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Interaction of Nucleolar Phosphoprotein B23 with Nucleic Acids†

Tamba S. Dumbar,‡ Glenn A. Gentry,§ and Mark O. J. Olson*,‡
Departments of Biochemistry and Microbiology, The University of Mississippi Medical Center, 2500 North State Street, Jackson, Mississippi 39216-4505

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ABSTRACT: The interaction of eukaryotic nucleolar phosphoprotein B23 with nucleic acids was examined by gel retardation and filter binding assays, by fluorescence techniques, and by circular dichroism. All studies utilized protein prepared under native conditions by a newly developed purification procedure. Electrophoretic gel mobility shift assays with phage M13 DNA suggested that protein B23 is a single-stranded nucleic acid binding protein. This was confirmed in competition binding assays with native or heat-denatured linearized plasmid pUC18 DNA where the protein showed a marked preference for the denatured form. In other competition assays, there was no apparent preference for single-stranded synthetic ribo- versus deoxyribonucleotides. Equilibrium binding with poly(riboethenoadenylic acid) indicated cooperative ligand binding with a protein binding site size of 11 nucleotides and an apparent binding constant ($K\omega$) of 5×10^7 M^{-1} which includes an intrinsic binding constant (K) of 6.3×10^4 M^{-1} and a cooperativity factor (ω) of 800. In circular dichroism (CD) studies, protein B23, when combined with the single-stranded synthetic nucleic acids poly(rA) and poly(rC), effected a decrease in ellipticity and a shift of the positive peak at 260-270 nm toward higher wavelengths, indicating helix destabilizing activity. No CD changes were seen with double-stranded poly(dA·dT). The change in ellipticity of poly(rA) was sigmoidal upon addition of protein, confirming the cooperative behavior seen with fluorescence methods. These studies indicate that protein B23 binds cooperatively with high affinity for single-stranded nucleic acids and exhibits RNA helix destabilizing activity. These features may be related to its role in ribosome assembly.

Phosphoprotein B23 (M_r/pI = 38K/5.1) is a eukaryotic RNA-associated nucleolar protein whose sequence has been recently determined in several species including rat (Chang et al., 1988), mouse (Schmidt-Zachmann et al., 1988), human (Chan et al., 1989), and *Xenopus laevis* (NO38) (Schmidt-Zachmann et al., 1987). The close association of the protein with RNA is suggested by the fact that it is localized to the granular region of the nucleolus (Ochs et al., 1983; Michalik

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‡ Department of Biochemistry.
§ Department of Microbiology.

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et al., 1981; Spector et al., 1984), which contains the more mature ribonucleoprotein particles (RNP)[1] on their way to becoming ribosomes. This is in contrast to nucleolin (C23), the other major nucleolar protein which is localized to the dense fibrillar regions which contain the nascent RNP complexes of the nucleolus (Escande et al., 1985). The RNA association of protein B23 is also suggested by the observation that it translocates to the nucleoplasm following treatment of cells with inhibitors of RNA synthesis such as actinomycin D (Yung et al., 1985a,b, 1986; Chan et al., 1988).

A number of RNA binding proteins, including the hnRNP proteins (Chung & Wooley, 1986; Cobianchi et al., 1988), the U1 RNA binding protein (Theissen et al., 1986), or the poly(A) binding protein (Adam et al., 1986; Sachs et al., 1986) and nucleolin (Lapeyre et al., 1986, 1987), share sequence elements in common. In fact, the RNA binding regions of nucleolin (Bugler et al., 1987; Sapp et al., 1989) contain segments called RNP consensus sequences (Adam et al., 1986) which are highly conserved among these proteins. Therefore, these proteins appear to constitute a gene family of RNA binding proteins. On the other hand, although protein B23 has many properties which are similar to those of nucleolin (e.g., phosphorylation, the presence of highly acidic segments, and the ability to bind single-stranded nucleic acids), it lacks a well-defined RNP consensus sequence, and the overall sequences of the two proteins appear to be unrelated. Thus, it seems likely that the mode of interaction of protein B23 with nucleic acids is different from the above RNA binding proteins.

