoctoral fellow of the U.S. Public Health Service. This research has been supported in part by grant RG-6965 from th U.S. Public Health Service.

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Complementary Strand Association between Nucleic Acids and Nucleic Acid Gels

Abstract. *Nucleic acid gels can be formed as a result of the cross-linking action of ultraviolet light or of nitrous acid. Such gels form duplex combinations with complementary nucleic acid strands.*

Deoxyribonucleic acid, immobilized on cellulose or in agar, has been used for the isolation of complementary ribonucleic acid (*1*, *2*) and also in studies of DNA-DNA interactions (*3*, *4*). That nucleic acids (*5–7*) themselves can be made into insoluble gels by ultraviolet-induced cross linkages suggests another general method for the preparation of materials suitable for complementary strand association studies. This report describes further observations on the formation of such gels by the actions of ultraviolet light or nitrous acid as well as tests of the resulting gels as practical experimental tools. It appears that any naturally occurring nucleic acid and most artificial nucleotide polymers, including oligonucleotides (*7*), can be readily converted to such gels.

A simple qualitative procedure has been used to test the formation of gels with a variety of nucleic acids. A drop of water containing about 50 μg of nucleic acid was dried at 100°C on a microscope slide. While drying the solution was distributed with a glass rod over an area of about 2 cm^2. The cover slip was irradiated for several minutes 5 cm from a General Electric 15-watt germicidal lamp. The slide was rinsed and scraped lightly in 3 ml of water to remove the gel and to dissolve any ungelled polymer. When DNA, ribosomal RNA, soluble RNA, or polyU (*8*) were treated in this way, visible fibers were formed. Upon irradiation for only a few seconds the gel structure was so open that it was barely visible in water, but the addition of 0.01*M* $MgCl_2$ condensed it into visible fibers. At higher doses the fibers appeared to be very dense even when suspended in distilled water. An estimate of the quantity of ungelled polymer could be made by filtering the gel and measuring the optical density of the filtrate at 260 mμ. The amount of irradiation required to form a gel depends on the molecular weight of the polymer, the optical thickness of the dried material, and the fraction of bases present capable of forming dimers.

Gels may also be formed on inert supporting materials such as polyvinyl beads (*9*), cellulose fibers and nylon thread. Figure 1 shows the degree of retention of polyU and polyC on Geon 101 resin grains after drying the polymers out of water solution and exposing them to ultra-violet light 22 cm from the 15-watt germicidal lamp. The curves are based on the reduction in optical density of the filtrate after the resin was suspended in water and washed thoroughly on a glass-wool filter. Other measurements have shown that for this dose range there is no significant reduction of the optical density of the total polyU or polyC resulting from dimer formation or other chemical effects of ultraviolet irradiation. At considerably higher doses such effects are significant. There was no evidence for gel-formation of polyA or polyI at these doses of irradiation. At a dose of 10^7 ergs/cm^2 there was a 30 percent reduction in the optical density of the polyA filtrate, which may have been due to chemical effects reducing the total optical density of the polyA.

The DNA gels could also be formed by treating dried films (previously made single-stranded by boiling in water solution) with nitrous acid (*10*). For this purpose 0.25*M* KNO_2 was adjusted to *p*H 4.2 by the addition of acetic acid and it was diluted with 2 volumes of ethanol. A few drops of this solution were placed on the dried film and allowed to remain for 10 minutes. The gel was then washed off in tris buffer, *p*H 7.4. Fibers were observed with properties comparable to those formed upon ultraviolet irradiation.

