Different Satellite Deoxyribonucleic Acids of Guinea Pig and Ox*

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ABSTRACT: By Ag^+–Cs_2SO_4 preparative centrifugation two new satellite DNAs, called satellite DNAs II and III, are isolated from guinea pig DNA, besides satellite DNA I which was previously isolated. Guinea pig satellite DNAs II and III have the same density of 1.704 g/cm³ in caesium chloride: they each account for approximately 2.5% of the total DNA and appear as distinct bands on the light side of the Ag^+–Cs_2SO_4 gradient. Their complementary strands differ in density one from the other in alkaline caesium chloride, respectively, by 0.031 and 0.022 g per cm³. They also differ in density in neutral caesium chloride. The two satellite DNAs previously isolated from calf thymus can be separated into their complementary strands. The two strands of calf thymus satellite DNA I (d 1.713 g/cm³) differ in density in alkaline caesium chloride by 0.019 g/cm³: those of calf thymus satellite DNA II differ by only 0.005 g/cm³. The kinetics of renaturation of the three guinea pig satellite DNAs and of calf thymus satellite DNA I were studied by determining the optical reassociation curves. On the basis of these results the repeated unit of guinea pig satellite DNA I would appear to be considerably longer than those of the other satellite DNAs.

In recent years studies on the kinetics of renaturation of DNA of eukaryotes and particularly of mammals led to the proposal that they contain repeated nucleotide sequences (Britten and Kohne, 1966, 1968).

In particular, in mouse (Waring and Britten, 1966; Flamm *et al.*, 1969a) and guinea pig (Flamm *et al.*, 1969b), it has been shown that the highly repeated DNA fractions correspond to the nuclear satellite DNAs.

The main properties of satellite DNAs, including the wide difference in the purine and pyrimidine content in the complementary strands, were described (Flamm *et al.*, 1967; Corneo *et al.*, 1968a,b).

However, the kinetics and extent of renaturation of isolated purified satellite DNAs have not been studied extensively and preliminary experiments (Corneo *et al.*, 1968b) indicate that there might be some significant difference among renaturation properties of different satellite DNAs.

As a thorough knowledge of the physicochemical properties of satellite DNAs might be a key to the study of their function, in the present work the guinea pig and calf satellite DNAs were obtained completely pure; then the separation and reassociation of their complementary strands have been studied, and the kinetics of renaturation of such satellite DNAs has been evaluated by determining the optical reassociation curves according to Britten and Kohne (1968).

Materials and Methods

Isolation of Satellite DNAs. DNA of 10–15 million dalton molecular weight was isolated from guinea pig liver and from calf thymus according to Marmur (1961). Guinea pig satellite DNAs were isolated from the main DNA by preparative ultracentrifugation in Ag^+–Cs_2SO_4 density gradients (Jensen and Davidson, 1966; Corneo *et al.*, 1968b).

Calculated quantities of DNA solution in 0.1 M Na_2SO_4, of borate buffer (0.1 M $Na_2B_4O_7$, pH 9.2), and of $AgClO_4$ (1×10^{-3} M) and 0.5 ml of Cs_2SO_4 (Suprapure, Merck, Darmstadt, Germany) saturated solution in 0.1 M Na_2SO_4 per ml of final volume were mixed to obtain a final DNA concentration of 30 μg/ml, a final borate concentration of 0.005 M and an Ag^+:DNA-P molar ratio equal to 0.27. The density of the solution was then adjusted to 1.5 g/cm³ approximately, as determined from the refractive index according to Vinograd and Hearst (1962). The solution obtained was centrifuged in the Spinco L2 preparative ultracentrifuge. Volumes of 25 ml were spun in each tube of a Spinco No. 30 fixed-angle rotor for 96 hr at 30,000 rpm; fractions of 0.2 ml were collected through a hole pierced on the bottom of the tube and their absorbance at 260 mμ was determined after a dilution to a volume of 0.7 ml. The fractions corresponding to each satellite DNA were pooled and dialyzed exhaustively against 5 M NaCl–0.01 M Tris-HCl (pH 7) and then against 0.1 × SSC[1] and concentrated in dialysis tubing by slow evaporation under a gentle stream of air.

Calf thymus satellite DNAs were isolated by CsCl density gradient preparative centrifugation, as previously reported (Polli *et al.*, 1966) or by fractionation of the DNA on a methylated albumin kieselghur column (Mandel and Hershey, 1960).

Separation of the Complementary Strands in Alkaline CsCl Density Gradients. The complementary strands of satellite DNAs were separated by preparative centrifugation in an alkaline CsCl density gradient. The gradient was prepared as follows: 0.3 ml of 1 M NaOH was added to 5 ml of 0.01 M Tris-HCl (pH 8.5) containing 100 μg of DNA and 500 μg of sodium lauryl sulfate. The density of the solution was brought to approximately 1.750 g/cm³ by adding solid CsCl. The final pH was about 12.5 (Flamm *et al.*, 1967).