Although the binding of nucleic acids to nucleolin has been extensively investigated (Olson et al., 1983; Sapp et al., 1986, 1989), little is known about the interaction of protein B23 with RNA or DNA. These studies were undertaken to elucidate the nature of association of protein B23 with nucleic acids, i.e., the relative affinity for duplex DNA versus single-stranded DNA and for RNA versus DNA, the thermodynamic parameters that describe the interaction, and whether the protein has nucleic acid helix destabilizing activity. It was found that protein B23 binds single-stranded nucleic acids cooperatively and with high affinity and that upon binding the protein may alter nucleic acid secondary structure.

Materials and Methods

Animals, Cells, and Nucleoli. Novikoff hepatoma ascites cells were propagated in male Sprague-Dawley rats, and the cells were harvested 6 days after transplantation and used for making nucleoli. Nucleoli were prepared by the magnesium–sucrose sonication procedure (Rothblum et al., 1977) and stored in 10 mM Tris-HCl, pH 7.9, 30% glycerol, 1 mM DTT, 1 mM PMSF, 10 μg/mL leupeptin, 1 μM pepstatin A, and 1 μM aprotinin (storage buffer) at −80 °C.

Purification of Protein B23. The following protease inhibitors at the indicated final concentrations were used in all buffers throughout the purification: 0.1 mM PMSF, 1 μM pepstatin A, 1 μM aprotinin, and 10 μg/mL leupeptin. A nucleolar extract was prepared under low ionic strength conditions as previously reported (Herrera & Olson, 1986) with the following modifications: the nucleoli were suspended by gentle homogenization in 5 mM Tris-HCl (pH 7.4), containing 1 mM EDTA, for 10 min at 4 °C and centrifuged at 5000*g* for 10 min. Prior to use, the supernatants (extracts) were stored at −80 °C after dilution with storage buffer. The extract was digested with 5 μg/mL RNase A at 4 °C for 1 h, diluted with 3 volumes of buffer H1 (50 mM Tris-HCl, pH 7.9, 5 mM $MgCl_2$, and 1 mM EDTA), and applied to an 8-mL heparin–Sepharose (Pharmacia) column (1.5 cm × 5 cm). The column was washed successively with 4 volumes of buffer H1, 2 volumes of buffer H2 [H1 + 20 mM $(NH_4)_2SO_4$], 2 volumes of buffer H3 [H1 + 50 mM $(NH_4)_2SO_4$], and 2 volumes of buffer H4 [H1 + 100 mM $(NH_4)_2SO_4$]. Protein B23 was eluted by a linear gradient of 20 mL of buffer H5 [H1 + 350 mM $(NH_4)_2SO_4$] and 20 mL of buffer H4. Near the end of this gradient, a portion of protein B23 coeluted with nucleolin. The latter fractions were dialyzed against buffer H1 and purified to homogeneity via chromatography on columns of double- or single-stranded DNA–cellulose obtained from Sigma Chemical Co. (see Results). Briefly, the fractions were loaded on DNA–cellulose columns (1.5 cm × 3 cm) followed by stepwise elution with buffer H1 containing increasing concentrations of KCl. The protein was concentrated via dialysis against 10% PEG 20000 (Sigma Chemical Co.) in buffer H1 or via ultrafiltration using Centriprep concentrators (Amicon). Protein concentrations were determined spectrophotometrically by using the Pierce BCA assay and/or by amino acid analysis.