It was possible to obtain specific complementary strand association between RNA from bacteria and DNA gels formed by cross linking as a result of ultraviolet irradiation. In one experiment 3.5 mg of DNA was dissolved in water and boiled for 10 minutes to separate the strands. The solution was brought to 0.15*M* NaCl containing 0.015*M* sodium citrate (SSC) and 100 mg of cellulose powder added to supply bulk for convenient handling. Two volumes of alcohol were added and the wet precipitate was exposed to ultraviolet light (2 minutes with stirring 5 cm from the 15-watt germicidal lamp). The bulk of the DNA did not precipitate in sufficiently close association with the cellulose fibers, and as a result only about 10 percent of the original DNA remained in the cellulose column after thorough

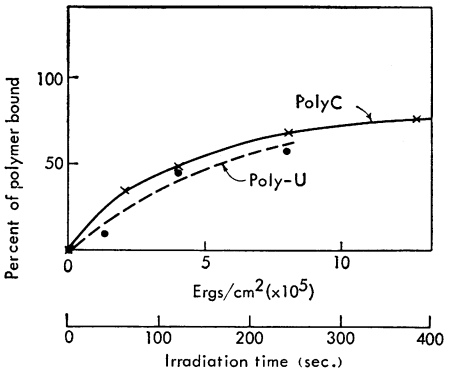

Fig. 1. Fraction of polyU and polyC immobilized on plastic beads as a function of total ultraviolet dose. Eight milligrams of polymer were dried on 2 g of Geon 101 resin. Samples (100 mg) were given four exposures with mixing in between each. The fraction bound was determined from the reduction in optical density of all material that could be washed from the resin. The scale showing ergs/cm^2 is based on a National Bureau of Standards calibration of the energy emitted in the Hg line at 254.7 mμ from an unfiltered lamp. The 254.7 line presumably is the effective radiation causing cross linking by dimer formation.

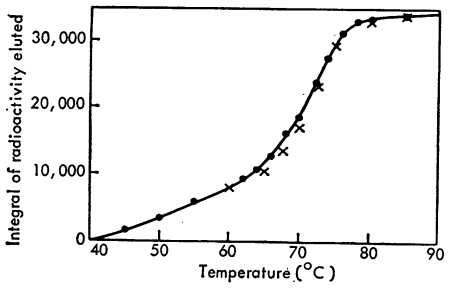

Fig. 2. Integral of the radioactivity from pulse-labeled *E. coli* RNA eluted from immobilized DNA as a function of temperature: (—·—) eluted from a DNA gel immobilized on a cellulose matrix; (×) eluted from DNA immobilized in agar.

washing. Even this small yield of nucleic acid gel was more than adequate for testing its capacity to form specific complementary strand association with labeled RNA. Following the procedure of Bolton and McCarthy (*2*), a small amount of pulse P^{32}-labeled RNA from *Escherichia coli* BB was incubated with the DNA-cellulose gel for 16 hours at 60°C in a mixture of 0.3*M* NaCl, 0.03*M* sodium citrate. The DNA-cellulose gel (about ½ ml) was placed in a water-jacketed column, washed at 40°C with the same buffer and with 0.015*M* NaCl containing 0.0015*M* sodium citrate. The temperature was raised in steps and the column washed with 5 ml cuts of this dilute buffer. Seventy-five percent of the radioactivity was removed by the washes at 40°C and the remaining 25 percent was eluted between 40° and 78°C. Figure 2 shows the integral of the radioactivity eluted from the column as a function of the temperature. Similar results were obtained with a column made by drying and ultraviolet cross linking of *E. coli* DNA on a length of cotton thread. In addition, Fig. 2 shows the results of elution of *E. coli* pulse-labeled RNA from DNA immobilized in agar (*4*). The curves are very similar over the range from 60° to 90°C and do not differ in form from elution curves obtained for sheared DNA or from DNA melting curves measured by hyperchromicity. Therefore, long lengths of the DNA in the cross-linked gel are available for specific strand association by complementary hydrogen bonding.