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[1] SSC = 0.15 M NaCl–0.015 M sodium citrate (pH 7.0).

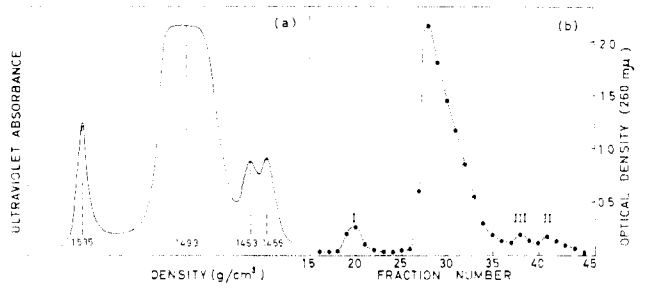

FIGURE 1: Analytical (a) and preparative (b) ultracentrifugation of guinea pig DNA in an Ag^+–Cs_2SO_4 equilibrium density gradient. The preparation of the gradients and the conditions of centrifugation are described in the text.

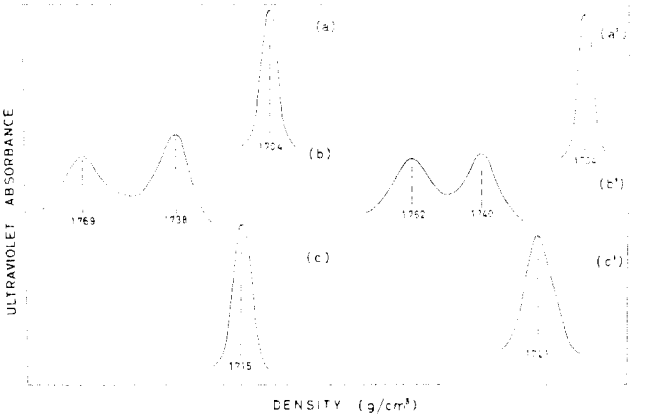

FIGURE 2: Microdensitometer tracings of isolated guinea pig satellite DNA II (a–c) and III (a'–c') centrifuged to the equilibrium in neutral (a and a'), alkaline (b and b'), and neutralized (c and c') CsCl density gradients in the analytical ultracentrifuge.

The solution was then centrifuged in a Polyallomer tube in the Spinco Model L2 preparative ultracentrifuge under a layer of mineral oil in the No. 40 fixed-angle rotor at 38,000 rpm for 48 hr. At the end of the centrifugation, 0.15–0.3-ml fractions were collected through a hole pierced on the bottom of the tube, and the absorbance of each fraction at 260 mμ was read in a Beckman DU spectrophotometer after a dilution to a volume of 0.7 ml.

Analytical CsCl Density Gradient Centrifugation. This was performed in a Spinco Model E ultracentrifuge, as previously reported (Schildkraut *et al.*, 1962). In neutral gradients 2–3 μg of DNA was centrifuged. The alkaline gradients were prepared in a similar manner as described above for preparative ultracentrifugation, except that no sodium lauryl sulfate was added to the solution, and contained 5–6 μg of DNA. Alkaline CsCl gradients prepared by adding 0.05 volume of 1 M NaOH were neutralized by adding 0.5 volume of 1 M KH_2PO_4. Ultraviolet absorption photographs, taken at equilibrium, were traced with a microdensitometer. The DNA densities were calculated from the density of the marker 2C phage DNA (*d* 1.742 g/cm³) according to Schildkraut *et al.* (1962) and were referred to the density of *Escherichia coli* DNA, taken to be 1.710 g/cm³ in neutral CsCl gradients. In alkaline gradients the buoyant densities were calculated from the initial density of the solution and the limiting isoconcentration distance according to Vinograd and Hearst (1962) as described by Nandi *et al.* (1965).

Determination of the "Cot" Curves. The kinetics of renaturation of satellite DNAs was evaluated by determining the cot curves according to Britten and Kohne (1968). In different experiments the size of DNA to be used was reduced to approximately 5000 nucleotides by passage of the satellite DNA solution in a 20-ml syringe through a No. 27 gauge syringe needle with maximum pressure applied by hand (Wetmur and Davidson, 1968), or to approximately 500 nucleotides by sonication (Walker and McLaren, 1965) for 8 min in a Kerby's sonicator. The molecular weight was then checked by determining the sedimentation velocity according to Eigner and Doty (1965).

The DNA in solution at a known DNA concentration and salt molarity was denatured by heating at 100° for 10 min in stoppered quartz cuvets adapted with a thermometer. Then the temperature was lowered rapidly in an ice bath, and the absorbancy of the DNA solution at 260 mμ was read at different time intervals in a Beckman spectrophotometer equipped with thermal spacers for circulating hot liquid, previously adapted at 60°.