Nucleic Acids. Poly(rA), poly(rC), poly(rU), poly(dA), and poly(dC) were obtained from Sigma Chemical Co.; poly(dA·dT) and poly(ethenoadenylic acid) were obtained from P-L Biochemicals. All polynucleotides were dissolved in 5 mM Tris–1 mM EDTA, pH 7.5, and the concentrations were determined spectrophotometrically by using the following extinction coefficients in units of M^{-1} cm^{-1}: poly(rA) = 9400 at 260 nm, poly(rC) = 6500 at 267 nm, poly(rU) = 9200 at 260 nm, poly(dA·dT) = 6600 at 262 nm, poly(dA) = 10000 at 257 nm, and poly(dC) = 7200 at 270 nm (Kowalzcykowski et al. 1981); poly(ethenoadenylic acid), EM(P) = 3700 at 257 nm (Ledneva et al., 1978). Single-stranded M13mp7 DNA was a gift from Dr. S. T. Case of this department, and pUC18 duplex DNA was purchased from Bethesda Research Labs and linearized by digestion with restriction enzyme *Eco*RI. Poly(A−) RNA was prepared by standard procedures via oligo(dT)–cellulose chromatography. rRNA (5 S) was obtained from Boehringer Mannheim. RNA was 5′ end labeled with [γ-^{32}P]ATP and polynucleotide kinase (Richardson, 1965) or 3′ end labeled with [^{32}P]pCp and T4 RNA ligase (England & Uhlenbeck, 1978).

Gel Retardation Assays. Protein B23–M13 ssDNA complexes were formed in 10 mM Tris-HCl, pH 7.5 40 mM KCl, 50 μg/mL BSA, and 1 mM DTT (Sapp et al., 1986). The DNA (0.5 μg) or 8 nmol (nucleotides) was heated at 65 °C for 5 min and quick-cooled on ice, and the binding reaction was quickly constituted with various concentrations of protein in the range of 0–10 μg. After incubation at 0 °C for 30 min, the reaction mixture was resolved on 1.2% agarose gels cast in 0.1 M Tris–acetate buffer, pH 8.0. Electrophoresis was for 90 min at 50 V. The protein–DNA complexes were visualized by ethidium bromide staining.

Filter Binding Assays. Binding was done by using conditions similar to those of Olson et al. (1983). The binding reaction was run in 0.200 mL of a binding buffer containing 20 mM Tris-HCl (pH 7.5), 0.1 mM DTT, 0.1 mM EDTA, 5 mM $MgCl_2$, 150 mM KCl, 50 μg/mL bovine serum albumin (Bethesda Research Laboratories), 5 nM labeled RNA, and 0.1 μM protein B23 plus varying concentrations of unlabeled

[1] Abbreviations: PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; CD, circular dichroism; RNP, ribonucleoprotein particle; hnRNP, heterogeneous nuclear ribonucleoprotein particle; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB, single-stranded nucleic acid binding protein; poly(rεA), poly(etheno-adenylate); PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid.

competitors. The binding mixtures were incubated at room temperature for 15 min prior to filtration through nitrocellulose membranes (S&S BA 0.45 μm). The filters were air-dried, and the fraction of RNA bound was quantitated by Cerenkov counting. Two kinds of plasmid DNAs were used as unlabeled competitors: native linearized pUC18 as duplex DNA and single-stranded DNA generated by heating the linearized double-stranded DNA at 100 °C for 5 min followed by rapid cooling on ice. In other experiments, the synthetic homopolynucleotides poly(rA), poly(dA), poly(rC), and poly(dC) were used as competitors.

Fluorescence Experiments. Fluorescence measurements were taken with a Perkin-Elmer 650-40 fluorescence spectrophotometer. The excitation and emission wavelengths (excitation 330 nm, emission 410 nm) were set by comparison to an etheno-AMP standard (P-L Biochemicals) (Cobianchi et al., 1988). Poly(riboethenoadenylic acid) at a concentration of 5 μM (nucleotides) was titrated at room temperature by additions of small volumes of protein to 300 μL of binding buffer (10 mM Tris, 1 mM EDTA, pH 7.5, and 10 mM NaCl). The measurements were corrected for volume changes due to protein addition. Analysis of the binding data was done within the PROPHET system,[2] a comprehensive scientific computing resource that was run in a UNIX environment on a Digital Equipment Corp. Microvax II computer.

