Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 1162–1166, March 1987
Biochemistry

Ribonucleotide-induced helical alteration in DNA prevents nucleosome formation

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Communicated by Richard E. Dickerson, October 29, 1986

ABSTRACT **Several polynucleotides that assume an A-form helical structure in solution are unable to form nucleosomes. We attempted to establish a relationship between the ease of the A-form → B-form helix transition and ease of nucleosome formation by reconstituting nucleosomes using ribosubstituted DNA containing various levels of ribonucleotides. Instead we discovered that, when riboadenosine is substituted for deoxyriboadenosine, even one ribonucleotide per 125 base pairs of DNA reduces nucleosome formation and that DNA containing >5% ribonucleotide is completely unable to form nucleosomes. Ribosubstituted DNA restriction fragments exhibited altered mobility on native 6% polyacrylamide gels, indicating an altered helical structure (probably bending). The effects on both nucleosome formation and gel mobility are nucleotide specific and are correlated, being greatest for riboadenosine and decreasing in the order riboadenosine > riboguanosine > ribocytosine. The results are consistent with the hypothesis that the rate of nucleosome formation can be drastically reduced by isolated local perturbations, such as kinking or bending, in the helical structure of DNA.**

We are interested in the manner by which DNA helix structure modulates its own mode of packaging by histones in chromatin. In some cases the effects on packaging have obvious physiological implications. For example, the positioning of artificial nucleosomes on *Escherichia coli lac* operator DNA (1) constrains the DNA in such a way that the repressor binding surface of the DNA always faces outward to bind repressor (2). Eukaryotic examples include the specific positioning of nucleosomes on 5S gene DNA (3) so as to facilitate binding by transcription factor IIIA (4) and the avoidance by nucleosomes of poly(dA)·poly(dT) tracts in DNA (5, 6), which probably accounts for the promoter-activating properties of this sequence in chromatin (7).

It now appears that the sequence-specific positioning of nucleosomes and the sidedness with which DNA binds the histone core may be explained by the tendency of certain short sequence elements (8) (or sequence junctions; ref. 9) to bend. However, there is as yet no explanation why certain sequences cannot be folded into nucleosomes. Why does poly(dA)·poly(dT) refuse packaging when its intrinsically preferred pitch (10, 11) actually matches the pitch of nucleosomal DNA (8, 12) better than does the pitch of normal DNA? Or why are the A-form helices of double-stranded RNA (13) and RNA·DNA hybrids (14, 15) unable to generate nucleosomes (5, 16) when poly(dG)·poly(dC) in the reverse-handed Z-form apparently succeeds (17)?

To approach this question we decided to construct DNA·RNA mixed helices containing progressively lower ratios of ribo to deoxyribo residues with the objective of examining both the canonical A-form structure (i.e., the asymmetric 50:50 hybrid) and various ambiguous forms having reduced proportions of ribo residues. We hoped to establish a relationship between ease of nucleosome formation and ease of the A → B transition in ribo-containing helices. Instead we discovered, using substitution of riboadenosine into DNA by nick-translation, that nucleosomes will not form, under our conditions, for any DNA containing >5% ribonucleotide. Lower percentages of riboadenosine incorporation, even down to 0.4% ribonucleotide, yielded DNAs with reduced abilities to form nucleosomes. These results suggest that ribonucleotides interfere with nucleosome formation by two different mechanisms, depending on whether the ribo residues are abundant and contiguous (thus forming a uniform A helix) or sparse and scattered (thus yielding an interrupted B helix).

