Role of the Double-Stranded Nucleic Acid Backbone Configuration in Adsorption Interactions†

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ABSTRACT: The chromatography of double-stranded nucleic acids on hydroxylapatite was investigated. It was found that double-stranded RNA and DNA–RNA hybrid both have less affinity for hydroxylapatite than double-stranded DNA. It was also found that there is a linear dependence on base composition of the affinity of double-stranded DNA for hydroxylapatite. These differences in affinity can be attributed to variations in the backbone configurations of the various nucleic acids; the phosphates of double-stranded DNA protrude from the helix whereas those of double-stranded RNA and DNA–RNA hybrid are relatively "buried." Among the possible mechanisms specifically excluded are direct interaction of the nucleic acid bases with hydroxylapatite and differences in affinity due to variations in the charge density of the nucleic acid helices. The mechanism of adsorption of DNA to methylated albumin kieselguhr is also considered in light of the proposed nucleic acid–hydroxylapatite adsorption mechanism. It is concluded that in the case of methylated albumin kieselguhr the bases of double-stranded DNA do interact significantly with the adsorbent.

In view of the fact that the bases of DNA are not much exposed to the environment it is interesting to find that several adsorbents for column chromatography can be used to distinguish between and fractionate various types of double-stranded DNA (ds-DNA)[1] (Kothari, 1970). Of particular interest is the fractionation on hydroxylapatite (Corneo *et al.*, 1970b; Oishi, 1971; Bernardi *et al.*, 1972; Piperno *et al.*, 1972) since only the phosphates of the DNA backbone are thought to interact with the hydroxylapatite crystal surface. This suggested that a study of the hydroxylapatite system might give insight to the way in which backbone configuration contributes to recognition of ds-nucleic acids.

Before being used to fractionate various ds-DNAs, hydroxylapatite came into widespread use for the separation of ss- from ds-nucleic acids when the technique was popularized by Bernardi (1965) and Miyazawa and Thomas (1965). The reduction in elution molarity which occurs when rigid double-stranded molecules are denatured is a special case in the generally observed tendency of more flexible molecules to have lower elution molarities than more rigid ones (Bernardi, 1971; Martinson, 1973c). It has been proposed that the unfavorable configurational entropy change imposed on flexible molecules as a result of adsorption accounts for their decreased affinity for hydroxylapatite (Martinson, 1973c). However in the case of fractionation of double-stranded molecules no clear pattern has emerged. In most of the cases which have been reported, the fractionation of ds-DNA species on hydroxylapatite has been associated with some unusual property such as glucosylation (Oishi, 1971) or repetitive base sequence (Corneo *et al.*, 1970b; Bernardi *et al.*, 1972; Piperno *et al.*, 1972). It is highly unlikely that configurational entropy could be a factor in these cases since all double-stranded nucleic acids must be quite similar in this respect. It was therefore decided to study the relationship between the affinities of various double-stranded nucleic acids for hydroxylapatite and their detailed secondary structures as determined by others through X-ray diffraction. The results indicate that the affinity of a rigid, helical nucleic acid for hydroxylapatite is governed by the steric availability of its backbone phosphates for adsorption interactions.

The implications of the nucleic acid–hydroxylapatite adsorption mechanism to other systems is discussed. It is suggested that, in contrast to the hydroxylapatite system, the binding of ds-DNA to methylated albumin kieselguhr (MAK) involves adsorption interactions of the DNA bases as well as the DNA phosphates.

Materials and Methods

Hydroxylapatite Chromatography. The hydroxylapatite used was either "HTP" or "DNA grade HTP," both produced by Bio-Rad, Richmond, Calif. Unless otherwise indicated the phosphate buffer used was an equimolar mixture of K_2HPO_4 and KH_2PO_4. The phosphate molarity gradients used were either of small steps, carried out and assayed as previously described (Martinson, 1973b), or continuous, as follows. Preheated "HTP" (0.5–2.0 M phosphate, 100°, 15 min) was loaded in a column and the sample was applied (Martinson, 1973a,b). The column was rinsed with the gradient starting buffer and then the gradient was fed into the column with a Milton Roy Minipump. For elutions conducted above room temperature the buffers and the hydroxylapatite were degassed by heating at 100° and the gradients were generated on a hot plate equipped with a magnetic stirring motor. Occasional fractions were collected under light mineral oil and these were used for phosphate concentration determination by means of refractive index measurements. The slope of the gradient is defined (Kawasaki and Bernardi, 1970) as $(dM/dV)D^2$ where M is the molarity of the phosphate buffer,

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[1] Abbreviations used are: M_E, the buffer molarity by which 50% of a nucleic acid has eluted during phosphate gradient chromatography; T_{mi}, the temperature by which 50% "irreversible" (Crothers *et al.*, 1965) strand separation has occurred; CPV, cytoplasmic polyhedrosis virus; MAK, methylated albumin adsorbed on kieselguhr; ds, double stranded; ss, single stranded.

V its volume in liters, and D the diameter of the hydroxylapatite bed in millimeters. Load ratios are expressed as micrograms of nucleic acid per gram of hydroxylapatite.

Thermal gradient chromatography (Miyazawa and Thomas, 1965) was done using 5-mm diameter columns of 70 mg of HTP loaded with the DNA sample. Each stepwise increase in the temperature of the gradient was followed by 5 min of temperature equilibration and then elution with 0.5 ml of preheated 0.03 M potassium phosphate (see Martinson, 1973b).

Preparative Centrifugation. Cesium sulfate density gradient isopycnic centrifugation was done in fixed-angle rotors (Flamm *et al.*, 1966). Fractions were collected by puncturing the bottom of the tube with a 20-gauge hypodermic needle and manually collecting a fixed number of drops per fraction. The A_{260} values of the fractions were determined in a Cary 14 spectrophotometer and aliquots were withdrawn from each fraction for radioactivity measurements by scintillation counting.

DNA. Bacillus subtilis [^{14}C]DNA (20,000 cpm/μg) was a gift of Dr. R. Burger. *B. subtilis* tritium-labeled radiolysed DNA (Rosenthal and Fox, 1970) (19,000 cpm/μg) was a gift of Dr. K. Drlica. Both of these preparations were about 1.5 years old when used and had been stored frozen. Freezing is known to favor the radiolysis of tritiated compounds (Evans and Stanford, 1963). Fresh *B. subtilis* [^{3}H]DNA (17,000 cpm/μg) was made shortly before use as previously described (Martinson, 1973a) and stored at 4° in 20% ethanol. This DNA was chromatographically indistinguishable from unlabeled DNA. Unlabeled *B. subtilis* DNA was a gift of Dr. K. Drlica. A portion, designated hydroxylapatite DNA, was sonicated (Martinson, 1973a), adsorbed to DNA grade HTP, washed with 0.08 M phosphate at 60° to remove any single strands and small fragments resulting from sonication and then eluted and stored at −20° until used. *Pseudomonas lemoignei* DNA was a gift of Dr. M. Doudoroff. Other bacterial DNAs were isolated from bacteria grown in nutrient broth as previously described (Martinson, 1973a).