Circular Dichroism. Circular dichroism (CD) spectra were measured with a Jasco J500A spectropolarimeter interfaced to and controlled by an IBM PC computer. All CD spectra were taken at 16 °C in water-jacketed cells. For each sample, a minimum of nine spectra were accumulated and averaged by the computer. The binding mixtures contained 10 μM (nucleotides) each polymer and varying concentrations of protein (0–10 μg) in a binding buffer of 5 mM Tris-HCl, pH 7.4, 10 mM NaCl, and 10% glycerol (Nowak et al., 1980; Karpel & Burchard, 1980).

Analytical Methods. SDS–polyacrylamide gel electrophoresis was performed according to Laemmli (1970) utilizing 12.5 or 15% acrylamide concentrations. Protein aliquots from column fractions were concentrated for electrophoresis via precipitation in 10% TCA, followed by washing with ether–ethanol to remove the TCA (Valenzuela et al., 1976). The protein samples were dried in a Speed Vac, dissolved in SDS sample buffer, and electrophoresed in a Bio-Rad Mini Protean Two apparatus.

Results

Purification of Protein B23. A low ionic strength extract of nucleoli was prepared from 6×10^{10} cells. This nucleolar fraction is enriched in protein B23 and nucleolin but also contains a variety of other polypeptides (Figure 1A, lane E). To release protein-bound RNA, the extract was digested with ribonuclease A (RNase) prior to being loaded on a heparin–Sepharose column (Figure 1A). Most of the RNA and a few proteins eluted in the flow-through and in the initial steps of increased ionic strength (data not shown). With a linear gradient, protein B23 eluted between 300 and 400 mM ammonium sulfate (fractions 11–14) essentially as a single polypeptide (Figure 1A). However, a portion of protein B23 coeluted with nucleolin between 400 and 500 mM salt. In order to increase the yield of protein B23, the fractions containing the two proteins were pooled, dialyzed against buffer