The ratio of the optical density at 60° to the initial value measured at 98° was plotted against the Cot, that is the product of DNA molar concentration and time of incubation expressed in seconds.

Low concentrations of DNA (about 10 μg/ml) and low salt molarities (0.04 or 0.072 M NaCl) were used in the case of rapidly reassociating satellite DNAs. The Cot curves were normalized to give the rate that would be observed at 0.18 M sodium ion concentration according to the plot reported by Britten (1969).

Absorbance–temperature profiles of the native, denatured, and reassociated DNA solutions were performed as described (Marmur and Doty, 1962).

Results

Figure 1a shows the pattern of guinea pig DNA centrifuged to the equilibrium in Ag^+–Cs_2SO_4 in the analytical ultracentrifuge. On the light side of the main DNA band two small bands are evident; on the heavy side there is the guinea pig satellite DNA I, the isolation and characterization of which was described in a previous paper (Corneo *et al.*, 1968b).

By collecting small volume fractions from a preparative Ag^+–Cs_2SO_4 gradient (Figure 1b) it is possible to isolate the two satellite DNAs banding on the light side. That of the two DNAs which is lighter in the Ag^+–Cs_2SO_4 gradient will be called satellite DNA II and the other will be called satellite DNA III. This definition is given for simplicity, and because these two DNA components appear as satellite bands in the Ag^+–Cs_2SO_4 gradient, although they do not appear clearly as distinct satellites in the neutral CsCl gradient.

When the whole guinea pig DNA, except satellite DNA I previously separated by Ag^+–Cs_2SO_4 preparative centrifugation, is centrifuged in an analytical CsCl gradient after extensive dialysis, a satellite DNA band is not clearly visible because the relative amount of the satellite DNAs II and III is not enough to be seen as a distinct band within the limits of the main DNA band.

As judged from the area of the different peaks in the

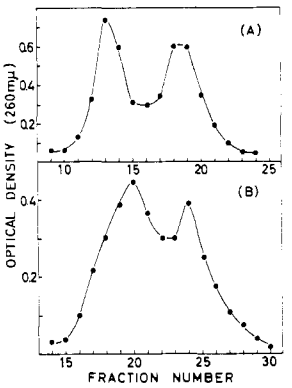

FIGURE 3: Fractionation of the isolated guinea pig satellite DNA II (A) and guinea pig satellite DNA III (B) in a preparative alkaline CsCl density gradient. The experimental conditions are described in the text.

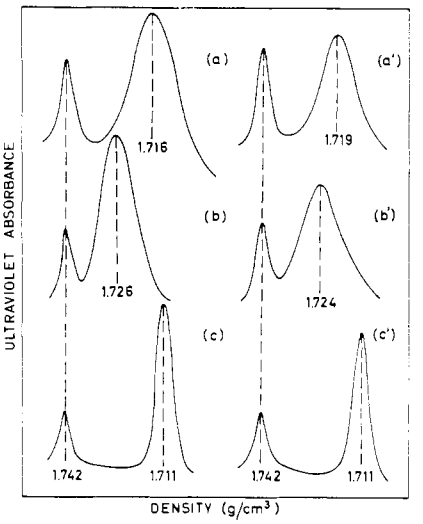

FIGURE 4: Microdensitometer tracings of guinea pig satellite DNA II (a–c) and III (a′–c′): isolated light strand (a and a′), isolated heavy strand (b and b′), and an equimolar mixture of the two strands submitted to renaturation (c and c′), centrifuged to the equilibrium in a neutral CsCl density gradient in the analytical ultracentrifuge. The peak on the left in each tracing corresponds to the density marker, 2C DNA (*d* 1.742 g/cm³).

analytical Ag^+–Cs_2SO_4 centrifugation (Figure 1a), guinea pig satellite DNAs II and III each account for approximately 2.5% of the total, while guinea pig satellite DNA I comprehends about 5.5% of the total DNA.

When the whole guinea pig DNA is centrifuged in an alkaline CsCl gradient in the analytical ultracentrifuge, the complementary strands of satellite DNA I separate widely because of the quite asymmetric distribution of purines and pyrimidines (Corneo *et al.*, 1968b). Also satellite DNAs II and III separate into their complementary strands in alkaline caesium chloride, as shown below in Figure 2. However, the resolution of these bands is poor in the microdensitometer tracing of the whole guinea pig DNA in alkaline CsCl, owing to the broadness of the main DNA band: their presence can only be guessed by observing the exposed film.