Reannealed *Escherichia coli* DNA [(ann)DNA] was prepared from denatured, sonicated DNA by standard procedures (Britten and Kohne, 1968), was adsorbed to DNA grade HTP, washed with 0.13 M phosphate at 50° to remove single strands and then eluted and stored at −20°. *E. coli* hydroxylapatite DNA was prepared from DNA kindly supplied by Dr. K. Drlica. It was sonicated, adsorbed to DNA grade HTP, washed with 0.14 M sodium phosphate at 60°, eluted, and stored at −20°.

ds-RNA. Cytoplasmic polyhedrosis virus (CPV) ds-RNA was a gift of Dr. L. Lewandowski. A portion, designated hydroxylapatite RNA, was sonicated, adsorbed to DNA grade HTP, washed with several bed volumes of 0.1 M phosphate at 60° to remove any ss fragments, and then eluted and stored at −20° until use.

ds-[^{32}P]RNA (3000 cpm/μg) complementary to bacteriophage ϕX174 DNA was kindly supplied by Dr. M. Chamberlin. The ϕX174 DNA had been transcribed with RNA polymerase as described by Chamberlin and Berg (1964) using [^{32}P]ATP as the labeled nucleotide. ds-RNA is one of the products of such a reaction (Tabak and Borst, 1971). The reaction mixture was extracted with water-saturated Tris–phenol (pH 6.3), dialyzed into 0.1 M NaCl–0.01 M Tris (pH 7.4), adsorbed to DNA grade HTP, washed with several bed volumes of 0.08 M phosphate at 75°, eluted with 0.25 M phosphate, diluted with 0.1 M NaCl, sonicated (Martinson, 1973a), resorbed to hydroxylapatite, washed with 0.1 M phosphate at 70°, and eluted and stored at −20° until used. The buoyant density of this RNA in Cs_2SO_4 (5-ml gradients were centrifuged in a Spinco 40.3 rotor at 40,000 rpm for 3 days at 20°) was found to be identical with that of CPV ds-RNA when the two were run together and is about 1.605 g/cm^3. The base composition of both RNAs is 43% G + C (Sinsheimer, 1959; Miura *et al.*, 1968).

RNA-DNA Hybrid. E. coli [^{3}H]RNA (with which to make hybrid) was prepared from bacterial strain DG 156 derivative B/r/1 (Hayes Dougan) growing in 200 ml of a minimal medium. When the A_{450} of the culture reached 1.2, 0.25 mCi each of [5-^{3}H]uridine and -adenine (26 Ci/mmole, Schwarz) was added. After 2 min the culture was chilled, and the bacteria were collected by centrifugation. The pellet was resuspended in 20 ml of 0.1 M KCl–0.01 M Tris (pH 7.4). Two milliliters of 25% sodium dodecyl sulfate was added and the mixture was frozen and thawed three times and then warmed to 40° to induce lysis. The mixture was then shaken with 20 ml of water-saturated sodium–phenol (pH 6.6). The aqueous phase was withdrawn and shaken with additional phenol after which it was again withdrawn and shaken with chloroform–octanol (10:1). The aqueous phase was then combined with two volumes of ethanol and put at −70° for 1 hr. The resulting nucleic acid precipitate was collected, dissolved in 5 ml of 10 mM $MgCl_2$–10 mM Tris-chloride (pH 7.4), and then incubated 2 min at 37° with 4 μg/ml of deoxyribonuclease 1 (electrophoretically purified, Worthington). The solution was then extracted twice with phenol as before, after which acid washed Norit was added to 0.1%, mixed in, and then removed by centrifugation. The RNA was precipitated with two volumes of ethanol (−20°, overnight), collected, washed in 95% ethanol, and redissolved in 2 ml of 10 mM NaCl–10 mM Tris-chloride (pH 7.4). One milliliter of ethanol was added to prevent freezing, and the RNA was stored at −20° at a concentration of 1.3 mg/ml. The specific activity was 10,000 cpm/μg.

To prepare the hybrid, 260 μg of the [^{3}H]RNA and 300 μg of *E. coli* DNA were combined in 2.6 ml of 40 mM Tris (pH 7.4) and heated in a boiling-water bath for 3 min. Two-tenths milliliter of 4 M KCl was added, and then the mixture was transferred to a 67° bath and incubated for 3 hr. Three milliliters of 0.3 M KCl and 4.23 g of Cs_2SO_4 were added giving a solution density of 1.528 g/cm^3. The solution was centrifuged at 40,000 rpm in a Spinco 40 rotor for 24 hr. Thirteen fractions were collected. Fractions 1–6 contained precipitated RNA while fractions 10–13 were noted to be viscous owing to the presence of the DNA. Fractions 8–11 were pooled and then sonicated at 0° in order to break up concatenated structures and release hybrid. The mixture was once again subjected to isopycnic centrifugation (9 ml at an initial density of 1.496 were centrifuged 4.5 days at 30,000 rpm in a Spinco 40 rotor). Twenty-six fractions were collected. The DNA peak (centered at fraction 23 and a density of 1.448 g/cm^3) contained within its leading edge a small peak of radioactivity which was collected by pooling fractions 11–18 (density 1.528–1.477 g/cm^3). The density of well formed *E. coli* RNA–DNA hybrid (50% G + C) is probably just slightly greater than the 1.506 g/cm^3 found by Chamberlin and Berg (1964) for ϕX174 (43% G + C).

The pooled Cs_2SO_4 hybrid fractions were diluted, loaded on to 75 mg of DNA grade HTP, and chromatographed at 40° with 0.5-ml steps and 20 mM increments of phosphate. Absorbance peaks, presumably corresponding to ss-DNA and DNA–RNA hybrid, were centered at 0.08 and 0.16 M phosphate and a single radioactivity peak, offset slightly from the second absorbance peak, was centered at 0.146 M phos-

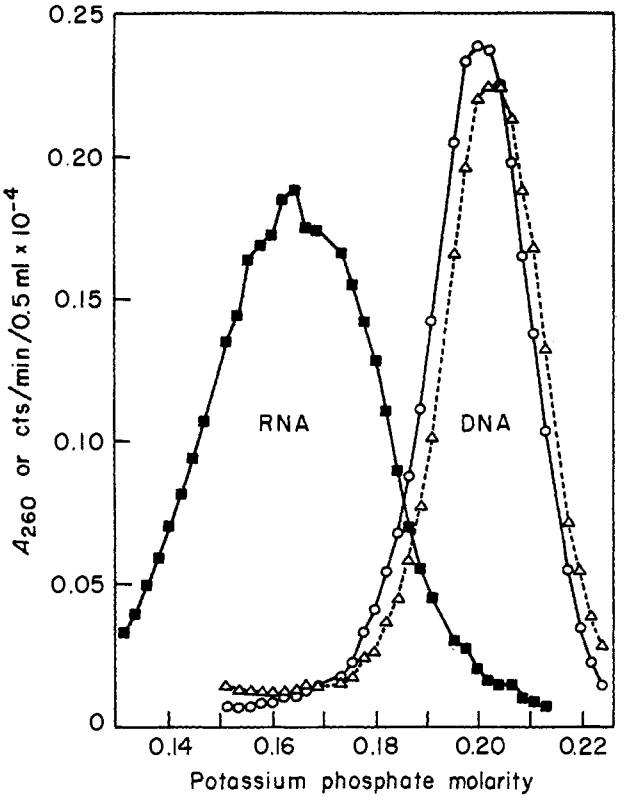

FIGURE 1: Chromatography of ds-RNA. Unlabeled CPV RNA (90 μg) (■), *B. subtilis* tritium-labeled radiolysed DNA (2 μg) (○), and *B. subtilis* [^{14}C]DNA (2 μg) (△) were mixed, sonicated (Martinson, 1973a), and loaded on a 5-mm diameter column of 140 mg of HTP. Elution was performed at room temperature with a 20-ml 0.10–0.25 M continuous phosphate gradient. The flow rate was 30 ml/hr and fractions were collected at 1-min intervals. The slope of the gradient was 125 mol (mm/l.)^2 and the load ratio was 640 μg/g.