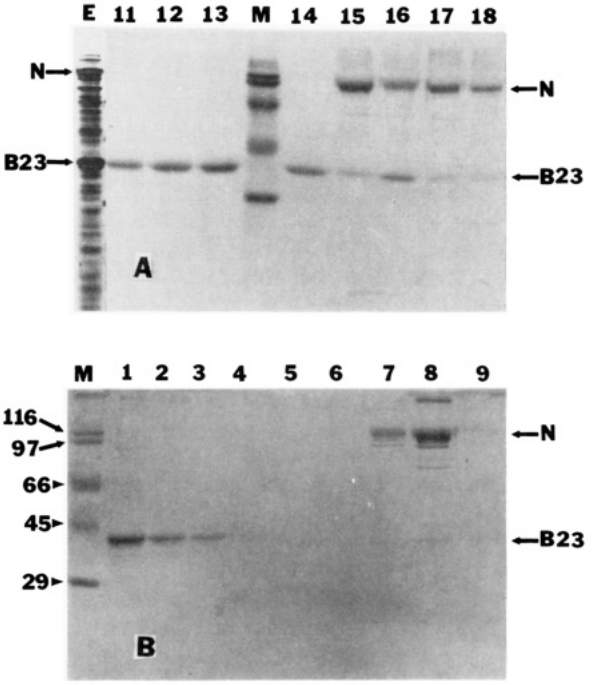

FIGURE 1: Purification of protein B23 by heparin–Sepharose and DNA–cellulose chromatography. (A) A nucleolar extract was applied to a heparin–Sepharose column (1.5 × 5 cm) and eluted with a gradient of ammonium sulfate. Fractions (2 mL) were collected, and the absorbance at 280 nm was measured. Aliquots of fractions from the heparin–Sepharose column were subjected to SDS–polyacrylamide gel electrophoresis. E, crude extract. Numbers at the top correspond to fraction numbers. (B) Fractions 15 and 16 were applied to a double-stranded DNA–cellulose column (1.5 × 3 cm) and eluted with a stepwise KCl gradient. Aliquots from the fractions were subjected to SDS–polyacrylamide gel electrophoresis. Fractions 1–3 (5 mL/fraction) eluted at 100–300 mM KCl. Fractions 7–9 eluted at 700–900 mM KCl. M, Sigma high molecular weight markers [molecular masses ($\times 10^{-3}$) are given in panel B]. The positions of nucleolin (N) and B23 are indicated.

H1, and applied to a double-stranded DNA–cellulose column. It was found that B23 and nucleolin bound either single- or double-stranded DNA–cellulose; although they bound more tightly to the single-stranded form, the duplex form gave a clearer separation. With stepwise elution, protein B23 eluted at 200 mM KCl, whereas nucleolin eluted at 700–800 mM KCl (Figure 1B). Routinely, fractions judged to contain virtually homogeneous protein B23 as analyzed by SDS–PAGE were used directly or concentrated for use in the experiments described below. The final yield was typically between 600 and 900 μg of protein from 6×10^{10} cells.

Interaction of Protein B23 with Nucleic Acids. Because of the apparent association of protein B23 with RNA and the fact that the other major nucleolar protein, nucleolin, prefers single-stranded nucleic acids, preliminary studies were done employing single-stranded M13mp7 DNA as the lattice in a gel retardation assay. Titration of this DNA with increasing concentrations of protein B23 generated protein–nucleic acid complexes as indicated by progressively decreasing mobilities (Figure 2). Since BSA was used in this assay to diminish nonspecific interactions, controls were performed in the absence of protein B23 and plus or minus BSA. The BSA did not cause a change in the mobility of the DNA when it was included in the DNA binding assay (Figure 2, lane 9). The gel retardation studies suggested that this protein is a single-stranded nucleic acid binding protein.

To determine whether protein B23 preferentially binds single-stranded DNA compared to duplex DNA, competition assays were performed using nitrocellulose filter binding. For

[2] The PROPHET system is supported by the Division of Research Resources, NIH, and operated by Bolt, Beranek, and Newman, Inc., Cambridge, MA.

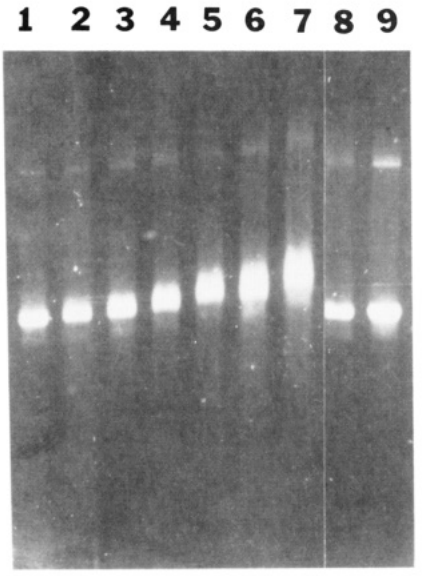

FIGURE 2: Gel retardation assay of single-stranded M13–B23 complexes. Increasing concentrations of protein B23 (in the range 1–10 μg) were added to 0.5 μg of single-stranded M13 DNA (lanes 2–7) and run on a 1.2% agarose gel. The complexes were visualized by ethidium bromide staining (lanes 1 and 8), M13 plus bovine serum albumin. Lane 9, M13 without added protein. BSA served as controls (lanes 1, 8, and 9).