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MATERIALS AND METHODS

Nick-Translations. DNA, nicked with DNase I to an average single-strand length of >700 bases, was phenol extracted, ethanol precipitated, and suspended at 40 ng/μl together with DNA polymerase I (0.5 unit/μl; P-L Biochemicals) in 67 mM Tris Cl, pH 8.0/10 mM 2-mercaptoethanol containing bovine serum albumin at 50 μg/ml. Manganese (67 μM $MnCl_2$) was used as the divalent cation to induce the polymerase to accept the ribonucleotides as substrates (18). dNTP concentrations were varied from 50 to 100 μM and the ^{32}P-labeled ribonucleotide concentrations were varied from 10 to 330 μM depending on the level of ribosubstitution desired. Tracer amounts of [^{3}H]dNTP were included in the ribosubstitution reaction mixtures to permit determination of the level of ribosubstitution by comparison of ribo- and deoxyribonucleotide specific activities. Nick-translated [^{3}H]DNA was prepared in parallel reactions containing only dNTPs. The reaction mixtures were incubated at 16°C for 12–16 hr. The total amount of DNA recovered from each nick-translation was quantitated and the extent of nick-translation (i.e., the amount of recovered DNA that had been nick-translated) was estimated by dividing the radioactivity recovered in the DNA by the specific activity of the nucleotides used. As described below and in Table 1, we have designed the experiments so that we can calculate the exact percentage of ribosubstitution in the actual patches of nick-translated DNA (i.e., not an average for the overall preparation). When the extent of nick-translation, as defined above, is low, we assume that only one of the two strands in any small double-stranded fragment receives ribonucleotides. In the poly(dG-dT)·poly(dC-dA) experiments, this assumption becomes a certainty. In such cases, the percentage substitution for substituted double-strand fragments is taken to be half the calculated level of substitution for the single-strand nick-translated patches.

S1 Nuclease Treatment and *Hpa* II Digestion. Ribosubstituted DNAs were treated with Bethesda Research Laboratories enzymes under the conditions described by the manufacturer.

Reconstitutions. Salt (0.45 M)-washed oligonucleosomes were prepared as described elsewhere (19). Ribosubstituted high molecular weight DNAs or restriction fragments were reconstituted by mixing with oligonucleosomes in 1.25 M

NaCl. The solution was diluted stepwise to 0.5 M NaCl by addition of 10 mM Tris Cl (pH 8). The oligonucleosome mixture was dialyzed exhaustively against 50 mM NaCl/10 mM Tris Cl, pH 8. Final DNA concentrations ranged from 0.05 to 0.1 μg/l. High molecular weight samples were digested to yield nucleosomes using micrococcal nuclease (0.05 unit/ml; Sigma) in 1 mM $CaCl_2$ for 5–10 min at 37°C and then EDTA was added to 10 mM.

Salt/urea dialysis reconstitution of DNA fragments was carried out as described (20) with some modifications. Normal DNA fragments and DNA fragments cut from ribosubstituted DNA were mixed with carrier DNA extracted from calf thymus mononucleosomes. An equal mass of acid-extracted histones (5) was added to the DNA in the presence of 2 M NaCl/5 M urea. Final DNA concentration was 0.25 mg/ml. Final dialysis was against 50 mM NaCl/10 mM Tris Cl, pH 8/1 mM EDTA.

Samples were loaded onto a 5–20% sucrose gradient containing 50 mM NaCl and 10 mM Tris Cl, pH 8, and centrifuged (SW 41 rotor, 32,000 rpm, 17 hr, 17°C) to separate digested chromatin and free DNA. The gradients were fractionated and the fractions were counted for ^3H and ^{32}P in a liquid scintillation counter. Raw scintillation counts were corrected for background and crossover between channels. The tracer ^3H counts associated with ^{32}P ribosubstituted DNA were subtracted from each fraction. The amount of nick-translated DNA recovered in each fraction, expressed in nanograms, was obtained by multiplying the corrected counts by the specific activity of that DNA. The use of these data to calculate the recovery of ribosubstituted DNA relative to normal DNA in these experiments is illustrated in Table 1.

RESULTS

DNAs containing various levels of ribonucleotide were prepared by nick-translation in the presence of [^{32}P]ATP and of [^3H]dATP at a much lower specific activity to monitor the reaction. Mn^{2+} was used as the divalent cation to induce the polymerase to accept the ribonucleotides as substrates. Radioactive normal DNA was prepared for use as a standard by running a parallel nick-translation reaction using [^3H]dATP and lacking ribonucleotides.

