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Nucleosome Reconstitution of Core-Length Poly(dG)·Poly(dC) and Poly(rG-dC)·Poly(rG-dC)†

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ABSTRACT: The double-stranded polypurine·polypyrimidines poly(dG)·poly(dC) and poly[d(A-G)]·poly[d(T-C)] and the mixed ribose–deoxyribose polynucleotide poly(rG-dC)·poly(rG-dC) have been successfully reconstituted into nucleosomes. The radioactively labeled particles comigrate in gel electrophoresis and sucrose density gradient experiments with authentic nucleosomes derived from chicken erythrocyte chromatin. These results show that nucleosomes are able to accommodate a wider variety of polynucleotides than was previously believed.

Nucleosomes are the basic organizational unit of chromatin in which 146 base pairs (bp) of DNA wrap around a core of eight histone proteins and a variable length of "linker" DNA is associated with a ninth histone, H1 (McGhee & Felsenfeld, 1980). Although the protein component of nucleosome cores is constant, the polynucleotide component is not. Since nucleosomes appear to organize almost all eukaryotic DNA, they have to accommodate a wide variety of DNA sequences. Previous experiments have shown that they have this ability. Nucleosomes cores have been reconstituted in vitro by using the synthetic polymers poly[d(A-T)·poly[d(A-T)] (Bryan et al., 1979), poly[d(G-C)]·poly[d(G-C)] (Simpson & Kunzler, 1979), and poly[d(G-m^{5}C)]·poly[d(G-m^{5}C)] (Nickol et al., 1982), as well as prokaryotic DNA (Bryan et al., 1979) and even bacteriophage T7 DNA, which contains glucosylated (hydroxymethyl)cytosine groups in place of normal cytosine residues, filling the major groove (McGhee & Felsenfeld, 1982).

There have been other reports, however, that some polynucleotides are unable to form nucleosomes. Failed in vitro attempts to reconstitute the synthetic polynucleotides poly(dG)·poly(dC) (Simpson & Kunzler, 1979; Rhodes, 1979), poly(dA)·poly(dT) (Simpson & Kunzler, 1979; Rhodes, 1979), DNA–RNA hybrids (Dunn & Griffith, 1980), plasmids substituted with ribonucleotides (Hovatter & Martinson, 1987), and the Z forms of poly[d(G-C)]·poly[d(G-C)] and poly[d(G-m^{5}C)]·poly[d(G-m^{5}C)] (Nickol et al., 1982; Ausio et al., 1987) have been published. This has usually been ascribed to the existence of the test polymer in a conformation other than the canonical B form, and thus the conclusion was drawn that nucleosomes cannot tolerate long regions of polynucleotide that deviate substantially from a normal B form.

In this paper we describe the attempted reconstitution of several polypurine·polypyrimidines and polymers containing ribonucleotides. The experiments show that nucleosomal particles can be formed by using polynucleotides that were resistant to reconstitution in other systems.

Materials and Methods

Poly[d(A-G)]·poly[d(T-C)], poly(dI), poly(rI), poly(dC), pdG_{10}, and pdC_{10} were purchased from Pharmacia. T4 polynucleotide kinase, Klenow fragment, and T4 polynucleotide ligase were obtained from U.S. Biochemicals. Micrococcal nuclease and bovine pancreatic DNase I were purchased from Bethesda Research Laboratories. Poly(rG-dC)·poly(rG-dC) was synthesized as described earlier (Wu & Behe, 1984) by using DNA polymerase I large fragment (Klenow fragment), rGTP, dCTP, and a poly[d(I-C)]·poly[d(I-C)] template in the presence of Mn^{2+}. Molecular size markers consisting of a ladder of fragments that are multiples of 123 bp were obtained from BRL.

Chicken blood was obtained in Alsever's solution from Mayer & Miles (Coopersburg, PA) and shipped on ice the same day. The time from bleeding of the animals to arrival of the blood in the laboratory was normally ~3 h.

Polymers of 150 Base Pair Length. Poly(rI)·poly(dC) and poly(rG-dC)·poly(rG-dC) [both 150 base pairs (bp) in length] were obtained by isolation of the appropriate size fragments from nuclease digests of longer starting material. Either polymer (250 μg) was digested at 37 °C with 100 units of micrococcal nuclease in 100 mL of 50 mM NaCl, 10 mM Tris·HCl, pH 8, and 2 mM $CaCl_2$. Time points were taken at 0.5, 1, 2, 3, and 4 min, the reaction was stopped by addition of EDTA to 5 mM, the solution was extracted three times with neutral phenol and chloroform, the digestion products were electrophoresed in a 6% polyacrylamide gel, and the gel was stained with ethidium bromide. For both polymers material of ~150 base pairs was excised, electroeluted with a Phar-

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macia Extraphor into a 3 M NH_4OAc salt barrier, extracted with 1-butanol, precipitated at −70 °C with 2 volumes of ethanol, and redissolved in 20 μL of 10 mM Tris, pH 8, and 0.1 mM EDTA.