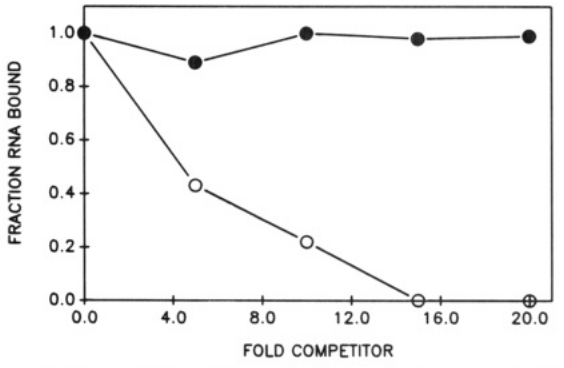

FIGURE 3: Competition of linear duplex or single-stranded pUC18 DNA with binding of ^{32}P-labeled poly(A–) RNA by protein B23. Assay conditions are described under Materials and Methods. The single-stranded form (open circles) was generated by heating the linearized form (filled circles) at 100 °C for 5 min and quick-cooling on ice. The ordinate (fraction of RNA bound) represents the ratio of labeled RNA bound in the presence of unlabeled competitor to labeled RNA bound in the absence of competitor.

these experiments, circular duplex pUC18 DNA was linearized by digestion with *Eco*RI endonuclease; the single-stranded form was generated by boiling the linearized plasmid at 100 °C for 5 min followed by rapid cooling and immediate utilization. The amount of protein B23 in the assay was capable of saturating 80% of the ^{32}P-labeled poly(A–) RNA lattice. The fraction of RNA retained when protein B23 alone was included in the assay served as the fully bound control. The binding assay was set up by first constituting the indicated amounts of radioactive template RNA and the unlabeled DNA in the reaction mixture, prior to addition of protein. As shown in Figure 3, single-stranded pUC18 DNA was an effective competitor for the labeled RNA. On the other hand, the double-stranded DNA was essentially incapable of competing with the labeled RNA for the protein. These results confirm that protein B23 preferentially binds single-stranded DNA. With the above findings at hand, it was of interest to know whether the protein exhibited preferential affinity to RNA versus DNA. Binding reactions were constituted as above,

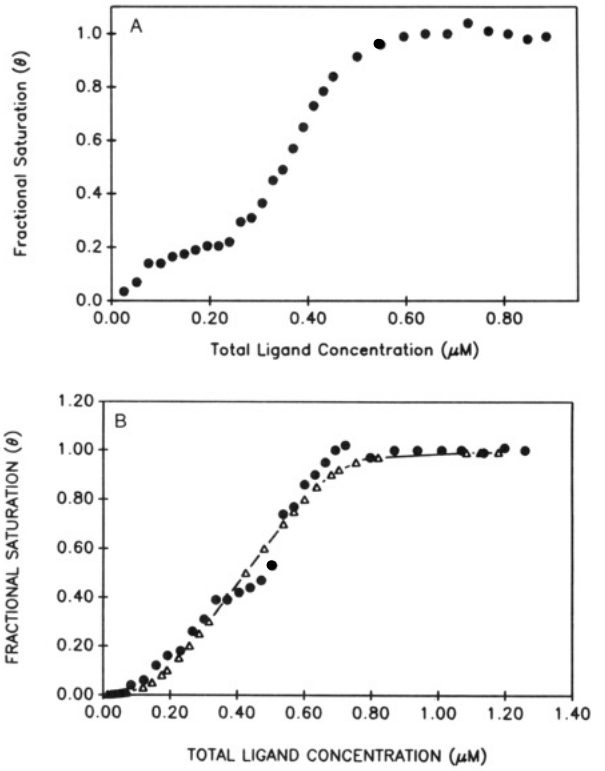

FIGURE 4: Fluorescence enhancement of poly(riboethenoadenylate) upon addition of protein B23. Titrations were performed in the presence of 10 mM NaCl (panel A) or 200 mM NaCl (panel B). Increasing concentrations of protein were added to 5 μM (nucleotides) nucleic acid lattice in binding buffer. (Panel B) Closed circles are experimental data points; open triangles represent a linear least-squares fit of the experimental data (see Results).

except that heat-denatured 5S rRNA served as the labeled template for competition between single-stranded synthetic polyribo- versus deoxyribonucleotides. The results (not shown) indicate that the protein has essentially no preferential affinity for either type of nucleic acid.