The structural integrity of the ribosubstituted DNAs was assessed by S1 nuclease challenge and by mobility of restriction fragments on polyacrylamide gels. Nick-translated DNAs and ribosubstituted DNAs were treated for various lengths of time with S1 nuclease to determine whether any significant single strandedness existed in the regions of ribosubstitution. As shown in Fig. 1*A*, S1 treatment for up to 20 min does not alter the size distribution of either the nonsubstituted or the ribosubstituted DNA. Note that the ribosubstituted DNA is labeled in the ribo moiety and, therefore, at these low levels of substitution, the labeled DNA will monitor any S1 cutting at the ribo positions very sensitively. Comparable digestion of denatured calf thymus DNA reduced the single-strand length by a factor of >7 in <5 min (data not shown). As a further check of structural integrity, ribosubstituted DNA was cut with a restriction enzyme and migration of the resultant fragments through a 6% polyacrylamide gel was compared to unsubstituted DNA. As shown in Fig. 1*B*, DNA restriction fragments substituted with [^{32}P]riboadenosine have a typical restriction pattern on the gel, confirming their intact double-stranded character. However, substitution with riboadenosine does alter the restriction pattern in slight but significant ways, reducing mobilities and causing blurring of the bands (compare the autoradiographic and ethidium bromide-stained bands in the "17" lanes; note that in these lanes riboadenosine represents 17% of total substituted "adenine"; thus, riboadenosine represents about 4% of the total bases in the substituted strand, which means only 2% ribosubstitution in the double-stranded fragments). The appearance of blurred bands rather than bands of discretely altered mobility presumably reflects the randomness of ribonucleotide location along the lengths of the restriction fragments (see ref. 21). Thus although ribosubstitution of DNA to the levels used in these experiments does not lead to gross abnormalities in the resulting DNA, it does give rise to some structural alteration in the DNA.

DNAs prepared as above were induced to form nucleosomes by exchange using a salt dilution protocol. Salt (0.45 M)-washed oligonucleosomes prepared from calf thymus were used as the source of histones and were mixed with the experimental DNAs in 1.25 M NaCl. The salt was then slowly diluted to 50 mM to allow histone–DNA reassociation and nucleosome formation. The resultant material was digested with micrococcal nuclease and sedimented through a 5–20% sucrose gradient, and the radioactivity in the fractions was determined by liquid scintillation counting. The results of such an experiment are shown in Fig. 2.

As shown in Fig. 2*A*, comparison of the recovery of ribosubstituted DNA with that of normal DNA in the nucleosomes demonstrates that DNA containing as few as one ribonucleotide per 8 base pairs (i.e., one ribonucleotide per 8 bases in the substituted strand) is unable to compete with carrier calf thymus DNA for histones to form nucleosomes. Moreover, as shown in Fig. 2*B*, formation of nucleosomes from ribosubstituted DNA (open symbols) is disfavored relative to normal DNA (closed symbols) even at the level of one ribonucleotide per 125 base pairs.

We considered carefully the method to use to calculate relative recoveries of DNA and ribosubstituted DNA in monosomes, as this bears significantly on the interpretation of the results. For example, the use of the average overall specific activity of the nick-translated preparation of DNA (Table 1, line IX) to calculate recoveries in reconstituted nucleosomes is potentially misleading if the extent of nick-translation varies significantly from molecule to molecule of DNA. Thus, in the extreme, if nick-translation under our conditions is highly processive, a minority of the DNA fragments might contain all of the enzymatically introduced ribonucleotides. Exclusion of these highly substituted fragments from nucleosomes would lead to the incorrect conclusion that a low *average* extent of ribosubstitution is not tolerated by nucleosomes. Therefore, to estimate correctly the degree of ribosubstitution we included low levels of [^3H]dATP in the ribosubstitution nick-translation reaction mixtures to allow us to monitor the reactions accurately by double-channel scintillation counting. The use of tracer levels of tritium during the ^{32}P ribosubstitution allows determination of both the actual amount of DNA nick-translated (Table 1, line X) and the actual percentage of ribosubstitution in those specific DNA molecules nick-translated (Table 1, line VIII). All calculations involving DNA specific activities are based on the corrected specific activities (Table 1, line XI) applicable to that portion of the DNA preparation that is actually nick-translated. The only assumption involved in this aspect of the work is that when riboadenosine and deoxyriboadenosine are present simultaneously during a nick-translation reaction, they are incorporated randomly with respect to each other. We believe that this assumption is almost certainly correct, especially for the poly(dG-dT)·poly(dC-dA) experiments (described below) in which adenines can never be incorporated adjacent to each other and there is therefore no opportunity for any processivity for either deoxyribo- or riboadenosine to the exclusion of the other.