Figure 2 shows the banding patterns of isolated guinea pig satellite DNAs II and III in CsCl. Satellite DNA II has a density of 1.704 g/cm³ in neutral CsCl (Figure 2a), and separates into the complementary strands which display densities of 1.738 and 1.769 g per cm³, respectively, in alkaline CsCl (Figure 2b). When it is brought back to neutrality, guinea pig satellite DNA II displays a density of 1.715 g/cm³ (Figure 2c).

Guinea pig satellite DNA III has a density of 1.704 g/cm³ in neutral CsCl (Figure 2a′) and separates into the complementary strands having densities of 1.740 and 1.762 g per cm³ respectively in alkaline CsCl (Figure 2b′). When it is brought back to neutrality, guinea pig satellite DNA III displays a density of 1.721 g/cm³ (Figure 2c′).

The complementary strands of these satellite DNAs were separated in preparative alkaline CsCl (Figure 3). The isolated single strands of guinea pig satellite DNA II display densities of 1.716 and 1.726 g per cm³, respectively (Figure 4a and b), and those of guinea pig satellite DNA III densities of 1.719 and 1.724 g per cm³ in neutral CsCl (Figure 4a′ and b′). When equimolar mixtures of the two complementary strands of each satellite DNA are heated at 65° in 2 × SSC for 5 hr, single bands with densities intermediate between those of denatured and native materials are obtained in CsCl (Figure 4c and c′). If each single strand alone is submitted to the same conditions favoring renaturation, it remains at the original density.

In Figure 5 are shown the patterns of isolated calf thymus satellite DNAs I and II in neutral (Figure 5a and a′), alkaline (Figure 5b and b′), and neutralized (Figure 5c and c′) CsCl.

Calf thymus satellite DNA I has a native density of 1.713 g/cm³. In alkaline CsCl the complementary strands separate and have densities of 1.750 and 1.769 g per cm³, respectively. After neutralization a single band with a density of 1.728 g/cm³ is obtained in CsCl.

Calf thymus satellite DNA II has a native density of 1.721 g/cm³: the preparation shown in Figure 5a′ is slightly contamined by a small amount of DNA which corresponds in density to satellite DNA I. Also calf thymus satellite DNA II separates in alkaline CsCl into two bands with densities of 1.768 and 1.773 g per cm³, respectively. The lightest peak, visible in Figure 5b′, is presumably due to contamination

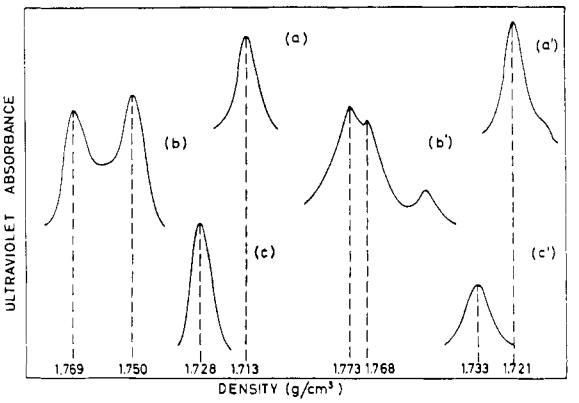

FIGURE 5: Microdensitometer tracings of isolated calf thymus satellite DNA I (a–c) and II (a′–c′), centrifuged to the equilibrium in a neutral (a and a′), alkaline (b and b′), and neutralized (c and c′) CsCl density gradient in the analytical ultracentrifuge.

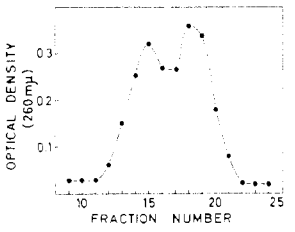

FIGURE 6: Fractionation of the isolated calf thymus satellite DNA I in a preparative alkaline CsCl density gradient. The experimental conditions are described in the text.

from DNA of lower density as shown in Figure 5a′. After neutralization a single band is obtained with a density of 1.733 g/cm³ (Figure 5c′).

The complementary strands of calf thymus satellite DNA I were separated in preparative alkaline CsCl (Figure 6) and were then recentrifuged separately in neutral CsCl in the analytical ultracentrifuge: the light strand displays a density of 1.722 g/cm³ and the heavy one a density of 1.730 g/cm³ (Figure 7a and b). If an equimolar mixture of the two strands is heated for 5 hr at 65° in 2 × SSC, a single band is obtained with a density of 1.716 g/cm³ (Figure 7c), which corresponds to the density previously found for the renatured satellite DNA (Polli *et al.*, 1966).

Data on the buoyant densities of guinea pig and calf satellite DNAs and their complementary strands are reported in Table I.

The optical reassociation curves of the satellite DNAs here studied are reported in Figure 8. The curves of mouse satellite DNA and of T4 phage DNA were also determined and are reported as reference. The curves in the experiments of Figure 8 were determined using DNA of molecular size of 5000 nucleotides. Mouse satellite DNA and T4 phage DNA yield curves which do not differ significantly from those reported by Britten and Kohne (1968) whose experiments were carried out on DNA of a size of 500 nucleotides.