Nucleic Acid Binding Parameters. To examine the thermodynamic parameters of interaction of single-stranded nucleic acids with protein B23, we employed an assay utilizing poly(riboethenoadenylate) as a fluorescent RNA lattice. Binding of this polymer to proteins leads to fluorescence enhancement, in a manner proportional to added protein. Spectra were accumulated using light excited at 330 nm and monitoring the emission at 410 nm. Titration of 5 μM poly(riboethenoadenylate) with protein B23 led to fluorescence increases which varied sigmoidally with protein concentration (Figure 4A), with a shift in fluorescence maxima from 410 to 415 nm (data not shown). The binding site size was estimated from the concentration of protein B23 where saturation of the lattice was achieved under low ionic strength or stoichiometric binding conditions as suggested by Newport et al. (1981). Under these conditions (Figure 4A), the binding site size was estimated to be 11 nucleotides. Extrapolation from the midpoint of the titration curve (Figure 4B) performed under equilibrium binding conditions at higher salt concentrations (0.2 M NaCl) allowed us to estimate a value of 5×10^7 M^{-1} for the net affinity binding constant, which is the product of the intrinsic binding constant (K) and the cooperativity factor (ω) (there was no change in the binding site under equilibrium binding conditions). The value of K was estimated as follows: A family of theoretical binding isotherms was generated by combining eq 2 and 3 of Kowalczykowski et al. (1986); for $K = 10^2$–10^5 M^{-1} in steps of 10, with θ (y axis) vs total ligand concentration in micromoles per liter (x axis), $K\omega = 5 \times 10^7$ M^{-1}, n (the site