Poly(dC) (150 base) was produced by ligation of pdC_{10} on a poly(dI) template. pdC_{10} (50 μg) was mixed with 50 μg of poly(dI) in 40 μL of 5 mM Tris·HCl, pH 7.5, heated to 90 °C, and slowly cooled to anneal the complementary strands. To this was added 5 μL of 10× ligation buffer (0.5 M Tris·HCl, pH 7.6, 0.1 M $MgCl_2$, 50 mM dithiothreitol, 1 mM spermidine, 1 mM EDTA, and 1 mM ATP). T4 polynucleotide ligase (100 units) was added, and the mixture was incubated at 4 °C overnight. The ligation products were extracted three times with neutral phenol and chloroform, precipitated with ethanol, redissolved in buffer, electrophoresed through 8% polyacrylamide/7 M urea, visualized by UV shadowing, excised, electroeluted, precipitated with ethanol, and redissolved in buffer, as described above. Poly(dG) (150 base) was synthesized analogously from pdG_{10} by using a poly(dC) template. To form double-stranded, 150 bp poly(dG)·poly(dC), the 150-base single strands were mixed in equimolar ratios in 50 mM NaCl, 10 mM Tris·HCl, pH 8, and 0.1 mM EDTA, heated to 90 °C, and allowed to anneal by slow cooling.

Radioactive labeling was carried out by kinasing 1 μg of polymer with [γ-^{32}P]ATP by using 50 units of T_4 polynucleotide kinase in 50 μL of 50 mM Tris·HCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, and 0.1 mM spermidine. Excess radioactive ATP was removed by chromatography through Sephadex-G50.

Reconstitution and Analysis of Core Particles. Nucleosome cores were prepared from chicken erythrocyte chromatin by limited digestion of erythrocyte nuclei with micrococcal nuclease (Simpson & Whitlock, 1976). The monomeric nucleosome cores were isolated by chromatography through Sepharose 6B and dialyzed against 10 mM Tris, pH 8, and 0.1 mM EDTA. The cores could be stored for up to 6 months at −20 °C as a 50% glycerol solution. SDS–polyacrylamide gel electrophoresis showed that no degradation of histones occurred during this time.

Radioactively labeled polynucleotides were reconstituted by the salt-exchange procedure of Ramsay et al. (1984) as modified by Drew and Travers (1985). Labeled 150 base pair polynucleotide (~0.5 μg) was incubated with 30 μg of core chicken nucleosomes in 20 μL of 800 mM NaCl, 20 mM Tris·HCl, pH 8, and 0.1 mM EDTA for 20 min at 37 °C. The mixture was subsequently diluted to 80 mM NaCl by the addition of 5-μL aliquots of 5 mM Tris·HCl, pH 8, and 0.1% Nonidet P-40, once every 20 min at 37 °C.

Aliquots of the diluted reconstitution mixture were analyzed by electrophoresis at low current (10 mA) on a preelectrophoresed 6% polyacrylamide gel containing a buffer of 50 mM Tris, 50 mM boric acid, and 1 mM EDTA, pH 8.3. Unreconstituted labeled polymer was also electrophoresed side by side with the reconstituted mixture. As a control, chicken nucleosomal DNA was also isolated, radioactively labeled, and put through the reconstitution procedure. A shift of the polynucleotide band in an autoradiogram of the gel from its position in the unreconstituted lane to a position close to that of reconstituted chicken cores was taken as indicative of successful reconstitution.

Aliquots of the diluted reconstitution mixture were also mixed with an additional 50 μg of unlabeled chicken nucleosome cores, further diluted to 500 μL with 50 mM Tris, pH 8, and 0.1 mM EDTA, layered on top of a 5%–20% linear sucrose gradient containing 10 mM NaCl, 10 mM Tris, pH 8, and 0.1 mM EDTA, and centrifuged at 22 000 rpm in a Beckman SW27 rotor at 4 °C for 40 h. The gradient was fractionated, the absorbance read at 260 nm, and the radioactivity of the samples determined by Cerenkov counting. The relative mobility of the labeled polynucleotide was then compared to the mobility of authentic, unlabeled chicken nucleosome cores. Apparently because of the length of time required to run the sucrose gradients, a lesser percentage of radioactively labeled polymer is found in nucleosomal peaks in these experiments than when the reconstitute is analyzed by gel electrophoresis.