We considered the possibility that micrococcal nuclease might preferentially attack ribosubstituted DNA so as to reduce recovery of nucleosomes containing ribonucleotides. To exclude this possibility, nucleosomes were formed direct-

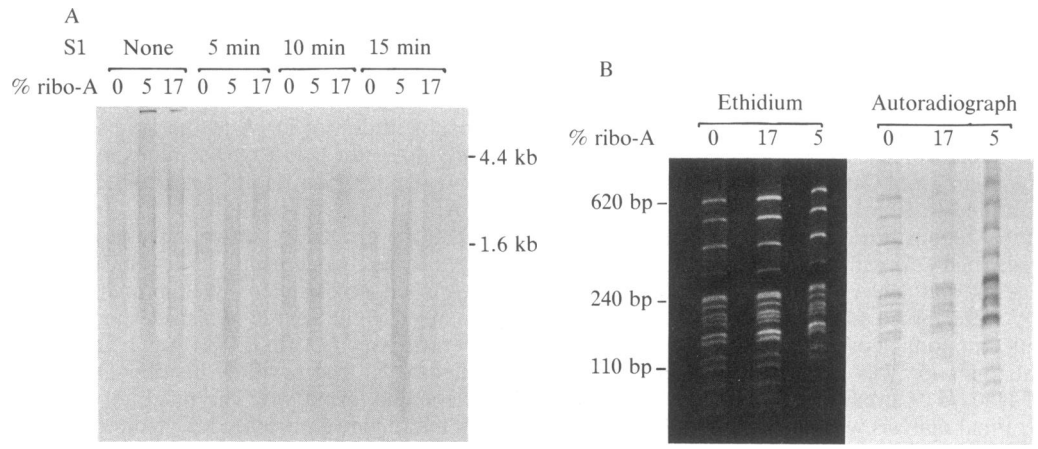

FIG. 1. Ribosubstituted DNA is structurally altered but is double stranded. Riboadenosine-substituted and nonsubstituted pBR322 plasmid DNAs were prepared and digested with either S1 nuclease or *Hpa* II. (*A*) DNAs were untreated or were treated with S1 nuclease for 5, 10, or 15 min as indicated and compared by electrophoresis through a native 1.5% agarose gel. Lanes: 0, deoxy[^{32}P]riboadenosine nick-translated DNA (i.e., nonribosubstituted DNA); 5, DNA in which 5% of the "substituted adenine" is [^{32}P]riboadenylate; 17, DNA in which 17% of the "substituted adenine" is [^{32}P]riboadenylate. (*B*) DNAs similar to those in *A* were digested with *Hpa* II and electrophoresed on a native 6% polyacrylamide gel. Ethidium bromide staining and an autoradiograph of the gel are shown. ribo-A, riboadenosine; kb, kilobases; bp, base pairs.

ly on monosome-length DNA restriction fragments, thus eliminating the micrococcal nuclease step. Monosome-length ^{32}P-labeled ribosubstituted DNA fragments were obtained by digestion of nick-translated pBR322 DNA with *Hpa* II followed by electrophoretic purification of the appropriate fragments. These were mixed with similarly prepared ^{3}H-labeled nonribosubstituted DNA and reconstituted by exchange with calf thymus nucleosomes. This method of reconstitution has been shown to produce normal core particles as determined by both sedimentation and DNase I digestion patterns (1, 22).