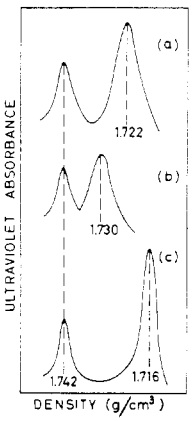

FIGURE 7: Microdensitometer tracings of calf thymus satellite DNA I: isolated light strand (a), isolated heavy strand (b), and an equimolar mixture of the two strands submitted to renaturation as described in the text (c), centrifuged to the equilibrium in a neutral CsCl density gradient in the analytical ultracentrifuge. The peak on the left in each tracing corresponds to the density marker, 2C DNA (*d* 1.742 g/cm³).

TABLE I: Buoyant Densities of Guinea Pig and Calf Satellite DNAs and of Their Complementary Strands.

	Guinea Pig Liver			Calf Thymus	
	I	II	III	I	II
Neutral CsCl	1.706	1.704	1.704	1.713	1.721
Alkaline CsCl	1.692	1.738	1.740	1.750	1.773
	1.778	1.769	1.762	1.769	1.768
Difference in densities between complementary strands in alkaline CsCl	0.086	0.031	0.022	0.019	0.005
Light strand in neutral CsCl	1.708	1.716	1.719	1.722	
Heavy strand in neutral CsCl	1.757	1.726	1.724	1.730	
T_m	86°	87°5′	88°	93°[a]	
Relative amount (%)	5.5	2.5	2.5	10[a]	2[a]

[a] Polli *et al.* (1966).

The cot value of guinea pig satellite DNA II is about a third of that of mouse satellite DNA derived from the curve reported by Britten and Kohne (1968) and that reported in the present paper (Figure 8). Guinea pig satellite DNA III and calf satellite DNA I have cot values which are a little higher (two or three times) than that of mouse satellite DNA. On the contrary the cot value of guinea pig satellite DNA I is about 100 times that of mouse satellite DNA.

The slope of the central two-thirds of the curves here reported has been evaluated according to Britten and Kohne (1968), and appears to be different for different satellite DNAs. Guinea pig satellite DNA II appears to be very homogeneous

TABLE II: Buoyant Densities of Denatured and Reassociated Guinea Pig, Calf, and Mouse Satellite DNAs.

	Guinea Pig Liver			Calf Thymus	
	I	II	III	I	Mouse[a]
Native	1.706	1.704	1.704	1.713	1.691
Heat denatured in 1 × SSC	1.726	1.713	1.715	1.728	1.698
Heat denatured in 0.1 × SSC	1.726	1.719	1.720		1.707
Renatured nucleotide pair					
50,000	1.723	1.711	1.711	1.718	1.697
5,000	1.718	1.707	1.710	1.716	1.694

[a] Corneo *et al.* (1968b).