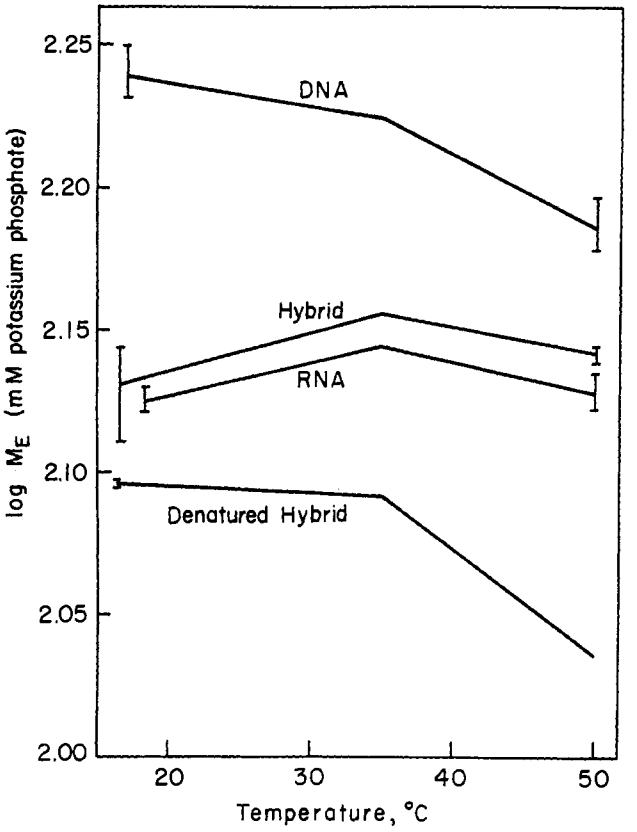

FIGURE 2: Dependence of log M_E on temperature for ds-DNA, ds-RNA, and DNA–RNA hybrid. DNA grade HTP (25–35 mg) and a step gradient format were used. The gradients were the same for each temperature and were started at 60 mM phosphate and developed in 20 mM phosphate increments (0.3–0.4 ml/fraction). Although load ratios varied from 4 to 160 μg per g, all elutions done at the temperature extremes were accompanied by one at 35°. This permitted effects such as load ratio to be normalized out as follows. First the log M_E values were determined for all of the chromatograms. Then, for each nucleic acid species, the average of the 35° log M_E values was determined. The difference of the average from the actual log M_E for each 35° determination was then subtracted from the log M_E values for the accompanying elutions at other temperatures. The ranges and averages of the resulting adjusted log M_E values at temperatures other than 35° are shown in the figure. Temperatures also varied 1 or 2° from run to run and those in the figure are the averages for above and below 35°. The ranges of the actual log M_E values at 35° are: DNA, 2.212–2.246; hybrid, 2.137–2.185; RNA, 2.127–2.172; denatured hybrid, 2.085–2.097. The number of determinations made at each temperature were: DNA—5 at low, 5 at intermediate and 3 at high temperature; hybrid—3, 4, and 3, RNA—3, 4, and 4; denatured hybrid—2,2, and 1. The nucleic acids used were *B. subtilis* hydroxylapatite DNA, *E. coli* hydroxylapatite DNA, *E. coli* (ann)DNA, *E. coli* hydroxylapatite hybrid, *E. coli* nitrocellulose hybrid, φX174 RNA, CPV hydroxylapatite RNA and hydroxylapatite and nitrocellulose hybrid denatured by heating at 100° for 5 min. Each time the tritium-labeled hybrid was used it was cochromatographed with unlabeled DNA or RNA to further reduce the effects of experimental variation.

phate. The peak of radioactivity (between 0.13 and 0.17 M phosphate) was pooled and is referred to as hydroxylapatite hybrid. The average specific activity of the hydroxylapatite hybrid was about 10,000 cpm/μg but ranged from an RNA-rich 16,000 cpm/μg to a presumably DNA-rich 5000 cpm/μg across the fractions pooled. These high specific activities are not inconsistent with the value of 10,000 cpm/μg found for the RNA alone (above) because pulse-labeled RNA was used, with the result that most of the RNA in the bulk preparation is unlabeled rRNA whereas most of the RNA in the hybrid is more highly labeled mRNA.

A second batch of hybrid was prepared similarly to that just described except that instead of adsorbing the hybrid pool to hydroxylapatite it was passed through three nitrocellulose membranes (Schleicher and Schuell, 25 mm, type B-6) which were then rinsed with 1 M Cs_2SO_4. The hybrid was eluted from the membranes with 10 mM Tris-chloride (pH 7.4) and then centrifuged for a third time in a Cs_2SO_4 density gradient (Spinco 40 rotor at 40,000 rpm for 4 days). The radioactivity peak was centered at a density of 1.500 g/cm^3 and fractions ranging in density from 1.537 to 1.484 g per cm^3 were pooled and designated nitrocellulose hybrid. Radioactive material which did not bind to the nitrocellulose membranes was found not to band in Cs_2SO_4 and had little affinity for hydroxylapatite.

Results

Figure 1 shows that at room temperature ds-RNA elutes at a significantly lower phosphate molarity than ds-DNA. Similar results have been obtained in other experiments with both sonicated and unsonicated nucleic acids, with both phosphate and sulfate as elution buffers, with salt concentrations up to 6.8 M (Cs^+) and with temperatures ranging from 20 to 85°. In short, ds-RNA of average base composition apparently has less affinity for hydroxylapatite than ds-DNA under all conditions. RNA also differs from DNA in the temperature dependence of its elution molarity (M_E). It has been shown that the M_E of the ds-DNA varies with temperature due to temperature dependent changes in the binding

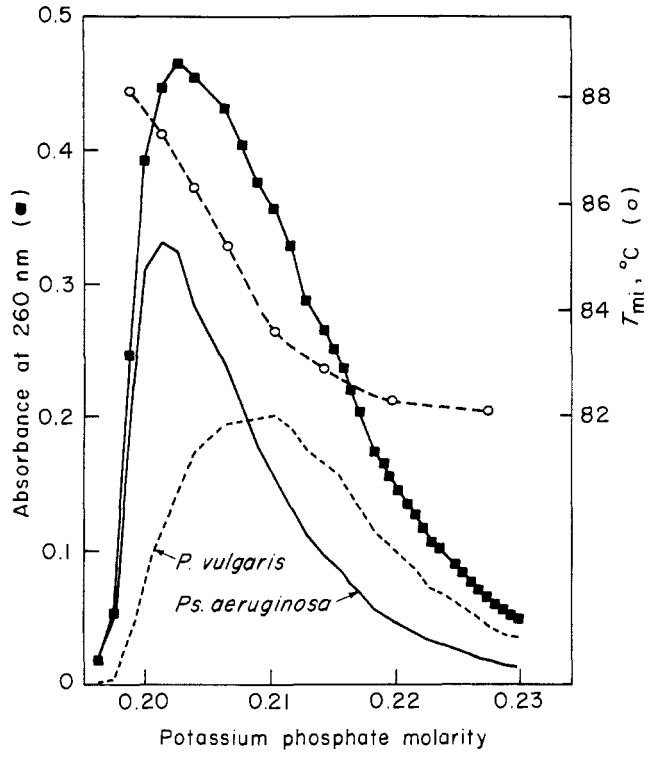

FIGURE 3: Fractionation of ds-DNA according to base composition. Nine-hundred micrograms each of sonicated native *Pseudomonas aeruginosa* and *Proteus vulgaris* DNAs were loaded on to an 8-mm diameter column of 24 g of HTP (the column length was more than 1 m). Elution was performed at room temperature with a 600-ml 0.14–0.24 M continuous phosphate gradient. The flow rate was 20 ml/hr and fractions were collected at 10- or 20-min intervals. The slope of the gradient was 12 mol (mm/l.)^2 and the load ratio was 75 μg/g. Pools of 60–70 μg of DNA from eight regions in the chromatogram were analyzed by thermal gradient chromatography. The T_{mi} values are plotted in the figure (open circles, right-hand scale). The thermal elution profile was clearly biomodal for each pool tested. Therefore, assuming the low- and high-temperature modes represented the denaturation of *P. vulgaris* and *Ps. aeruginosa* DNAs, respectively (Marmur and Doty, 1962), it was possible to calculate the contribution from each DNA to the overall absorbance profile.