Reconstituted [^{32}P]poly(dG)·poly(dC) was further digested at 37 °C with 1 unit of DNase I in 200 μL of 20 mM Tris·HCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$, pH 8, time points were taken, and the reaction was stopped by addition of EDTA to 5 mM. The reaction mixture was deproteinized by three extractions with neutral phenol followed by three extractions with chloroform. The sample was precipitated with ethanol, redissolved in 20 μL of 80% formamide, 10 mM NaOH, and 1 mM EDTA, and electrophoresed through 8% polyacrylamide containing 7 M urea, 50 mM Tris, 50 mM boric acid, and 1 mM EDTA, pH 8, and the gel autoradiographed.

Poly[d(A-G)]·poly[d(T-C)] was reconstituted with chicken histones by a direct mixing procedure in 200 mM NaCl using a histone/DNA mass ratio of 0.8 (Ruiz-Carrillo et al., 1979). Histones were isolated from chicken chromatin by chromatography on hydroxyapatite (Simon & Felsenfeld, 1979). Digestion of the reconstituted polymer was done at 37 °C with 30 units of micrococcal nuclease in a buffer of 50 mM Tris, pH 8, 2 mM $CaCl_2$, and 0.1 mM EDTA. The reaction was stopped by addition of EDTA to 5 mM. The reconstitution mix was deproteinized, the DNA fragments were electrophoresed through a nondenaturing 6% polyacrylamide gel, and the gel was stained with ethidium bromide.

RESULTS

Reconstitution of Poly(dG)·Poly(dC). Recently, our laboratory showed that genomic and viral eukaryotic DNA sequences are strongly biased in favor of long tracts of contiguous purine residues (Behe, 1987; Beasty & Behe, 1988). We analyzed the 67-kilobase DNA sequence of the human β-globin region and over 80 kilobases of other human DNA sequences for the occurrence of strings of contiguous purine or pyrimidine residues. Tracts of 10 or more contiguous purine or pyrimidine residues were found four times more frequently than would be expected for a random distribution of bases so that a long string occurs at an average of every 250 base pairs and accounts for a total of ~5% of the DNA. Since the frequency of occurrence of these strings corresponds closely to the nucleosomal repeat unit and since some synthetic polydeoxynucleotides in which one strand is composed completely of purine residues were reported to not form normal nucleosomal structures (Simpson & Kunzler, 1979; Rhodes, 1979), we speculated that these elements may play a role in influencing the structure of chromatin in vivo.

In an attempt to quantitatively examine the effect of the sequence bias, we are employing the salt-exchange method of nucleosome reconstitution (Ramsay et al., 1984; Drew & Travers, 1985) to measure the affinity of approximately core-length (140–160 bp) synthetic polymers containing oligopurine tracts for nucleosome formation in the presence of competing DNA. In initial control experiments we synthesized core-length poly(dG)·poly(dC) by ligation of oligo$(dG)_{10}$ and oligo$(dC)_{10}$ on poly(dI) and poly(dC) templates, respectively, elution of the 150-residue, single-stranded polymers from

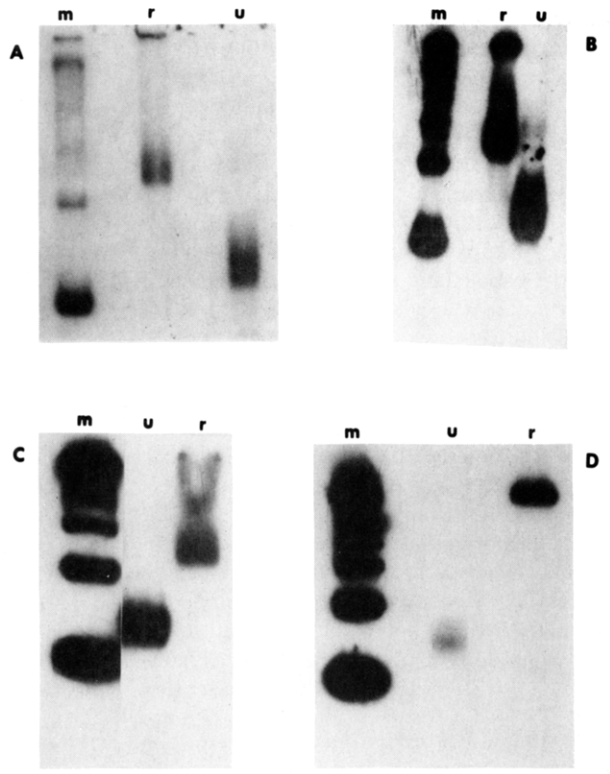

FIGURE 1: Electrophoresis of reconstituted synthetic polynucleotides. Lanes marked "m" contain 123 bp ladder (i.e., −123, 246, 369, etc.) molecular size standard; lanes marked "u" contain unreconstituted, naked polynucleotide; lanes marked "r" contain reconstituted polynucleotide running as a nucleoprotein complex. Reconstituted nucleosome cores run at ~300 bp in this gel system, with mobility intermediate between the second and third bands in the size standard lanes. (A) Chicken DNA; (B) poly(dG)·poly(dC); (C) poly(rG-dC)·poly(rG-dC); (D) poly(rI)·poly(dC).