Polyuridylic acid gel supported on polyvinyl beads was used for a number of experiments. It was formed by mixing 3 mg of polyU with 0.5 g of Geon 101 beads, drying at 100°C in a silicone-treated dish, and irradiating the resulting powder (spread thinly in a large glass dish) for six 15-second intervals 7.5 cm from the 15-watt germicidal lamp with stirring between exposures. About 1 mg of the polyU remained associated with the beads after washing. Fluorescent miscroscopy after acridine orange staining showed that the gel was distributed over the surface of most of the grains although it was not uniform in thickness. Very little of the polyU gel was removed from the beads by washing with water or salt solution at 80° to 90°C.

A sample of this poly-U gel, in a water-jacketed column, was saturated with polyadenylic acid by passing a solution (20 μg/ml of commercial polyA dissolved in 0.1*M* NaCl containing .01*M* sodium citrate, *p*H 7.4) at 40°C slowly over the column until the effluent concentration rose to that of the input solution. The column was then washed at the same salt concentration at 40°C and 1-ml samples of effluent were collected as the temperature was raised in steps. The resulting elution curve is shown in Fig. 3 along with the melting curve for a 1:1 mixture of the polymers measured by hyperchromicity at 257 mμ. The similarity of the two curves is striking, indicating that long lengths of polyA can form hydrogen bonds with the polyU gel and that the binding energy per unit length is similar to that in free solution.

The same polyU gel column was also used to separate oligonucleotides from a hydrolysate of polyadenylic acid (5 hr, 25°C, 1*M* KOH). A sample of the

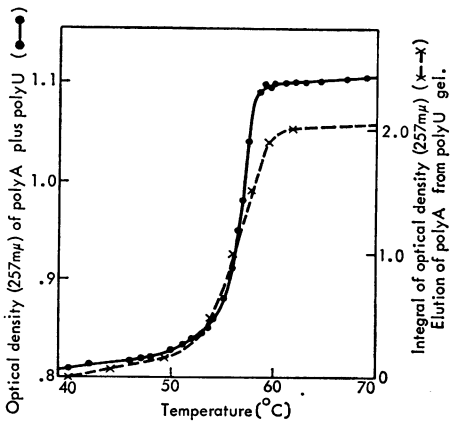

Fig. 3. Melting curves for polyA–polyU strand dissociation. (—·—), optical density of a 1:1 mixture of polyA and polyU; (–×–) sum of the optical density of polyA eluted with increasing temperature from a polyU gel immobilized on plastic beads.

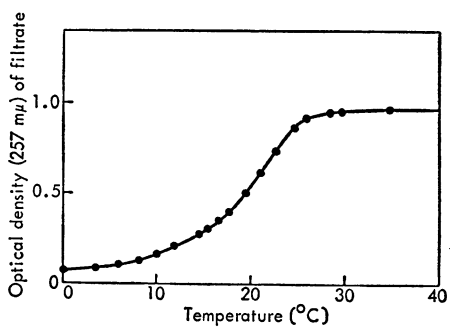

Fig. 4. Concentration of 5′ pApApA in equilibrium with a polyU gel immobilized on polyvinyl beads. The optical density of the filtrate is plotted as a function of the temperature.

hydrolysate was neutralized with carboxylic ion-exchange resin, brought to 1*M* NaCl containing 0.01*M* sodium citrate, *p*H 7.2, and passed over the column at 0°C. Adenylic acid and diadenylic acid were not retained by the column. The column was slowly flushed with 1*M* NaCl containing 0.01*M* sodium citrate while the temperature was steadily raised by a motor driven thermostat. Resolved peaks of tri-, tetra-, penta-, and hexaadenylic acids were successively eluted and the temperatures at which the peaks appeared agreed with the values for the melting points of these oligonucleotides interacting with polyU in solution (*11*). Since the cuts taken (1 ml) were twice the column volume, the column was operating as a single-plate fractionating system. If conditions were properly adjusted, chromatographic resolution of much longer oligonucleotides could be achieved.