equilibrium of the DNA phosphates to hydroxylapatite (Martinson, 1973b). Figure 2 shows that for RNA the temperature of maximum log M_E is higher than for DNA. (Log M_E, is the appropriate quantity to use when comparing elution molarities, Martinson, 1973b.)

The chromatographic behaviors of some types of DNA–RNA hybrid molecules on hydroxylapatite have been reported (see in particular Becker *et al.*, 1970, and Siebke and Ekren, 1970). However the hybrids of Becker *et al.* (1970) contained only 25% or less RNA and the studies of Siebke and Ekren (1970) involved T2 phage DNA which chromatograms atypically on hydroxylapatite (Oishi, 1971). Therefore the chromatographic characteristics of their structures cannot be taken as representative of hybrid helices in general. For the following studies DNA–RNA hybrid was prepared by annealing denatured *E. coli* DNA with *E. coli* [^{3}H]RNA, sonicating the mixture to break up concatenated structures and then isolating material banding isopycnically at hybrid density in a Cs_2SO_4 gradient. Such hybrid presumably possesses ss tails but is otherwise perfectly paired. One preparation of hybrid was further purified by hydroxylapatite chromatography (hydroxylapatite hybrid) and another by adsorption to nitrocellulose (nitrocellulose hybrid). Figure 2 shows that with respect to both the absolute log M_E as well as the temperature dependence of log M_E these hybrid preparations chromatograph essentially like ds-RNA rather than intermediate to ds-RNA and ds-DNA. Although only the average behavior of several chromatograms is illustrated in Figure 2 it is important to point out that both hybrid preparations showed a temperature dependence of log M_E similar to that of ds-RNA in all trials whether cochromatographed with unlabeled DNA or unlabeled RNA. The chromatographic characteristics of the hybrid cannot be assigned to some effect of reannealing, such as the presence of ss tails, since reannealed *E. coli* DNA chromatographs similarly to native DNA and not like hybrid. Furthermore the chromatographic characteristics of the hybrid can be seen to be the result of its ds structure and not due to the mere presence of RNA since the [^{3}H]RNA of denatured hybrid chromatographs quite differently from native hybrid (Figure 2).

These results allow the following conclusions to be drawn. First, ds-RNA and DNA–RNA hybrid have less affinity for hydroxylapatite than ds-DNA and, second, DNA–RNA hybrid is chromatographically nearly identical with ds-RNA. The relationship between these observations and the fact that ds-RNA and DNA–RNA hybrid exist in the A form in solution (Tunis and Hearst, 1968) while DNA exists in the B or C form (Bram, 1971a; Tunis-Schneider and Maestre, 1970) will be discussed later.

The dependence of M_E on the base composition of DNA has also been investigated. Corneo *et al.* (1970b) have reported that mammalian ds-DNA is fractionated on hydroxylapatite according to buoyant density in CsCl. Examination of their results shows the dependence of DNA buoyant density on phosphate molarity of elution to be strongly nonlinear and somewhat different for the two samples tested. But the interpretation of their results is complicated somewhat by the fact that mammalian genomes contain many subpopulations of sequences (Yunis and Yasmineh, 1971; Hatch and Mazrimas, 1970) some of which give rise to unusual physical properties in the DNA (Corneo *et al.*, 1968) including anomalous chromatography on hydroxylapatite (Corneo *et al.*, 1970b). Since the detection of such subpopulations in the genome depends on which techniques are used (Corneo *et al.*, 1970a, 1972; Maio, 1971; Botchan *et al.*, 1971), it appeared possible that the hydroxylapatite fractionation found by Corneo *et al.* (1970b) could have been influenced by hitherto undetected subpopulations of unusual sequence. Therefore, in order to determine the effect of base composition *per se* on the affinity of DNA for hydroxylapatite, the chromatographic behavior of bacterial DNAs of varying base composition was studied.

Figure 3 illustrates the elution profile (solid squares) of a mixture of *Pseudomonas aeruginosa* (66% G + C) and *Proteus vulgaris* (37% G + C) DNAs which were cochromatographed on hydroxylapatite. The thermal stability of DNA eluting at increasing phosphate concentrations across the chromatogram was determined. The results (open circles) show (Miyazawa and Thomas, 1965), consistent with the finding of Corneo *et al.* (1970b), that ds-DNA is fractionated on hydroxylapatite according to base composition, with DNA rich in G + C being eluted first. The initial slope of the thermal stability curve (open circles) yields a relationship of about 2.7 mM phosphate/1° change in thermal stability. This gives a calculated difference of 0.8% G + C for a 1 mM difference in phosphate molarity (based on 2.2% G + C/1° change in T_{mi}, see end of footnote 2).

Based on the thermal stability data it was also possible to resolve (see legend to Figure 3) the single elution absorbance

profile (Figure 3, solid squares) into the individual contributions of the *Ps. aeruginosa* and *P. vulgaris* DNAs. The shapes of the two derived profiles differ considerably from each other presumably because of competition between the DNA molecules for adsorption sites during chromatography as described for MAK by Mandell and Hershey (1960). This effect tends to exaggerate slightly the displacement to higher phosphate concentrations of the *P. vulgaris* profile but the displacement is not great because of the low DNA to hydroxylapatite load ratio (see below). In any event the M_E of *P. vulgaris* DNA is calculated to be about 5.6 mM phosphate higher than that of the *Ps. aeruginos* DNA giving a difference of 5.2% G + C/1 mM difference in phosphate molarity. This result is in sharp contrast to the 0.8%/mM value obtained if the T_{mi} data (Figure 3, open circles) are interpreted directly (above). This apparent disagreement is explained by the fact that the elution profiles strongly overlap so that individual fractions which are sampled are heavily contaminated with DNA molecules whose profiles are centered at neighboring fractions. Such cross-contamination serves to reduce the observed differences in per cent G + C from fraction to fraction.[2] Consequently fractionation according to base composition is best studied by observing the extent to which entire elution profiles are shifted relative to some standard. This has been done for several additional bacterial DNAs in Figure 4A. For convenience, and in order to conserve the available DNA, much shorter columns (about 1% as long) were used than the one for the above experiment but precision was maintained by chromatographing each DNA to be tested together with two radioactively labeled DNAs included as markers (the same markers as in Figure 1). The differences in M_E between the unlabeled DNA samples and the marker [^{14}C]DNA are shown in Figure 4A for DNAs ranging from 37 to 72% in G + C content. Because the M_E range is so small, M_E can be taken as proportional to log M_E in the present context and it is not necessary to obtain logarithms of the M_E values. The results confirm that the M_E of DNA steadily decreases as its G + C content increases.