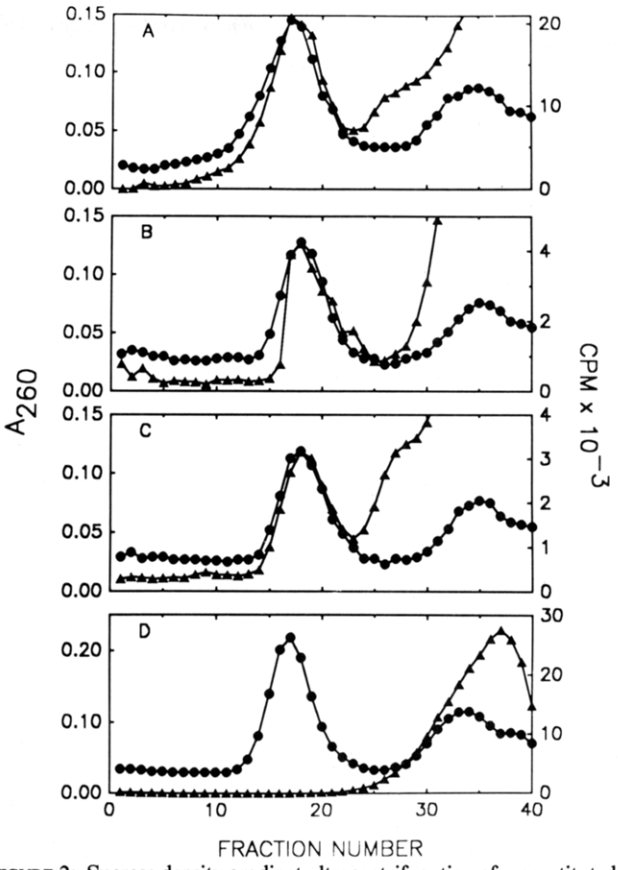

FIGURE 2: Sucrose density gradient ultracentrifugation of reconstituted, labeled synthetic polynucleotides. The top of the gradient is to the right. (●) Absorbance at 260 nm; (▲) Cerenkov counts. (A) Reconstituted chicken DNA (area under nucleosome peak represents 31% of total radioactivity on gradient); (B) reconstituted poly(dG)·poly(dC) (area under nucleosome peak represents 26% of total radioactivity on gradient); (C) reconstituted poly(rG-dC)·poly(rG-dC) (area under nucleosome peak represents 22% of total radioactivity on gradient); (D) poly(rI)·poly(dC) put through reconstitution procedure.

denaturing gels, and annealing of the two strands in solution. After subjection of the radioactively labeled poly(dG)·poly(dC) to the salt-exchange procedure by incubating it in the presence of chicken erythrocyte nucleosomes in a solution of 0.8 M NaCl, the mixture, diluted with buffer to 80 mM NaCl, was electrophoresed in a nondenaturing gel. Contrary to what we expected, the bulk of the labeled poly(dG)·poly(dC) migrates very similarly to chicken nucleosomes, and both are well separated from 150 bp naked DNA. Figure 1A, lane r, shows that, in this gel system, reconstituted, labeled chicken nucleosomes migrate with an electrophoretic mobility intermediate between the 246 and 369 bp standards shown in lane m. Figure 1B, lane r, shows that reconstituted poly(dG)·poly(dC) has a very similar mobility. Comparison of the bands in lanes u of Figure 1A,B with the molecular size markers in the lanes marked "m" shows that, as expected, naked, unreconstituted poly(dG)·poly(dC) and chicken DNA have a mobility of ~150 bp.

The labeled poly(dG)·poly(dC) also comigrates with chicken nucleosomes as measured by ultracentrifugation in sucrose density gradients. Figure 2B shows that the peak of radioactivity from poly(dG)·poly(dC) coincides with the peak of A_{260} absorbance from cosedimenting bulk chicken nucleosomes (centered at fraction 18). Unassociated DNA is well separated from the nucleosomal peaks (centered at fraction 35). In a control experiment a similar profile is obtained simply by using labeled 145 bp chicken DNA that is put through the reconstitution procedure (Figure 2A). Labeled poly(dG)·poly(dC) that is mixed at low salt concentration where exchange does not occur does not complex with nucleosomes under the conditions of the sedimentation (data not shown).