Figure 4 shows the results of a measurement of the quantity 5′ pApApA (*12*) in solution in equilibrium with a given quantity of a polyU gel as a function of temperature. For this purpose about 0.5 mg of polyU gel bound to a coarser grade (for rapid filtering) of polyvinyl beads was used. A sample of 5′ pApApA disolved in 1*M* NaCl containing .01*M* sodium citrate *p*H 6.8 was added to the washed resin in a test tube immersed in a temperature-controlled bath at 0°C. Samples were displaced out through a filter and drawn back again into the test tube after measuring their optical density. The temperature was raised in steps and the approach to a steady optical density was followed at each temperature. Check measurements made as the temperature was lowered indicated that equilibrium had been achieved. This

method is suitable for determining the heat of reaction per hydrogen bond between oligonucleotides and polymers. Some complexity is introduced in this case by the apparent formation of a triple-stranded structure (*11*) between one 5′ pApApA molecule and two polyU strands in the gel.

The degree of cross linking is important in the use of gels for studies of complementary relationships between nucleic acids. A tightly cross-linked gel retards the diffusion of nucleic acid strands into position for hydrogen bond formation. In addition, the presence of an interstrand cross link certainly would prevent hydrogen bonding in its immediate neighborhood and might have longer range effects. It was observed that one sample of polyU dried on Geon 101 beads and exposed to about 5×10^6 ergs/cm^2 of radiation at 254.7 mμ had a very much reduced capacity to hydrogen bond with even so small a molecule as pApApA. This was perhaps, in part, due to ultraviolet induced hydration of the uracil residues and other possible chemical effects. Opposed to the requirements for a low degree of cross linking is the need to prevent the polymer from going into solution at high temperatures where some thermal strand cissions will occur. The gels should therefore be made from as high molecular weight material as possible and irradiated only enough to provide stability. One solution to these conflicting requirements currently being tested is to form an extremely tightly cross-linked gel of polyU or a DNA unrelated to the nucleic acids otherwise participating in the experiment (*13*). The nucleic acid of interest then can be stably bound to this matrix with minimal irradiation and a small number of cross links.

The nature of the interstrand cross links which give rise to insoluble nucleic acid gels after ultraviolet irradiation is not yet fully understood. It has been clearly demonstrated that thymine dimers are induced by ultraviolet irradiation in frozen solution (*14, 15*) and that they occur in irradiated DNA (*16, 17*). Insoluble polyU gels have been previously reported (*11*) and evidence for a uracil dimer has been published (*17*). The formation of polyC gels by ultraviolet irradiation has not been previously reported. The irradiation of cytosine or cytosine C^{14}-labeled DNA in solution appear to give rise to a uracil dimer rather than a cytosine dimer (*17*). However, the irradiation doses were much higher than those used here and the product might be different with the high concentrations of cytosine residues in the dried polyC films. Some evidence has been presented for the existence of mixed dimers of uridine and thymidine (*17*). The conclusion can hardly be avoided as previously suggested (*15*), that dimers can form between any pair of pyrimidines. This would account for the relative ease with which insoluble gels of naturally occurring nucleic acids can be induced by ultraviolet irradiation It is very unlikely that purine-purine dimers play a part in the process. However, there is no evidence for or against purine-pyrimidine dimers. It remains to be determined which of the possible dimers are important in a particular situation.