As was the case for Figure 3, the competition for adsorption sites between DNA molecules again becomes a factor in the interpretation of Figure 4A and can account for the pronounced curvature of the M_E: (% G + C) relationship (Mandell and Hershey, 1960). The cause of this curvature can also be considered in terms of load ratio. Desorption of molecules becomes easier as the load ratio increases because of interference between adsorbed molecules. For example, the logarithm of the ratio of the M_E value of the DNA in the chromatogram in Figure 3 (load ratio = 75 μg/g) to the average M_E value of the DNAs in the chromatograms used to construct Figure 4 (load ratio = 640 μg/g) is about 0.07. In

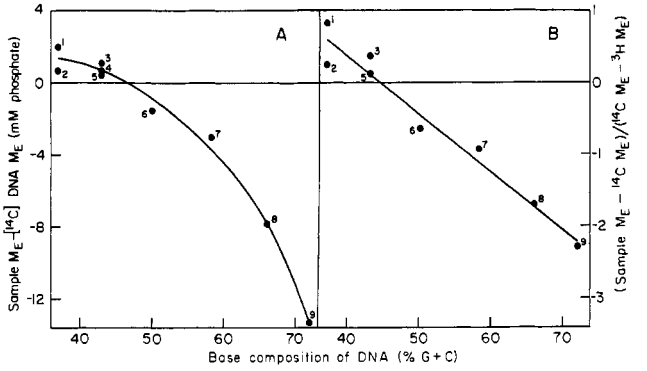

FIGURE 4: Effect of base composition on the M_E of native bacterial DNAs. Various unlabeled DNAs were mixed with *B. subtilis* tritium-labeled radiolysed DNA and *B. subtilis* [^{14}C]DNA, cosonicated and then cochromatographed on HTP. The points in the figure refer to chromatograms using unlabeled DNAs from various bacteria as follows: 1, *Bacillus megaterium;* 2, *P. vulgaris;* 3, 4, and 5, *B. subtilis;* 6, *E. coli;* 7, *Ps. lemoignei;* 8, *Ps. aeruginosa;* 9, *Micrococcus lysodeikticus.* The chromatograms for points 2, 4, 5, 7, and 8 were similar to that of Figure 1 except that the one for 4 lacked the tritium-labeled radiolysed DNA. The chromatograms for points 1, 3, 6, and 9 were also like that of Figure 1 except that half as much DNA and HTP were used and the gradient was 25 ml extending from 0.05 to 0.25 M phosphate. The base composition of *Ps. lemoignei* DNA was given by Dr. M. Doudoroff (personal communication) and the base compositions of the other DNAs were taken from Marmur and Doty (1962). In part A of the figure the differences in M_E between the unlabeled DNAs and the [^{14}C]DNA marker are plotted *vs.* the base compositions of the unlabeled DNAs. In part B the same M_E differences are expressed as multiples of the differences in M_E between the [^{14}C]- and [^{3}H]DNA markers in each chromatogram.

Figure 4 the contribution of the labeled DNAs to the load ratio in each chromatogram amounted to only about 2–3% of the total. Nevertheless, if the labeled and unlabeled DNAs eluted with the same M_E, they experienced the same load ratio throughout the elution process. However if the unlabeled DNA had a lower M_E value than (and therefore eluted slightly ahead of) the radioactive markers the effective load ratio that the latter experienced during elution became less. This in turn enhanced the affinity of these minor components for the hydroxylapatite. Therefore, up to the point of complete resolution, M_E differences become more exaggerated as the M_E of the major component is decreased relative to the minor components. Furthermore at a high overall load ratio as in Figure 4A, the difference in M_E is greater (about 9 mM phosphate between 37 and 66% G + C) than at a low ratio as in Figure 3 (5.6 mM phosphate between 37 and 66% G + C).

It was in anticipation of this load ratio effect that two radioactive marker DNAs were included in each chromatogram for Figure 4. One of them, the tritium-labeled radiolysed DNA, has a slightly lower intrinsic M_E than the other, [^{14}C]DNA (see open symbols, Figure 1). Using this M_E difference as an internal standard, load ratio effects can be normalized out. This has been done in Figure 4B where the ordinate value for each point in Figure 4A has been divided by the ^{3}H to ^{14}C M_E interval obtained in the same chromatogram. It can now be seen that the dependence of M_E on base composition is a linear relationship within experimental error.

A quantitative interpretation of Figure 4B may be obtained by making use of the actual M_E difference calculated for the two radioactive markers under conditions of very low load ratio (Figure 1). This value is 2.3 mM phosphate.

[2] This effect can be observed in the thermal elution data of Miyazawa and Thomas (1965) but it was ignored by them because cross contamination of fractions is less important in their experiments owing to the relative sharpness of thermal denaturation transitions. However considering only points above 80° in their Figure 6, two observations can be made. First, if the points in their figure are segregated according to the species of DNA (because different DNAs have different degrees of heterogeneity) it can be seen that in each case those points which were obtained by analysis of individual chromatographic fractions fall on a line of slope less than 2%/°C. On the other hand, if only the points based on the thermal chromatography of entire genomes (their solid points) are considered, the slope is about 2.2%/°C which is the value used in the text of the present paper. This value is in remarkably close agreement with the value of 2.36%/°C for DNA in solution which can be calculated from the equation of Owen *et al.* (1969) for 0.12 M Na^+ (phosphate).

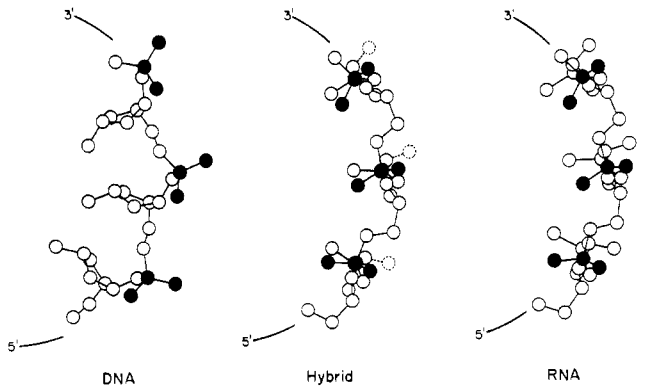

FIGURE 5: A view of three repeat units in the sugar–phosphate backbones of the probable solution configurations of ds-DNA, DNA–RNA hybrid, and ds-RNA, projected along the helix axis. The PO_2^- atoms are represented by filled circles. The atomic coordinates are taken from Arnott (1970). DNA in solution is normally in a configuration similar to the B form found in fibers (Tunis-Schneider and Maestre, 1970; Bram, 1971a); therefore it is B-DNA which is illustrated above. In solutions of high salt concentration DNA apparently assumes the C configuration (Tunis-Schneider and Maestre, 1970). However the PO_2^- groups of C-DNA (Marvin *et al.*, 1961) are just as prominent as those of the B-DNA illustrated above. ds-RNA in solution probably adopts the A′ configuration (Arnott, 1970; Bram, 1971b) so it is A′-RNA which is illustrated above. All known configurations of ds-RNA are of the A type whether in fiber (Arnott, 1970) or solution (Tunis and Hearst, 1968). DNA–RNA hybrid of balanced base composition apparently also has a conformation of the A type in solution (Tunis and Hearst, 1968). X-Ray diffraction studies on fibers of natural DNA–RNA hybrid show that its configuration is similar to that of A-DNA (Milman *et al.*, 1967). It is therefore the A-DNA backbone which is illustrated above for hybrid. However, since the RNA strand in a hybrid double helix contains an oxygen which the DNA strand does not have, this oxygen has been added to the hybrid illustration in dashes. The coordinates for this oxygen (O_6) as well as the O_6 of A′-RNA were estimated from data in Arnott *et al.* (1967). [After the preparation of this figure slightly revised atomic coordinates appeared for A- and B-DNA (Arnott and Hukins, 1972) and for A- and A′-RNA (Arnott *et al.*, 1972). Furthermore Arnott *et al.* (1972) suggest that perhaps DNA–RNA hybrid is most similar to A′-RNA. These revisions would be inconsequential to the discussion of the figure.]