When a reconstituted mixture containing labeled poly(dG)·poly(dC) is digested with DNase I and electrophoresed on a denaturing gel, discrete subnucleosomal bands are seen (Figure 3). This, of course, is similar to the pattern obtained with authentic nucleosomes (Noll, 1974). The fact that discrete bands are obtained shows that the polynucleotide must be associated with protein in a defined structure. Figure 3 is inconsistent with a nonspecific association of histones with poly(dG)·poly(dC) to give an aggregate that happens to migrate in electrophoresis experiments with normal nucleosomes.

Other Polypurine·Polypyrimidines. We have also successfully reconstituted another polypurine·polypyrimidine, poly[d(A-G)·poly[d(T-C)], by direct mixing of heterogeneous-length polymer with chicken histones in 0.2 M NaCl (Ruiz-Carrillo et al., 1979). Limited digestion of the reconstitute yields a discrete band at ~145 base pairs in gel electrophoresis (Figure 4A). Although the band occurs on a high background of nonspecific fragments, a densitometer trace of the negative of the gel photograph (Figure 4B) clearly shows a single band, absent at zero time, that increases in size over the background with time of digestion. The high background is probably due to the inefficiency of the direct mixing procedure when used with poly[d(A-G)]·poly[d(T-C)]. The ability of synthetic poly[d(A-G)·poly[d(T-C)] to form nucleosomal particles was predicted by Satchwell et al. (1986) when they observed that 1 of 177 cloned chicken erythrocyte

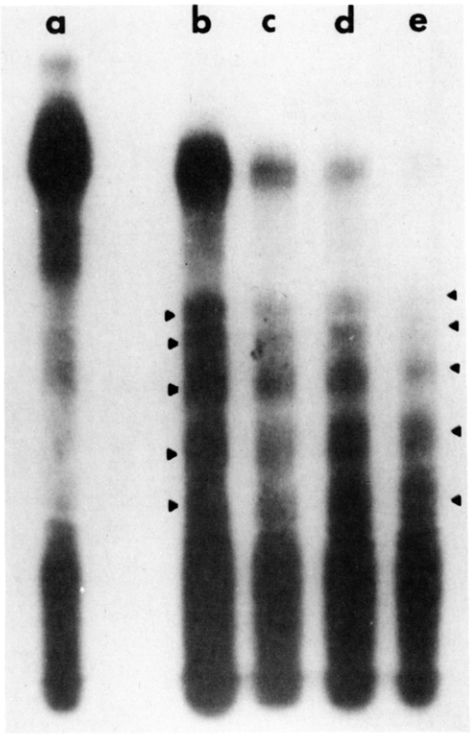

FIGURE 3: Autoradiograph of a DNase I digest of end-labeled poly(dG)·poly(dC) core particles. (Lanes a, b, c, d, and e) Digestion for 0, 1, 2, 5, and 10 min, respectively. The intense band near the top of lane a, and in decreasing intensity in lanes b–e, is core-length poly(dG)·poly(dC). The arrowheads point to subnucleosomal fragments, released upon DNase I digestion, that are readily observable in the original autoradiogram. The origin of the smear at the bottom of the autoradiogram in all lanes is not known.

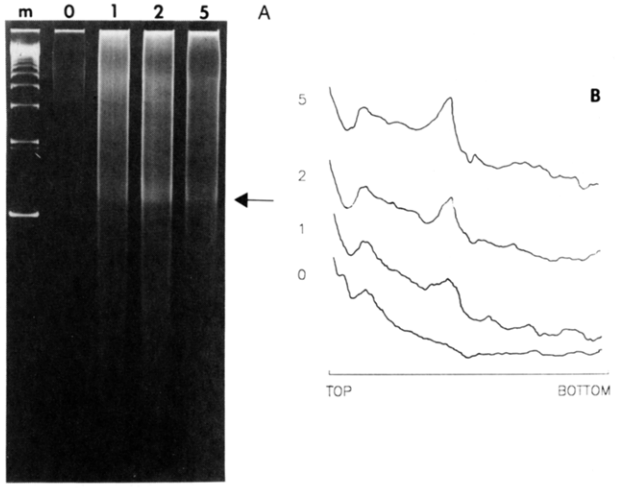

FIGURE 4: (A) Electrophoresis of a nuclease digest of poly[d(A-G)]·poly[d(T-C)] reconstituted by the direct mixing precedure. (Left lane) 123 bp ladder size standard; (right lanes) digestion for 0, 1, 2, and 5 minutes, respectively. Note the appearance above the background of the nucleosomal-length band (arrow) running slightly slower than the 123 bp standard. (B) Densitometer trace of the four sample lanes of panel A. The small arrow points to the nucleosomal-length DNA peak and corresponds to the band indicated by the large arrow in panel A.

nucleosomal DNAs consisted predominantly of alternating A-G residues.