Sucrose gradient profiles of two such experiments are shown in Fig. 3. These results, using restriction fragments, confirm the results obtained by micrococcal nuclease digestion. As shown in Fig. 3*A*, DNA ribosubstituted to the level

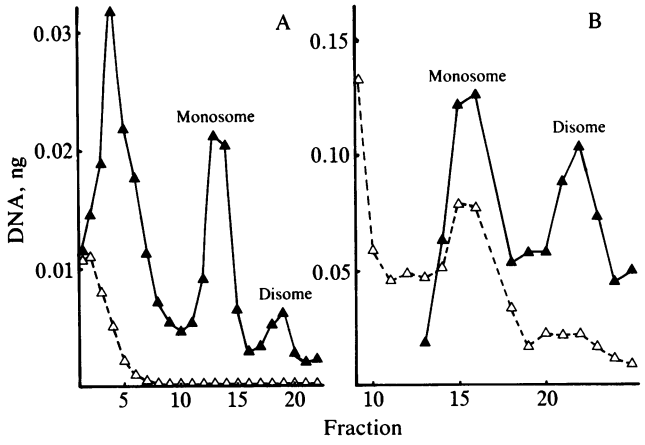

FIG. 2. Plasmid DNA that contains ribonucleotides is greatly disfavored in nucleosome formation. Riboadenosine-substituted DNAs were prepared, exchange reconstituted, micrococcal nuclease digested, and separated on sucrose gradients. (*A*) △, ^{32}P-labeled plasmid DNA containing 6.5% ribonucleotides (13% in the substituted strand); ▲, ^{3}H-labeled nonribosubstituted plasmid DNA. Ribosubstituted DNA was recovered <0.1% as well as unsubstituted DNA in monosomes. (*B*) △, ^{32}P-labeled plasmid DNA containing 0.4% ribonucleotides (0.8% in the substituted strand); ▲, ^{3}H-labeled nonribosubstituted plasmid DNA. Ribosubstituted DNA was recovered 60% as well as unsubstituted DNA in monosomes. Amounts of nick-translated DNA, as plotted, are normalized to represent equal loads of ribosubstituted DNA and normal DNA onto the sucrose gradients.

of approximately two or three ribonucleotides per double-stranded fragment (open symbols) is recovered as monosomes only 50% as well as a similar amount of unsubstituted DNA (closed symbols; see Table 1 for a sample calculation of how the relative recovery of ribosubstituted DNA in monosome was determined). Similarly (Fig. 3*B*), DNA containing one or two ribonucleotides per fragment (open symbols) was recovered as monosomes only 80% as well as normal DNA (closed symbols). Moreover, the experiments illustrated in Fig. 3, as well as additional experiments (not shown), demonstrate the quantitative reliability of the exchange method of reconstitution: there is a clear inverse relationship between the amounts of normal DNA and of ribosubstituted DNA recovered in the monosome versus free DNA peaks of the gradients. For example, in the case of ribosubstituted DNA the decrease in recovered monosomes is mirrored, though not always to a quantitatively exact extent, by an increase in the free ribosubstituted DNA peak. This inverse relationship clearly indicates that reduction in recovery of nucleosomes containing ribonucleotides is due to a reduced ability of such nucleosomes to form and not due primarily to loss of ribosubstituted DNA in the experimental protocol (e.g., loss due to aggregation).

To determine whether the particular method of reconstitution chosen might have an effect on recovery of ribosubstituted DNA in monosomes, DNA fragments similar to those used above were reconstituted using a salt/urea dialysis protocol in which DNA and denatured histones are mixed in 2 M NaCl and 5 M urea. The salt and urea are then slowly dialyzed out, allowing nucleosomes to form. Despite the reduced overall efficiency of reconstitution in the salt/urea method, comparison of recovery for ribosubstituted DNA (Fig. 4, open symbols) and unsubstituted DNA (closed symbols) for the two reconstitution methods shows that ribosubstituted DNA (containing about seven ribonucleotides per fragment) is impaired to similar extents in nucleosome formation whether assayed by exchange (Fig. 4*A*) or by salt/urea dialysis reconstitution (Fig. 4*B*).