Each ordinate unit in Figure 4B is therefore equivalent to an intrinsic M_E difference of 2.3 mM phosphate. Interestingly, according to this relationship, a difference of 29% in G + C content gives a difference of 5.4 mM phosphate in M_E, just slightly less than the 5.6 mM calculated directly from Figure 3 (see above). However presumably the extent of fractionation of DNA according to base composition will vary according to the type of hydroxylapatite used.

Piperno *et al.* (1972) have shown that hydroxylapatite fractionates DNA between molecular weights of 10^5 and 10^6 according to size, with larger molecules being retarded relative to smaller ones. This is the size range within which the DNA used above falls. In order to determine if size differences were somehow responsible for the separations being observed here, extensively sonicated *B. subtilis* DNA (43% G + C) was cochromatographed with lightly sonicated *Ps. aeruginosa* DNA (66% G + C). Despite the fact (verified by sucrose density gradient sedimentation) that the *Ps. aeruginosa* DNA had the larger average piece size, it nevertheless eluted slightly ahead of the smaller *B. subtilis* DNA. Piece size did have an effect however, the M_E difference being less than that predicted on the basis of Figure 4A (Martinson, 1971). These data show that fractionation according to base composition is not the result of some greater susceptibility of (G + C)-rich DNA to fragmentation. Numerous other control experiments involving various conditions have been done, and no condition has been found under which (G + C)-rich DNA does not precede (A + T)-rich DNA during phosphate elution chromatography (Martinson, 1971).

Discussion

The main findings which have been reported in the previous section are that ds-RNA and DNA–RNA hybrid are very similar, possibly identical, in their chromatographic behavior on hydroxylapatite. Both have less affinity for hydroxylapatite than DNA, and both have their maximum elution molarity at a higher temperature than DNA. It has also been shown that ds-DNA is fractionated according to base composition on hydroxylapatite; DNA rich in G + C has slightly less affinity for hydroxylapatite than DNA rich in A + T, and the relationship between G + C content and M_E (or log M_E) is linear.

Nucleic Acid–Hydroxylapatite Interaction. In searching for an explanation for the observed variations in affinity of the various double-stranded nucleic acids for hydroxylapatite a number of possible mechanisms have been considered. These included changes in adsorption affinity due to (1) variations in the interactions of the nucleic acid bases with hydroxylapatite, (2) variations in the charge densities of the different nucleic acids, (3) variations in the distributions of phosphates on the nucleic acids, (4) variations in the redistribution of ions and water molecules during adsorption of different nucleic acids, and (5) variations in the steric availability of the nucleic acid backbone phosphates for surface interactions. On the basis of arguments to be presented below, possibilities 1–4 are considered to be of minor significance except perhaps in special cases. On the other hand, variations in the configurations of the sugar–phosphate helix exterior (number 5 above) appear to be principally involved in modifying the adsorption affinity of double-stranded nucleic acids.

Figure 5 shows portions of the sugar–phosphate backbones in hypothetical cross-sections of the probable solution configurations of DNA, RNA, and DNA–RNA hybrid helices. In each repeat unit, the two oxygens which share the negative charge, as well as the intervening phosphorus atom, are represented by filled circles. It can be seen that the PO_2^- groups of DNA protrude from the helix and are apparently relatively accessible for surface interactions while the PO_2^- groups of both ds-RNA and DNA–RNA hybrid appear "buried" in the surface of the helix. These observations correlate well with the findings that ds-RNA and DNA–RNA hybrid both have less affinity for hydroxylapatite than does DNA and that furthermore ds-RNA and DNA–RNA hybrid chromatograph on hydroxylapatite very similarly to each other.

Unfortunately X-ray data of comparable detail are not available for other helix types whose chromatography on hydroxylapatite have been studied. Nevertheless some interesting parallels can be drawn. The data of Figure 4 indicate that a DNA of 100% A + T content would be expected to have slightly more affinity for hydroxylapatite than *E. coli* DNA. However Bernardi *et al.* (1972) have reported that while the affinity of poly[d(A-T)·d(A-T)] is indeed slightly higher than that of *E. coli* DNA, poly[d(A·T)] has a much greater affinity for hydroxylapatite [log (M_E ratio) about $^1/_4$; see Martinson, 1973c] than would be predicted on the basis of Figure 4. On the other hand, the elution molarities of poly(A·U) and poly(I·C) (Bernardi, 1971) appear to be

lower than that of DNA (Bernardi, 1969). X-Ray diffraction data on these synthetic polymers show that the structure of poly(A·U) and poly(I·C) are like that of natural RNA (Arnott, 1970) and that the structure of poly[d(A-T)·d(A-T)] can be essentially identical to that of natural DNA but that the structure of poly[d(A·T)] differs considerably from either (Langridge, 1969). Thus of these polymers, only the ones which are structurally similar to natural ds-DNA and ds-RNA have the chromatographic behavior expected of the respective natural nucleic acids.

Implicit in the discussion which has been presented so far is the assumption that the configurations of the double-stranded nucleic acids as determined in fibers and in solution are relevant to the adsorbed states of the nucleic acids. This assumption seems to be reasonable since the geometrical limitations governing the contact of a rigid cylinder with a plane surface would prevent the simultaneous adsorption of more than just a small proportion of the helix phosphates, and the solution environment would therefore remain the predominant influence on the detailed geometry of the adsorbed double helix. Of course the "solution environment" near a hydroxylapatite crystal differs from that of the bulk solution (Pak and Skinner, 1968) but the difference, one of ionic strength, is quantitative, not qualitative. Within this context a reduction in the affinity of a nucleic acid for hydroxylapatite can be viewed as being the result of a combination of two factors. First, the PO_2^- groups may be unfavorably disposed for adsorption interactions as already discussed, and second, when PO_2^- groups become involved in an adsorption interaction it is likely that some localized helix backbone distortion occurs and this would be more important in the case of "buried" PO_2^- groups than for more exposed ones. Both of these factors would serve to reduce the free energy of adsorption of a nucleic acid.

Earlier in this discussion four other possible explanations for the observed variations in double-stranded nucleic acid affinity for hydroxylapatite were mentioned. These will now be individually evaluated.

Base interactions. It has been assumed above, based on evidence presented by Bernardi (1971), that the backbone phosphates basically account for the affinity of nucleic acids for hydroxylapatite. This seems reasonable since it would require severe helix distortion (much more than the changes in PO_2^-orientation discussed above) for such to occur in the case of double-stranded nucleic acids. No studies have been done on the effect of hydroxylapatite on the conformation of adsorbed double helices. However two lines of evidence suggest that double-stranded nucleic acids retain their native configuration when in the adsorbed state. First, the effects of changes in cation concentration (Martinson, 1973a) and base composition (see footnote 2) on the thermal stability of ds-DNA are quantitatively the same for adsorbed DNA and DNA in solution. Second, hydroxylapatite actually increases the thermal stability of DNA (Martinson, 1973a), thus decreasing the likelihood that its bases could be exposed. These considerations appear to rule out direct involvement of the bases with hydroxylapatite as a factor in the fractionation of any double-stranded nucleic acids.