Poly(dA)·poly(dT) (~150 bp) was also subjected to the salt-exchange reconstitution procedure. Unlike poly(dG)·poly(dC), however, reconstituted poly(dA)·poly(dT) runs near the top of the gel, perhaps indicating a nonspecific histone–polynucleotide aggregate (data not shown). In sedimentation experiments labeled poly(dA)·poly(dT) is found at the bottom of the centrifuge tube (data not shown).

Double-Stranded Polynucleotides Containing Both Ribose and Deoxyribose. Poly(rG-dC)·poly(rG-dC), a mixed ribose–deoxyribose polynucleotide that exists in the A conformation in solutions of moderate ionic strength (Wu & Behe, 1984), was exposed to chicken nucleosomes in the salt-exchange procedure. As seen in Figure 1C, lane r, the bulk of the reconstituted product migrates through 6% polyacrylamide with the same mobility as that of chicken nucleosomes (Figure 1A), running between the second and third molecular standard bands (lane m). Similarly, reconstituted poly(rG-dC)·poly(rG-dC) sediments to the same position as unlabeled chicken nucleosomes (Figure 2C).

When the DNA–RNA hybrid poly(rI)·poly(dC) is subjected to the salt-exchange reconstitution procedure, no band is seen to run similarly to chicken nucleosomes in a gel electrophoresis of the mixture (Figure 1D, lane r). Most of the labeled material runs at a position indicating a high molecular weight, probably a nonspecific aggregate. Neither is there any labeled material cosedimenting with unlabeled chicken nucleosomes when the poly(rI)·poly(dC) reconstitution mixture is sedimented through a 5%–20% sucrose gradient (Figure 2D).

Discussion

Polypurine·Polypyrimidines. We have shown here that the synthetic polypurine·polypyrimidines poly(dG)·poly(dC) and poly[d(A-G)]·poly[d(T-C)] can be reconstituted in vitro into nucleosomal structures. The fact that a synthetic polypurine·polypyrimidine sequence, and especially a homopurine·homopyrimidine sequence, can occur over the entire length of a nucleosome, including the central dyad, is surprising. Purine–purine sequences in DNA are thought to bend less readily and thus resist packaging into nucleosomes more than mixed sequences (Zhurkin, 1985; McCall et al., 1985). Apparently, however, the binding energy of these sequences in a nucleosome is sufficient to overcome their greater resistance to bending. One interesting implication of this is that the nucleotide sequence of the nucleosome-free region near the 5′ end of the chicken β-globin gene (McGhee et al., 1981), which contains a run of 19 contiguous guanosine residues, cannot by itself exclude nucleosomes but rather must rely at least in part on other mechanisms to keep it nucleosome free.

A question that arises is why the salt-exchange procedure is successful in reconstituting a polynucleotide that was resistant using other procedures (Simpson & Kunzler, 1979; Rhodes, 1979). Several factors that seem to be to the advantage of the salt-exchange procedure are the relatively low salt concentration used here for the exchange and the relatively short length and low concentration of the labeled poly(dG)·poly(dC) used here. Possible aggregation and triple-strand formation would be minimized by decreased polymer and salt concentration and decreased polymer length. Additionally, assaying for nucleosome formation by limited nuclease digestion, as was done previously (Simpson & Kunzler, 1979; Rhodes, 1979), of a long reconstituted polynucleotide presents a potential problem when the polynucleotide has an unusual structure. Since the assay depends on the differential susceptibility to digestion of histone-bound and unbound polynucleotide, it is possible that the nuclease used to probe the structure of the reconstitute will attack the bound and unbound polynucleotide regions at comparable rates, due to either the unusual structure of the polynucleotide per se or a looser binding of the polymer under investigation as compared to bulk DNA in chromatin, and thus miss detecting nucleosomes even when present.