To determine whether the dramatic effects of ribosubstitution are attributable solely to conformational effects localized at the sugar residue or whether other DNA helix conformational changes are involved, we conducted experiments similar to those of Fig. 2 but using ribocytosine and riboguanosine. The alternating copolymer poly(dG-dT)·poly(dC-dA) was used in these experiments to eliminate effects of heterogeneous sequence. As shown in Fig. 5, all ribosubsti-

Table 1. Sample data manipulation

	DNA	Ribosubsti-tuted DNA
Level of ribosubstitution and specific activity of nick-translated DNAs		
Raw data		
I. Specific activity of deoxy[^{3}H]riboadenosine, cpm/ng	1.58×10^4	2.2×10^2
II. Specific activity of [^{32}P]riboadenosine, cpm/ng	—	1.02×10^5
III. Deoxy[^{3}H]riboadenosine incorporated, cpm	4.78×10^5	8.12×10^3
IV. [^{32}P]Riboadenosine incorporated, cpm	—	8.84×10^4
V. Total DNA, ng	455	455
Calculated data		
VI. Deoxyriboadenosine recovered in DNA (III/I), ng	30.3	36.6
VII. Riboadenosine recovered in data (IV/II), ng	—	0.87
VIII. Substituted riboadenosine as percentage of total substituted adenosine [VII × 100/(VI + VII)]	—	2.3
IX. Specific activity of total DNA [(III or IV)/V], cpm/ng	1050	194
X. Amount of DNA actually nick-translated [(VI + VII) × 3.95], ng	121	150
XI. Specific activity of DNA actually nick-translated [IX × (V/X)], cpm/ng of nick-translated DNA	3950	589
Relative recovery of DNAs in monosomes		
XII. Input nick-translated DNA, cpm	4.78×10^5	8.84×10^4
XIII. Input nick-translated DNA (XII/XI), ng	121	150
XIV. Pooled monosome fractions, cpm	8.45×10^4	2.38×10^3
XV. Nick-translated DNA recovered in monosomes (XIV/XI), ng	21.4	4.04
XVI. % recovery of nick-translated DNA [(XIV/XII) × 100 or (XV/XIII) × 100]	17.7	2.7
XVII. Recovery of ribosubstituted DNA relative to normal DNA [(2.7/17.7) × 100], %	—	15.3

tuted DNAs tested (open symbols) were recovered in nucleosomes less well than normal DNA (closed symbols). However, the effect of riboadenosine (open diamonds) is considerably more pronounced than that of riboguanosine (open triangles) or ribocytosine (open squares). We therefore conclude that the effects of ribosubstitution are mediated through fundamental localized alterations in double-helical DNA structure and are not the simple consequence of histone–ribose interactions *per se*.

DISCUSSION

We have shown that a low level of ribosubstitution in DNA profoundly reduces the ability of DNA to form nucleosomes.