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References and Notes

1. E. K. F. Bautz and B. D. Hall, *Proc. Natl. Acad. Sci. U.S.* **48**, 400 (1962); B. Hoyer, B. J. McCarthy, E. T. Bolton, *Science* **140**, 1408 (1963); B. J. McCarthy, E. T. Bolton, *Proc. Natl. Acad. Sci. U.S.* **50**, 156 (1963); G. Attardi, S. Naono, F. Gros, G. Buttin, F. Jacob, *Comp. Rend.* **256**, 805 (1963).
2. E. T. Bolton and B. J. McCarthy, *Proc. Natl. Acad. Sci. U.S.* **48**, 1390 (1962).
3. D. B. Cowie and B. J. McCarthy, *ibid.* **50**, 537 (1963); B. J. McCarthy and E. T. Bolton, unpublished.
4. E. T. Bolton and B. J. McCarthy, *J. Mol. Biol.*, in press.
5. R. Setlow and B. Doyle, *Biochim. Biophys. Acta* **12**, 508 (1963); J. Baranowska and D. Shugar, *Acta Biochim. Polon.* **7**, 505 (1960).
6. R. Setlow and B. Doyle, *Biochim. Biophys. Acta* **15**, 117 (1954).
7. D. Shugar and J. Baranowska, *Nature* **185**, 33 (1960).
8. The following abbreviations have been used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; polyU, polyC, polyA, polyI, commercialy available (Miles Laboratory) long chain polymers of uridylic, cytidylic, adenylic, and inosinic acids; 5′ pApApA, oligonucleotide with three adenylic acid residues, the terminal phosphorus linked to the 5 carbon of the ribose.
9. Geon 101, The Goodrich Co. Akron, Ohio.
10. E. P. Geidushek, *Proc. Natl. Acad. Sci. U.S.* **47**, 950 (1961).
11. M. N. Lipsett, L. A. Heppell, and D. F. Bradley, *J. Biol. Chem.* **236**, 857 (1961).
12. I thank Drs. L. A. Heppel, M. N. Lipsett, and M. Singer for helpful discussion and samples of oligonucleotides.
13. E. T. Bolton and T. J. Byers (unpublished) have successfully used ultraviolet cross-linked pure DNA gels, without supporting material, for complementary strand association studies. The small (less than 1 mg) samples of gel were washed by centrifugation rather than in a column assembly.
14. R. Beukers and W. Berends, *Biochim. Biophys. Acta* **41**, 550 (1960).
15. ———, *ibid.*, **49**, 181 (1961).
16. J. Marmur and L. Grossman, *Proc. Natl. Acad. Sci. U.S.* **47**, 778 (1961).
17. A. Wacker, *Prog. in Nucleic Acid Res.* **1**, 369 (1963) (Academic Press, New York).

30 September 1963

Lactate Dehydrogenase Isozymes: Lability at Low Temperature

Abstract. *A change in the lactate dehydrogenase isozyme pattern can arise when human tissue homogenates are kept at −20°C. This change is most pronounced in a homogenate with a* p*H of about 7.9 in barbital buffer and is not seen when nicotinamide adeninedinucleotide is added to the homogenates before freezing, nor when the homogenates are kept at room temperature, nor when the tissue alone is kept at −20°C.*

Although differences in heat lability of the lactate dehydrogenase (LDH) isozymes (*1*), have been reported, little information is available on the influence of low temperatures on the stability of LDH.

In our laboratory, LDH isozymes of human tissues were studied with the aid of electrophoresis in agar gel on microscope slides (*2*). Tissue homogenates were prepared by grinding the fresh tissues in a Potter-Elvehjem glass homogenizer with about 1 g of tissue to 4 ml of barbital buffer, *p*H 8.4, ionic strength 0.1, and then by centrifuging for 30 minutes at 3000*g*. The actual *p*H of the homogenates thus obtained is about 7.9. Prior to electrophoresis the homogenates were diluted with buffer to a convenient LDH activity. Five active LDH fractions are found in most tissue extracts, in different relative proportions depending on the tissue.

Total loss of activity in the slower moving isozymes LDH-4 and LDH-5 occurred when the concentrated tissue homogenates were kept overnight at −20°C. This loss of activity was not seen when the extracts were kept at room temperature, nor when the tissue itself was kept at −20°C. When the isozyme pattern of a tissue homogenate kept at room temperature (Fig. 1*a*) was compared with that of the same homogenate kept at −20°C (Fig. 1*b*), (i) the activity of LDH-1, the fastest moving anodal fraction, is the same in both samples; (ii) the activity of LDH-2 is the same or probably a little less in the −20°C sample; (iii) the activity