The fact that polypurine·polypyrimidine sequences can form nucleosomes in vitro does not mean that they are as stable in such structures as heterogeneous-sequence DNA. Satchwell et al. (1986), upon sequencing 177 separate nucleosomal DNA sequences, observed that, although some oligo(dA) tracts appeared near the central dyad region, on average long tracts of oligo(dA) residues were underrepresented there. This indicates that such sequences bind less well to the dyad region than do other sequences. A reconstituted synthetic homopolymer, however, since it is constrained to bind to the nucleosome in places requiring a flexible sequence, must bind with a smaller free energy change than a flexible sequence. This implies that there is a range of binding energies in a nucleosome, depending on the exact sequence and nucleosomal location of the bound DNA.

Polymers Containing Ribonucleotides. The mixed ribo–deoxyribopolynucleotide poly(rG-dC)·poly(rG-dC) is a very versatile polymer. It is known to exist in the A conformation at moderate salt concentrations and to convert to left-hand helical Z forms at low or high monovalent cation concentration (Wu & Behe, 1984). In solutions containing tetrabutylammonium ions and lacking any competing cations, the polymer can even assume a conformation that yields a circular dichroism spectrum characteristic of B-form DNA (Jayasena & Behe, 1987). Under the solution conditions employed here, however, the unassociated polymer should be firmly in the A form, at least prior to association with histones. As shown in Figures 1C and 2C, the polymer can be reconstituted into a nucleosomal particle, comigrating with chicken nucleosomes in both electrophoretic and sedimentation experiments. Therefore, a double-stranded polynucleotide containing 50% ribonucleotide residues can form a nucleosome.

Is the polymer in an A conformation when bound to the surface of the nucleosome? This question is not easily addressed experimentally, but we think the answer is likely to be yes for several reasons. First of all, the results with poly(dG)·poly(dC) support the contention. From single-crystal studies of the self-complementary oligodeoxynucleotide d(GGGGCCCC) McCall et al. (1985) have shown that the sequence has a structure very similar to an A conformation, with a deep major groove and a helical repeat of ~11 bp/turn. Subsequently, from their crystal data they generated a model for poly(dG)·poly(dC) that fits a number of pieces of data from other experiments on the polymer, including X-ray fiber diffraction data (Langridge, 1969) and solution X-ray scattering studies (Bram, 1971). Thus they presented a strong case that poly(dG)·poly(dC) itself is in an A-like conformation in solution. Our reasoning here, then, is that if an A-like poly(dG)·poly(dC) can form a nucleosome (Figures 1B and 2B), then it is possible that poly(rG-dC)·poly(rG-dC) can form such a structure while in the A form.

Second, we consider the alternative conformations accessible to the polymer. The low- and high-salt Z forms are unattractive candidates for the conformation of the polymer while on the nucleosome since it has been shown that the Z form of poly[d(G-m^{5}C)]·poly[d(G-m^{5}C)] resists such packaging (Nickol et al., 1982; Ausio et al., 1987). The B form of the polymer also looks unattractive. This conformation is only seen in solutions of the polymer containing tetrabutylammonium ion as the *sole* counterion (Wu & Behe, 1984). Small concentrations of Na^+ immediately convert the polymer back to the A form. Additionally, tetraalkylammonium ions with shorter chain lengths (methyl, ethyl, etc.) and primary amines (methylamine, butylamine) do not support the B form of the polymer. Thus the lysine or arginine side chains of the core histones by themselves would not be expected to cause an A to B transition, and the high Na^+ concentrations in the exchange solution would inhibit it.

Perhaps poly(rG-dC)·poly(rG-dC) is induced into a state intermediate between A and B when bound in a nucleosome? Although a possibility, this line of reasoning does not strike us as having much force as an argument against either non-B-form polymers or DNA–RNA hybrids binding to nucleosomes. The argument immediately admits the existence of a non-B, intermediate conformation on the nucleosome and says nothing about the possibility of other polymers containing ribonucleotides being induced into similar hybrid architectures that could form nucleosomes.

Recently, a report was published indicating that DNA containing 5% or more ribonucleotides is "completely unable to form nucleosomes" (Hovatter & Martinson, 1987). It may be possible to reconcile that report with the present one by noting that the polymer we have studied is composed of a regularly repeating, self-complementary dinucleotide sequence, rG-dC. Hovatter and Martinson (1987), on the other hand, synthesized polymers containing ribonucleotides by nick-translation of DNA. Thus those polymers contained randomly placed ribonucleotides which apparently induce kinks into the DNA (Hovatter & Martinson, 1987). Perhaps when the polynucleotide is being bent around the nucleosome, kinks induced by randomly placed ribonucleotides are less easily accommodated than a smooth helix containing regularly spaced ribose residues.