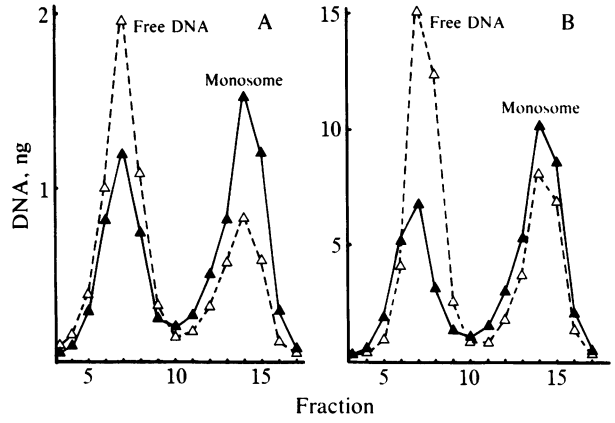

FIG. 3. Ribonucleotide-substituted restriction fragments resist being folded into nucleosomes. Riboadenosine-substituted pBR322 plasmid DNA was digested with *Hpa* II and fragments ranging from 147 to 201 base pairs were recovered from a native 6% polyacrylamide gel. DNAs were exchange reconstituted and separated on sucrose gradients. (*A*) △, DNA fragments containing 0.8% ^{32}P-labeled ribonucleotides (1.6 ribonucleotides per 100 base pairs); ▲, ^{3}H-labeled unsubstituted DNA. The ribosubstituted DNA was recovered 50% as well as unsubstituted DNA in monosomes. (*B*) △, DNA fragments containing 0.4% ^{32}P-labeled ribonucleotides (1 ribonucleotide per 125 base pairs); ▲, ^{3}H-labeled unsubstituted DNA. The ribosubstituted DNA was recovered 80% as well as the unsubstituted DNA in monosomes. Amounts of nick-translated DNA, as plotted, are normalized to represent equal loads of ribosubstituted DNA and normal DNA onto the sucrose gradients.

The reconstitution experiments presented here indicate that 2 or 3 ribonucleotides per 150 base pairs are sufficient to reduce nucleosome formation, in competition with normal DNA, by >50%. Moreover, the presence of even a single ribonucleotide per 125 base pairs of DNA measurably reduces nucleosome formation.

Both salt/urea dialysis and exchange reconstitution give similar results (Fig. 4), suggesting either that nucleosomes containing ribosubstituted DNA are of drastically decreased stability or that a fundamental step during assembly is being curtailed. Although the structural basis of this ribosubstitution effect is obscure, we note the surprising fact that very low levels of ribosubstitution give rise to changes in helix structure that can be detected on polyacrylamide gels (Fig. 1). These helical changes in ribosubstituted DNA are produced in the absence of any increase in sensitivity to S1 nuclease cleavage and are therefore not due to partial denaturation of the ribosubstituted fragments. These results

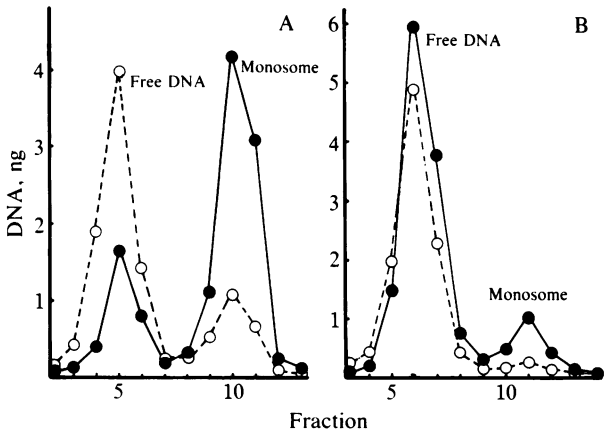

FIG. 4. Method of reconstitution has little effect on recovery of ribosubstituted DNA in monosomes. (*A*) Exchange reconstitution. The ribosubstituted DNA is recovered 33% as well as the unsubstituted DNA. (*B*) Salt/urea dialysis reconstitution. The ribosubstituted DNA is recovered 39% as well as unsubstituted DNA. ○, DNA restriction fragments containing about 7 ribonucleotides per fragment; ●, unsubstituted DNA. Amounts of nick-translated DNA, as plotted, are normalized to represent equal loads of ribosubstituted DNA and normal DNA onto the sucrose gradients.