Charge density. Bernardi (1965) has suggested that the charge density of nucleic acids is an important determinant of adsorption affinity. This suggestion was based on the observation that ds-DNA has both more affinity for hydroxylapatite and a higher charge density than denatured DNA. However in the case of flexible, single-stranded molecules it seems unlikely that a property, such as charge density, which is characteristic of the solution conformation, can have much effect on an adsorption interaction during which the nucleic acid configuration is undoubtedly altered considerably (Roe, 1965; Martinson, 1973c).

In the rigid double-stranded nucleic acids the relative concentrations of PO_2^- groups over the surfaces of the various helix types can be calculated from the helix parameters given by Arnott (1970). The charge densities of ds-RNA and DNA–RNA hybrid (see legend to Figure 5) calculated relative to DNA (taken as 1) are then about 1.1 and 1.4, respectively. Also recent electrophoretic results of Zeiger *et al.* (1972) suggest that (G + C)-rich DNA may have a radially more compact helical structure than (A + T)-rich DNA. This would likely result in a higher charge density for the former. However if charge density were a significant factor in hydroxylapatite chromatography, molecules of higher charge density would be expected to have greater affinity for hydroxylapatite (Bernardi, 1965). Therefore, since both ds-RNA and RNA–DNA hybrid have less affinity for hydroxylapatite than ds-DNA, and (G + C)-rich DNA is less strongly adsorbed than (A + T)-rich DNA, charge density can be ruled out as a determining factor in their relative adsorption affinities.

Phosphate distributions. Although the charge density *per se* of nucleic acids does not appear to have much influence on their relative affinities for hydroxylapatite, it might seem likely that the specific *arrangement* of charges (phosphates) on the surface of a double helix would be of some importance. According to this view the nucleic acid possessing the arrangement of phosphates which best matches the arrangement of adsorption sites on a hydroxylapatite crystal would be expected to adsorb most firmly (Bernardi, 1971). Except for superhelices which present different geometrical considerations (see Bourgaux-Ramoisy *et al.*, 1967) it is clear that in the case of rigid double-stranded nucleic acids the tighter the winding of the helix (the fewer the number of base pairs per turn), the greater is the number of adsorption contacts per strand which can occur with the hydroxylapatite. It is therefore interesting to find that ds-DNA does indeed have fewer base pairs per turn of the helix than ds-RNA or RNA–DNA hybrid (for references, see legend to Figure 5), both of which have less affinity for hydroxylapatite. However this correlation is apparently not of general significance since poly[d(A·T)], which has an exceptionally high affinity for hydroxylapatite (Bernardi *et al.*, 1972), nevertheless has 10 residues/turn (Langridge, 1969), the same number as normal B-DNA (Arnott, 1970). Furthermore, triple-stranded poly(A)·2poly(U), which also has a very high elution molarity (Bernardi, 1971), has 12 residues/turn (Arnott *et al.*, 1968), no fewer than for poly(A·U) (Arnott, 1970). (It should be pointed out that since charge density is apparently not an important factor in adsorption it is by no means a foregone conclusion that a triple-stranded molecule should have more affinity for hydroxylapatite than a double-stranded one because although the number of PO_2^- groups per molecule is increased, likewise is the number of strands, so that the number of phosphates per unit mass of the molecule remains constant.)

An aspect of helix geometry related to the number of residues per turn is the actual pitch (linear repeat distance) of the helix. Taves and Reedy (1969) have proposed a model for the binding of ATP to hydroxylapatite. They suggest that binding sites occur along the *C* axis of a hydroxylapatite crystal at 3.44-Å intervals. If DNA also adsorbs along the *C* axis (or along any other preferred direction), it might be proposed that the binding affinities of nucleic acids having different characteristic helix pitches would be affected by how

TABLE I: Densities of Double-Stranded Nucleic Acids in CsCl.

Nucleic Acid	Density (g/cm³)	% G + C	References
DNA	1.712	53	Schildkraut *et al.* (1962)
DNA	1.702	43	Schildkraut *et al.* (1962)
Hybrid	1.80	53	Spadari *et al.* (1972); Mahler and Cordes (1966)
Hybrid	1.79	44	Becker *et al.* (1970)[a]; Schildkraut *et al.* (1962)
RNA	1.868	52	Kelly *et al.* (1965); Billeter *et al.* (1966)

[a] The density of pure 1:1 hybrid was extrapolated from their data.

closely the helix pitch matches the spacing of the hydroxylapatite adsorption sites. However in view of the variability of helix pitch (Bauer, 1972; Arnott, 1970; Bode and MacHattie, 1968), it seems quite unlikely that the necessity of slight adjustments of less than 2 Å in pitch during adsorption would have much effect on the strength of binding to hydroxylapatite.

In this connection it should be mentioned that the observation that nucleotide triphosphates and trinucleotides differ dramatically in their characteristic elution molarities [the nucleotide triphosphate:trinucleotide log (M_E ratio) is greater than 2] has been cited as evidence in favor of the importance of phosphate distribution to adsorption affinity (Bernardi, 1971). However there does not appear to be any reason why trinucleotides could not adsorb to the same sites that are proposed for the adsorption of triphosphates (Taves and Reedy, 1969). These sites occur at 3.44-Å intervals along the hydroxylapatite surface, a distance which is very close to that normally separating the bases in nucleic acid helices (Arnott, 1970). It seems more likely that the difference in adsorption affinities between a trinucleotide and a triphosphate is due to the greater unfavorable configurational entropy change which must occur when the comparatively large and flexible trinucleotide becomes immobilized on the hydroxylapatite surface (see Martinson, 1973c). A similar line of reasoning has been used to explain the relatively strong association of complementary tRNA anticodons in solution (Eisinger, 1972).

SMALL ENTITY INTERACTIONS. The adsorption of DNA to hydroxylapatite is actually more properly viewed as an ion-exchange reaction. Thus during adsorption, helix counterions are displaced by hydroxylapatite adsorption sites while phosphate ions on the surface of the hydroxylapatite are displaced by the PO_2^- groups of the nucleic acid. In addition to the ion exchanges which take place during adsorption, a redistribution of water molecules can be expected as the DNA penetrates the hydration layer of the hydroxylapatite and counterions are liberated into solution. Doubtless slight differences in affinity of the ions and water molecules for different types of nucleic acid would affect the strength of the nucleic acid–hydroxylapatite adsorption interaction. However available data suggest that such effects are not sufficient to account for the observed differences in affinity of the various nucleic acids for hydroxylapatite. First, although Latt and Sober (1967) have shown that simple cations can exhibit specific nucleic acid binding preferences which apparently depend on base composition, (G + C)-rich DNA has less affinity for hydroxylapatite than (A + T)-rich DNA in both Na^+ and K^+ buffers and RNA has less affinity for hydroxylapatite whether Na^+, K^+, or Cs^+ buffers are used. No evidence of any effect caused by the cation specificity differences reported by Latt and Sober (1967) have been found. Second, Shapiro *et al.* (1969) could detect no base composition dependent specificity of binding of Ca^{2+} ions to ds-DNA. Thus the fractionation of ds-DNA according to base composition is probably not the result of any specific competition for DNA phosphates by the free Ca^{2+} ions which are known to exist in the neighborhood of the hydroxylapatite crystals (Pak and Bartter, 1967).[3] Third, the differential release of water of hydration during adsorption could possibly account for the relative adsorption affinities as has been suggested in different contexts by Latt and Sober (1967) and Von Hippel and McGhee (1972). However, the variations in nucleic acid affinity for hydroxylapatite are not well correlated with differences in the strength of hydration (and perhaps also in ion binding) as estimated by buoyant density in cesium salts (Hearst and Vinograd, 1961; Tunis and Hearst, 1968). Thus while ds-RNA and DNA–RNA hybrid have similar affinities for hydroxylapatite, their buoyant densities differ from each other considerably in both Cs_2SO_4 (see Materials and Methods section) and CsCl (Table I) with the hybrid having a density intermediate to those of ds-DNA and ds-RNA. Thus it can be concluded that, in general, differences in the redistribution of ions and water molecules during adsorption do not contribute appreciably to the observed variations in adsorption affinity of nucleic acids for hydroxylapatite.