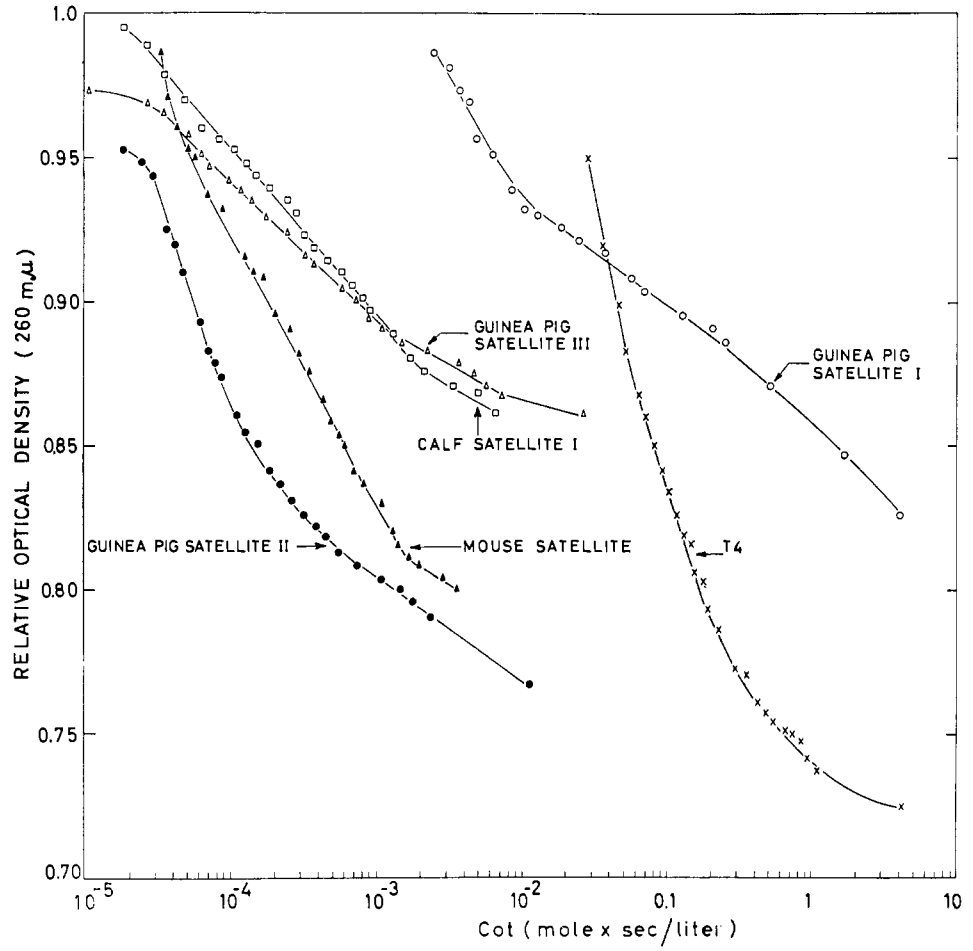

FIGURE 8: Optical reassociation curves of guinea pig satellite DNAs and of calf thymus satellite DNA I. Mouse satellite DNA and T4 phage DNA curves are also reported as references. The left scale gives the ratio of the optical density at 60° to the initial value measured at 98°. All the satellite DNAs, except guinea pig satellite DNA I, were reassociated at a salt concentration of 0.04 M NaCl. Guinea pig satellite DNA I and T4 phage DNA were reassociated at a salt concentration of 0.18 M NaCl. All the curves were normalized to give the rate that would be observed at 0.18 M sodium ion concentration, according to the curve reported by Britten (1969).

with regard to this property, the slope of its curve being rather steep as in the case of mouse satellite DNA. Guinea pig satellite DNA III and calf satellite DNA I have less steep curves. Guinea pig satellite DNA I has an even less steep curve, which also regarding this property is quite different from that of mouse satellite DNA.

CsCl equilibrium centrifugation measurements of denatured and reassociated satellite DNAs are reported in Table II. Guinea pig satellite DNA II increases in density to 1.713 g/cm³ when heat denatured in 1 × SSC and fast cooled, and to 1.719 g/cm³ when heat-denatured in 0.1 × SSC and fast cooled. When it is denatured and then heated at 65° for 5 hr in 2 × SSC, its density decreases to 1.711 g/cm³. A further decrease in density to 1.707 g/cm³ is obtained if the molecular weight of the sample is reduced to 5000 nucleotides and the renaturation is carried out for prolonged time (48 hr). Also guinea pig satellite DNA III, when heated in 1 × SSC and fast cooled, increases in density to 1.715 g/cm³, and a greater increase in density, to 1.720 g/cm³, is obtained by heat denaturation in 0.1 × SSC. Upon renaturation for prolonged time in the conditions described for the determination of the cot curves, it displays a smaller decrease of DNA density than that obtained with guinea pig satellite DNA II. Similar results were obtained in the case of calf satellite DNA I.

Guinea pig satellite DNA I shows densities of the reassociated material which are still rather higher than the native density also after heating at 60° for 48 hr. This is in agreement with the optical reassociation curve obtained for this satellite DNA. Because of its reassociation features, which differ from those of mouse satellite DNA, guinea pig satellite DNA I was further investigated. Its size was reduced to 500 nucleotides by sonication and an optical reassociation curve was then carried out. The curve obtained did not differ significantly from that reported in Figure 8, determined on a sample with a size of 5000 nucleotides, and the density of the reassociated material was only slightly decreased by the reduction in molecular weight.

In Figure 9 the ultraviolet absorbance–temperature curves of guinea pig satellite DNAs II and III are reported. Also, the same curves of guinea pig satellite DNA I as previously published (Corneo *et al.*, 1968b) are reported for comparison. For each satellite DNA the absorbance–temperature curves were made on native, heat-denatured, and fast-cooled, and reassociated DNA. The melting temperatures were 87°5′ for native