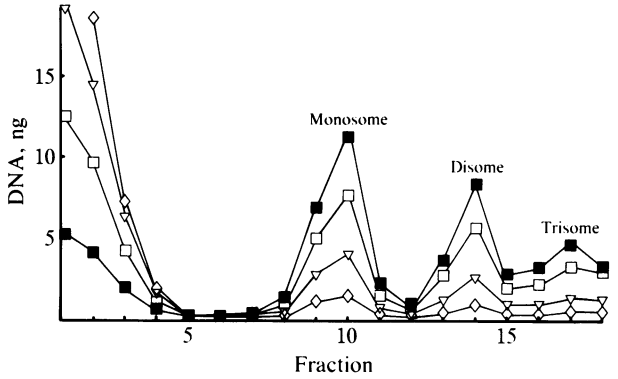

FIG. 5. The effect of ribosubstitution is nucleotide specific. The alternating copolymer poly(dG-dT)·poly(dC-dA) was nick-translated with different ribonucleotides. DNAs were exchange reconstituted, digested with micrococcal nuclease, and separated on sucrose gradients as described for Fig. 2. ■, Unsubstituted DNA; □, DNA substituted with ribocytosine (0.5% ribonucleotide or 1 ribonucleotide per 100 base pairs); ▽, DNA substituted with riboguanosine (1% ribonucleotide or 1.9 ribonucleotides per 100 base pairs); ◇, DNA substituted with riboadenosine (0.6% ribonucleotide or 1.2 ribonucleotides per 100 bp). Amounts of nick-translated DNA, as plotted, are normalized to represent equal loads of ribosubstituted DNA and normal DNA onto the sucrose gradients.

are reminiscent of the analysis by Wu and Crothers (21) showing a relationship between DNA bending and mobility on polyacrylamide gels. Perhaps ribosubstituted DNA is bent.

How could substitution of a single ribonucleotide lead to a structural consequence (bending?) of such significance? The phosphodiester backbone of DNA is generally regarded as being a relatively passive and elastic structural component of the double helix (23–25). However, the results of ribosubstitution suggest that this may not be the case since introduction of a single hydroxyl group into the backbone of a long DNA can have such a dramatic effect. On the other hand, perhaps we should view the apparent ease with which ribosubstitution distorts the helix as an actual manifestation of helix flexibility, possibly in a manner similar to that proposed by Sobell *et al.* (26) for spontaneous kinking of DNA at thermal energies. Indeed, Sobell *et al.* suggested that such kinking would involve a mixed sugar pucker in the DNA backbone of a type resembling the C3′ endo pucker preferred by ribonucleotides both in solution (27, 28) and in RNA (13) (but shunned by deoxyribonucleotides, which tend to adopt a C2′ endo pucker).

One might predict that the extent to which ribo residues stabilize such kinks would vary depending on the sequence environment around the ribonucleotide as well as on the specific identity of the ribonucleotide itself. Indeed, low level substitution of riboadenosine in DNA fragments reduces mobility and produces significant band blurring in polyacrylamide gels (Fig. 1), whereas riboguanosine produces little and ribocytosine produces none under similar conditions (data not shown). As shown in Fig. 5, the effect of the different ribonucleotides on the ability of DNA to reconstitute varies similarly. It is clear from these results that the effect of the 2′ hydroxyl is not localized merely to the sugar moiety of the nucleotide. Rather, changes in base stacking must be the principal source of the effects we observe. As one would expect, pyrimidine ribonucleotides, which produce a smaller interchain steric clashing effect (25), are better tolerated in nucleosomes than purines (Fig. 5).

Helical alterations in DNA of the type we have proposed would also involve an angular unwinding (26) at the site of the ribonucleotide. Possibly both of these effects in concert account for the surprising ability of even a single ribo residue in DNA to impair nucleosome formation. However, it seems unlikely to us that ribosubstituted DNA forms nucleosomes poorly because ribo-containing nucleosomes are of markedly reduced stability. Indeed, even Z-DNA has been reported to form stable nucleosomes (17). Rather we hypothesize that, at some critical step in the pathway toward nucleosome formation, histones are unable to interact productively with DNA when it is in the altered, ribonucleotide-stabilized structure. There would thus be a large activation energy for nucleosome formation, corresponding to a requirement for overcoming these local helical alterations in the DNA prior to productive interaction with the histones, and ribo-containing DNA would therefore compete poorly with normal DNA for nucleosome formation.

This work was supported by National Science Foundation Grant DMB 83-16920.

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