In the above discussion it has been shown that the nucleic acid–hydroxylapatite adsorption interaction is, at least in most cases, not appreciably influenced by variations in base interactions, charge density, phosphate distribution or small entity interactions. Therefore, it appears that the PO_2^- steric availability hypothesis presented at the beginning of this section offers the best general explanation for the observed differences in adsorption affinity between the various double-stranded nucleic acids.

The linear dependence of DNA backbone structure on base composition proposed here is probably quite different from the DNA polymorphism described by Bram and Tougard (1972) which is prominent only in DNA of very high A − T content. Also it should be emphasized that if PO_2^- steric availability is indeed the factor of predominant importance to variations in adsorption affinity, then changes in other helix parameters (*e.g.*, pitch, tilt, twist, *etc.*) would be expected to affect adsorption affinity primarily (or only) insofar as the prominance or ease of reorientation of the helix phosphates is altered. Therefore changes in the structure of double-stranded nucleic acids do not necessarily imply variations in affinity for hydroxylapatite.

In summary, the data presented above suggest that the strength of the double-stranded nucleic acid–hydroxylapatite adsorption interaction is sensitive to certain types of structural change in nucleic acids such that double-stranded nucleic acids having sugar–phosphate backbone configurations in which the PO_2^- groups are either at, or can be induced to,

[3] To view the hydroxylapatite adsorption sites themselves as being calcium ions (Bernardi, 1971) is a considerable oversimplification (for example, see Taves and Reedy, 1969). The lack of base compositional selectivity by Ca^{2+} in solution is therefore not at all inconsistent with the presence of selectivity by the adsorption sites in the hydroxylapatite crystal surface.

greater prominence have more affinity for hydroxylapatite than nucleic acids with less prominent phosphates or less flexible backbones.

DNA–MAK INTERACTION. In the course of the foregoing discussion it has been possible to consider the nucleic acid–hydroxylapatite adsorption interaction in fairly straightforward terms, owing to the defined geometry of the crystalline hydroxylapatite. However most other procedures for nucleic acid chromatography involve considerably more heterogeneous adsorbents (Kothari, 1970), rendering the mechanisms of adsorption correspondingly more complex. It is therefore worthwhile investigating whether an understanding of the nucleic acid–hydroxylapatite interaction, considered as a model for the ionic component of a more complex adsorption interaction, can aid in determining the mechanism by which nucleic acids are fractionated in other systems. For the sake of brevity, only the MAK system (Mandell and Hershey, 1960) will be discussed. (For a brief discussion of another system, see Mindich and Hotchkiss, 1964.)

In MAK column chromatography ds-DNA is eluted *before* ss-DNA when an increasing salt concentration gradient is imposed (Sueoka and Cheng, 1962). It has been suggested that this is the result of the increased number of phosphates available for adsorption interactions when a nucleic acid helix is disrupted (Lichtenstein and Shapot, 1971). However the proportion of nucleic acid phosphates available for adsorption interactions is probably a factor of minor importance since in the hydroxylapatite system denaturation of a nucleic acid *reduces* its adsorption affinity (see Martinson, 1973c, for discussion). Therefore the great affinity of single-stranded nucleic acids for MAK must be the result of adsorption interactions not present in the hydroxylapatite system. Ellem and Rhode (1969) have shown that there are important hydrophobic interactions between single-stranded nucleic acids and MAK. It therefore seems likely that the greater affinity of single-stranded- compared to ds-DNA for MAK is attributable to the increased opportunity of the DNA bases to interact directly with the methylated albumin when the DNA is in the single-stranded form.

Other differences between the chromatography of nucleic acids on MAK and hydroxylapatite appear to have similar explanations. For example, ss-DNA rich in A + T has *more* affinity for MAK than ss-DNA rich in G + C (Sueoka and Cheng, 1962) presumably because the folded configurations of the former are less stable (Eigner and Doty, 1965), thus providing easier access of the methylated albumin side chains to the DNA bases. Of course the methylated albumin may also have greater affinity for the A and T bases themselves. (In the hydroxylapatite system ss-DNA richer in G + C is adsorbed more strongly; Martinson, 1973c.)

Only for ds-DNA do the hydroxylapatite and MAK systems yield similar results. In both cases a gradient of increasing salt concentration elutes G + C rich ahead of (A + T)-rich DNA. However an inverse affinity relationship between the two adsorbents has been found for DNA satellite in chromatograms of mouse DNA (Corneo *et al.*, 1970). On hydroxylapatite mouse satellite DNA elutes late whereas on MAK it elutes early compared to the bulk of mouse DNA. Thus the adsorption interactions of MAK and hydroxylapatite are sensitive to different aspects of DNA structure, notwithstanding the parallel fractionations according to base composition. It was previously argued (see above) that the plane geometry of the hydroxylapatite surface and the enhanced stability of the adsorbed DNA helix precluded the interaction of the bases of ds-DNA with hydroxylapatite. MAK, in contrast, has an irregular surface with amino acid side chains, and has been shown to destabilize the nucleic acid helical structure (Raué and Gruber, 1970). Direct interaction of MAK with the bases even of ds-DNA is therefore probable. Such interactions could give rise to the observed fractionation according to base composition either because of a greater affinity of methylated albumin for the A and T bases *per se* or because of regions of localized exposure of the bases induced by the MAK in the very (A + T)-rich segments which would be more numerous in (A + T)-rich DNAs. However it should be pointed out that low helix stability is apparently not a factor in facilitating the kind of backbone conformational adjustments which have been proposed to increase affinity of nucleic acids for hydroxylapatite. DNA–RNA hybrid and poly[d(A-T)·d(A-T)] are more easily denatured than ds-DNA (Chamberlin and Berg, 1964; Bolton and McCarthy 1964; H. G. Martinson, unpublished data) and poly(dA·dT) (Felsenfeld and Miles, 1967), respectively. Yet their respective affinities for hydroxylapatite follow the reverse order: DNA > hybrid; poly[d(A·T)] > poly[d(A-T)·d(A-T)].

Not much can be said about the exact nature of the adsorption interactions between the nucleic acid bases and MAK. However hydrophobic interactions have been shown to be involved (Ellem and Rhode, 1969) and may include intercalation in the DNA by the aromatic residues of albumin, as suggested for protein–nucleic acid interactions by Hélène (1971) and Gabbay *et al.* (1972). Consistent with this interpretation is the observation that the organic resin, IRC-50, which has no aromatic substituents, has, like hydroxylapatite, less affinity for ss- than ds-DNA (Mindich and Hotchkiss, 1964).

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Added in Proof

Stutz and Bernardi (1972) have recently reviewed the growing list of natural DNAs which can be preparatively fractionated according to base composition on hydroxylapatite.

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