The RNA–DNA hybrid poly(rI)·poly(dC) could not form, a nucleosome in the salt-exchange reconstitution system (Figures 1D and 2D). Since the results with poly(rG-dC)·poly(rG-dC) show that a polynucleotide containing 50% ribose residues can be accommodated in a nucleosome, there must be another explanation for the failure of poly(rI)·poly(dC) to reconstitute. One possibility is that the occurrence of contiguous ribonucleotides on one strand of the duplex makes it too stiff to bend around the histone octamer. Another possibility is that poly(rI)·poly(dC) is not intrinsically resistant to nucleosome formation but that the salt-exchange method is not able to provide the correct mechanism for reconstitution. We wish to emphasize this last point, noting that, like the polymers that were resistant to reconstitution in other systems but not to the salt-exchange method, an RNA–DNA hybrid might eventually be found to be able to reconstitute under other conditions.

Registry No. Poly(dG)·poly(dC), 25512-84-9; poly[d(A-G)]·poly[d(T-C)], 53351-74-9; poly(rG-dC)·poly(rG-dC), 84536-45-8.

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Specificity of Arginine Binding by the *Tetrahymena* Intron†

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ABSTRACT: L-Arginine competitively inhibits the reaction of GTP with the *Tetrahymena* ribosomal self-splicing intron. In order to define this RNA binding site for arginine, K_i's have now been measured for numerous arginine-like competitive inhibitors. Detailed consideration of the K_i's suggests a tripartite binding model. The dissociation constants of the inhibitors can be consistently interpreted if the guanidino group of arginine binds in the GTP site by utilizing the H-bonds otherwise made to the N1-H and 2-NH_2 of the guanine pyrimidine ring. The positive charge of the arginine guanidino group also enhances binding. A second requirement is for the precise length of the aliphatic arm connecting the guanidino with the α-carbon. The positive charge of the α-amino group is the third feature essential to effective inhibition. The negative carboxyl charge of arginine inhibits binding, and the substituents on the α-carbon are probably oriented, with the α-amino group near the phosphate backbone of the RNA. This orientation contributes strongly to the L stereoselectivity of the amino acid site on the RNA. When spaced optimally, net contribution to the free energy of binding is of the same order for the guanidino group and for the arginine α-carbon substituents, but the guanidino apparently contributes more to binding free energy. Taken together, these observations extend the previous binding model [Yarus, M. (1988) *Science (Washington, D.C.) 240*, 1751-1758]. The observed dependence of binding on universal characteristics of amino acids suggests that RNA binding sites with other amino acid specificities could exist.

Among the 20 biological amino acids, L-arginine alone is an effective inhibitor of the group I self-splicing reaction carried out by the *Tetrahymena* ribosomal intron (Yarus, 1988). L-Arginine inhibits more strongly than D-arginine, and inhibition is competitive with the attack of GTP on the RNA precursor. Arginine appears to block the guanosine (rG) site, without further effects on catalysis. Arginine could bind in the rG site, using the structural similarity between the guanidino group of the arginine side chain and an isosteric set of atoms on the guanosine six-membered ring (Yarus, 1988).

Such specific binding of an amino acid by an RNA has not been previously studied. The nature of the binding interaction bears on the origin of the genetic code, on the possibility of amino acid substrates for RNA enzymes, and on potential regulatory interactions between RNAs and amino acids (Yarus, 1988). Therefore, the arginine binding site on the intron has been probed with a set of arginine analogues which exemplify or alter all parts of the arginine molecule. The thermodynamic dissociation constants of these materials (the K_i's) are presented here. These data distinguish two possible binding modes for arginine and further resolve the sources of the binding free energy and L stereoselectivity shown by the RNA.

Experimental Procedures

Inhibitors. Materials of the highest commercially available purity were used, after storage in vacuo over P_2O_5. Solutions were adjusted to pH 8 if addition of inhibitor to splicing reactions at maximal concentrations changed the pH > 0.1.

L- and D-arginine from Chemical Dynamics, Fluka, and Sigma were compared without detectable differences. Guanidinoformic acid, guanidinoacetic acid, methylguanidine hydrochloride, ethylguanidine hydrochloride, butylguanidine hydrochloride, N^{α}-acetyl-L-arginine, N^{α}-benzoyl-L-arginyl ethyl ester, 6-guanidinocaproic acid, and 5-guanidinovaleric acid were obtained from Aldrich or the Bader Library of Rare Chemicals. L-2-Amino-3-guanidinopropionic acid and L-2-amino-4-guanidinobutyric acid were from Chemical Dynamics. Guanylurea sulfate was obtained from Eastman Kodak. Agmatine, L-argininamide, L-arginine methyl ester, L-arginine ethyl ester, L-leucyl-L-arginine, L-argininyl-L-leucine, L-homoarginine, L-canavanine, L-citrulline, creatine, creatinine,

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