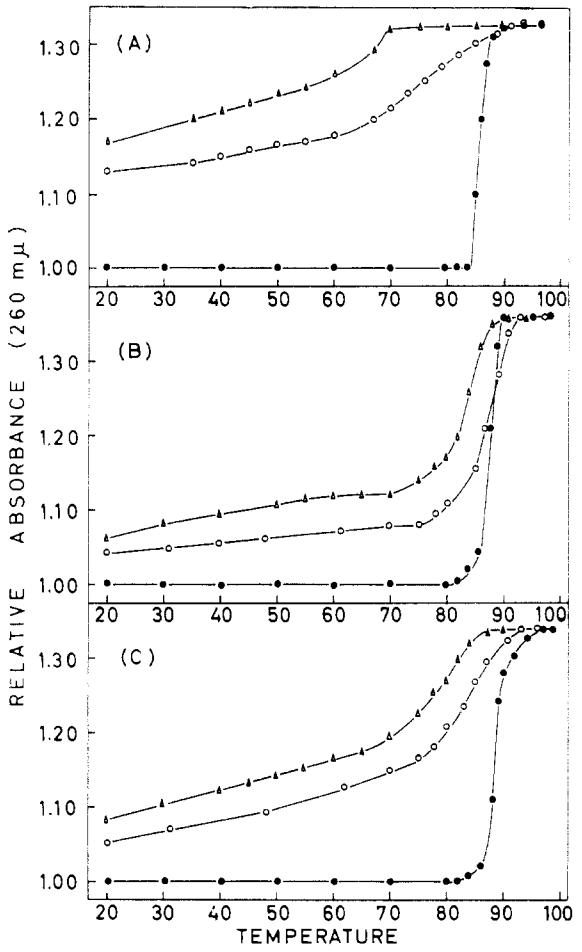

FIGURE 9: Absorbance-temperature curves of guinea pig satellite DNA I (A), II (B), and III (C), in 1 × SSC: native (·–·–·–), previously heat denatured and fast cooled (–▲–▲–) and previously heat denatured and renatured as described in the text (○-○-○). Experimental conditions as reported by Marmur and Doty (1962).

guinea pig satellite DNA II and 88° for native guinea pig satellite DNA III. Both these values differ slightly from the T_m of guinea pig satellite DNA I, which was previously found to be 86° (Corneo *et al.*, 1968b). In the case of guinea pig satellite DNAs II and III the base compositions derived from the melting points according to Marmur and Doty (1962) correspond approximately to the values calculated from the CsCl buoyant densities according to Schildkraut *et al.* (1962). A discrepancy between melting point and CsCl density was found in the case of guinea pig satellite DNA I (Corneo *et al.*, 1968b). The hyperchromicities of the melting curves of guinea pig satellite DNAs II and III, previously denatured and then reassociated (Figure 9), indicate that a sequence matching had occurred for a considerable extent after heat denaturation, particularly when this was followed by heating at 60° for prolonged time. A greater sequence matching is obtained with guinea pig satellite DNA II.

Discussion

The three guinea pig satellite DNAs isolated in the present work have approximately the same densities in neutral CsCl, but bind different amounts of Ag^+, at least in the conditions here used: therefore, they display different densities in an Ag^+–Cs_2SO_4 gradient and can be separated from the main DNA. Studies carried out on the nature of the interaction between Ag^+ and DNA (Jensen and Davidson, 1966) would not seem to exclude the hypothesis that different interactions between Ag^+ and DNAs with the same overall base composition might be due to differences in nucleotide sequences. Other unpredictable behaviors of satellite DNAs, as the discrepancies found between chemically determined base compositions, buoyant densities in CsCl and melting points of mouse and guinea pig satellite DNA I (Corneo *et al.*, 1968b) might also be ascribed to a specific base sequence.

Guinea pig satellite DNAs II and III, now isolated, can be separated into their complementary strands in alkaline CsCl. The difference in density between the two strands in alkaline CsCl is 0.031 g/cm^3 for guinea pig satellite DNA II, and 0.022 g/cm^3 for guinea pig satellite DNA III. The strands of these satellite DNAs also differ in densities in neutral CsCl. These results indicate a difference between these satellite DNAs in the distribution of purines and pyrimidines in the two strands. Guinea pig satellite DNAs II and III differ also having different cot values and different density decreases and hyperchromicities of the reassociated materials.

Also calf satellite DNAs have complementary strands which separate in alkaline CsCl; the difference in density between the strands is 0.019 g/cm^3 in the case of calf satellite DNA I and appears to be only 0.005 g/cm^3 in the case of calf satellite DNA II.

The cot curves here reported indicate that, according to the correlation established by Britten and Kohne (1968), the length of the repeated unit should vary from 100 nucleotides for guinea pig satellite DNA II to a few 100 nucleotides for guinea pig satellite DNA III and calf satellite DNA I. These values are in the same order of length as the repeated unit of mouse satellite DNA.

The only exception is guinea pig satellite DNA I, which renatures more slowly than the other satellite DNAs. It is evident that guinea pig satellite DNA I has not such a high repetition frequency as mouse satellite DNA. It has some of the properties of the very highly repetitive fractions: in fact it is very homogeneous, forms a very sharp band in the analytical ultracentrifuge, has a very steep thermal transition and has a wide difference of base composition among the complementary strands. However one would calculate from the cot value that its repeated unit is about 100 times longer than that of mouse satellite DNA.

To explain this result the following hypothesis could be proposed. This satellite could be made up of repeated units shorter than predicted from the cot value, but having a very high degree of divergence between them.

Alternatively this satellite might contain clusters of very short repeated sequences interspersed among nonrepeated sequences. The very short repeated sequences might cause the rapid reassociation of the complementary strands with base pairing only in the cluster regions, and the noncomplete sequence matching might be due to the nonrepeated sequences. The wide asymmetry in the distribution of the bases among the complementary strands and particularly the high content of G in one of the strands and of C in the complementary one (Corneo *et al.*, 1968b) could favor the hypothesis of the presence of clusters of very simple polymers.

Also, the wide difference in the slope of the curves of mouse satellite DNA and guinea pig satellite DNA I indicates that the latter might contain sequences of DNA with rather different rates of reassociation. The presence of sequences with different rates of reassociation in satellite DNAs could be in agreement with the fact that in general a complete sequence matching of the complementary strands of satellite DNAs is not obtained upon reassociation after denaturation.

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References

Britten, R. J. (1969), *Yearbook Carnegie Instn. 68*, 332.
Britten, R. J., and Kohne, D. E. (1966), *Yearbook Carnegie Instn. 65*, 78.
Britten, R. J., and Kohne, D. E. (1968), *Science 161*, 529.
Corneo, G., Ginelli, E., and Polli, E. (1968a), *J. Mol. Biol. 33*, 331.
Corneo, G., Ginelli, E., Soave, C., and Bernardi, G. (1968b), *Biochemistry 7*, 4373.
Eigner, J., and Doty, P. (1965), *J. Mol. Biol. 12*, 549.
Flamm, W. G., McCallum, M., and Walker, P. M. B. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 1729.
Flamm, W. G., Walker, P. M. B., and McCallum, M. (1969a), *J. Mol. Biol. 40*, 423.
Flamm, W. G., Walker, P. M. B., and McCallum, M. (1969b), *J. Mol. Biol. 42*, 441.
Jensen, R. H., and Davidson, N. (1966), *Biopolymers 4*, 17.
Mandel, J. D., and Hershey, A. D. (1960), *Anal. Biochem. 1*, 66.
Marmur, J. (1961), *J. Mol. Biol. 3*, 208.
Marmur, J., and Doty, P. (1962), *J. Mol. Biol. 5*, 109.
Nandi, U. S., Wang, J. C., and Davidson, N. (1965), *Biochemistry 4*, 1687.
Polli, E., Ginelli, E., Bianchi, P., and Corneo, G. (1966), *J. Mol. Biol. 17*, 309.
Schildkraut, C. L., Marmur, J., and Doty, P. (1962), *J. Mol. Biol. 4*, 430.
Vinograd, J., and Hearst, J. E. (1962), *Progr. Chem. Org. Natl. Prod. 20*, 395.
Walker, P. M. B., and McLaren, A. (1965), *J. Mol. Biol. 12*, 394.
Waring, M., and Britten, R. J. (1966), *Science 154*, 794.
Wetmur, J. G., and Davidson, N. (1968), *J. Mol. Biol. 31*, 349.

Interactions of Hormonal Steroids with Nucleic Acids. III. Role of Polymer Structure*

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ABSTRACT: The role of polymer structure in the guanine-specific binding of progesterone, testosterone, and estradiol under equilibrium conditions has been examined by the use of particular nucleic acids. Possible steroid associations with chain-terminal guanine residues have been excluded by the following observations: binding occurs with circular, single-stranded *M13* and *φX174* DNAs; binding occurs with denatured T_7 DNA lacking terminal guanine residues; the numbers of binding sites on sheared and unsheared *Pseudomonas aeruginosa* DNA having a 50-fold difference in average chain length are not greatly different; the fluorescence quantum yield and fluorescence emission spectrum of poly-7-methylguanylic acid are unchanged in the presence of estradiol.

These data together support the model of steroid association internally along polynucleotide chains.

A number of hormonal steroids bind to ribo- and deoxyribopolynucleotides under equilibrium conditions in aqueous buffer (Cohen and Kidson, 1969a). There is a specific requirement for guanine residues, except in the case of estradiol, which will also bind to inosine (Cohen and Kidson, 1969b). The observation that two functional groups of each steroid are necessary for binding to guanine residues in polyguanylic acid, the fact that these functional groups can act as proton donors or acceptors and the finding that slightly protic solvents have minimal effects on the binding of the steroids to polynucleotides suggest that two hydrogen bonds are formed in each case between functional groups of steroid and purine (Cohen *et al.*, 1969), although hydrophobic forces are undoubtedly involved in these associations.

Several attributes of polynucleotide structure essential to the binding of steroids have already been ascertained.

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