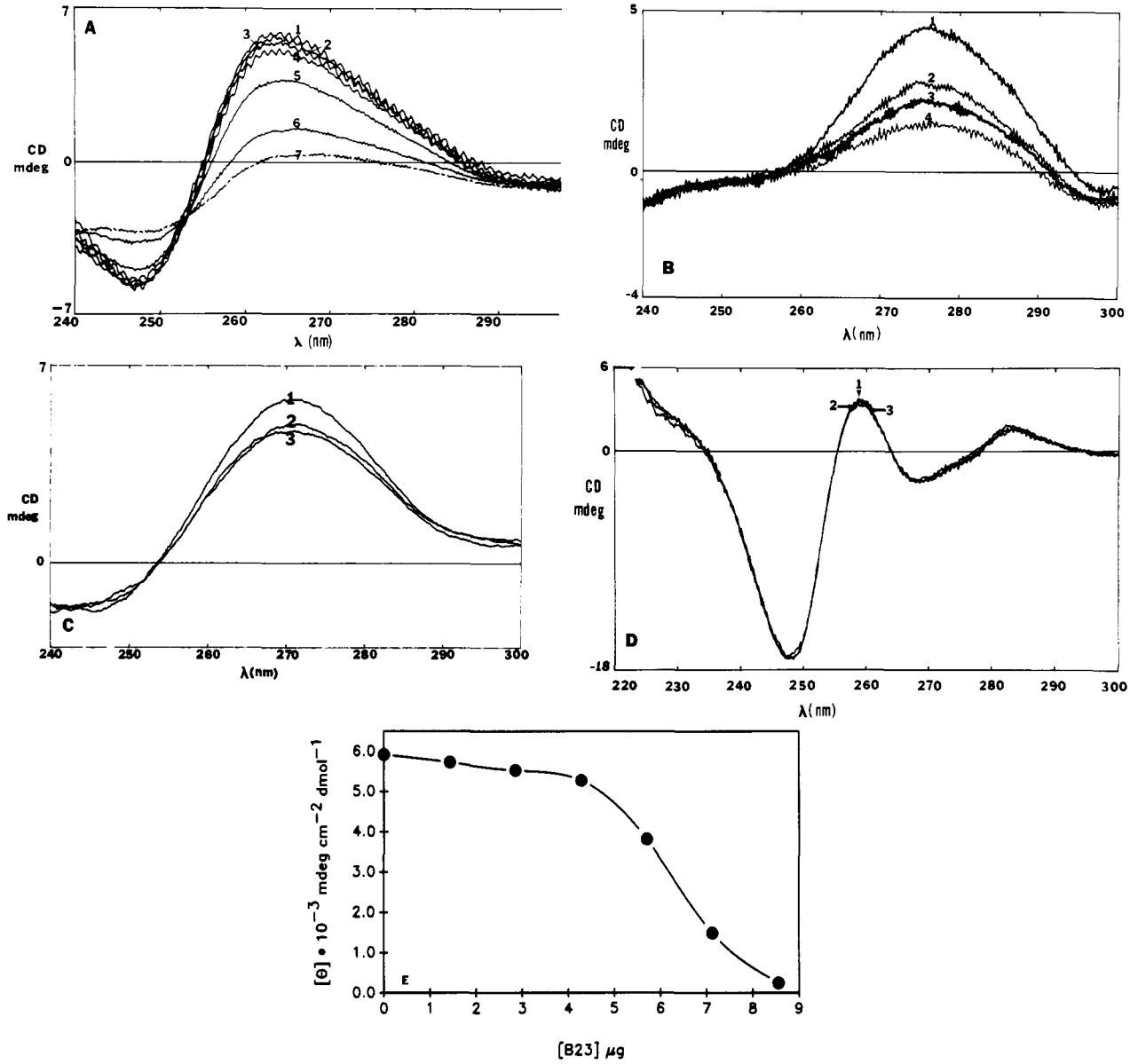

FIGURE 5: Circular dichroism (CD) of the interaction of protein B23 with synthetic polynucleotides. (A) Poly(rA); (B) poly(rC); (C) poly(rU); (D) poly(dA·dT); (E) plot of poly(rA) ellipticity as a function of protein concentration. Titrations were done in 5 mM Tris-HCl, pH 7.4, 10 mM NaCl, 10% glycerol, 10 μM (nucleotides) each polynucleotide, and increasing concentrations of protein B23 in the range of 0–10 μg. (Panel A) Curves 1–7: additions of 0, 1.5, 2.9, 4.3, 5.7, 7.1, and 8.6 μg of protein, respectively. (Panel B) Curves 1–4: additions of 0, 2, 5, and 10 μg of protein, respectively. (Panel C) Curves 1–3: additions of 0, 5, and 10 μg of protein, respectively. (Panel D) Curves 1–3: additions of 0, 5, and 10 μg of protein, respectively.

size) = 11, and N (the total lattice concentration) = 5 μmol/L. When the data obtained from the titration under equilibrium binding conditions were added (Figure 4B), it was seen that the points generally overlay the central part of the family of theoretical curves. Because of the inherent imprecision in approximating $K\omega$ values (Kowalczykowski et al., 1986), it was necessary to offset the experimental curves slightly in order to superimpose them on the theoretical curves. Accordingly, a line was fit by the least-squares method (PROPHET program "fit") to the linear portion of the curve (θ values from 0.26 to 1.02). The resulting equation was $y = 1.809x - 0.195$; the significance of the intercept was $p = 0.002$ and of the slope $p = 0.0001$. The slope thus obtained was compared to the slopes of the theoretical curves calculated between $\theta = 0.5$ and 0.6. The value of K thus obtained was 6.3×10^4 M^{-1}; from the relationship $K\omega = 5 \times 10^7$ M^{-1}, ω was found to be 800. Thus, protein B23 binds poly(riboethenoadenylate) with relatively high affinity and with a high degree of cooperativity.

Effect of Protein B23 on Nucleic Acid Secondary Structure. Circular dichroism (CD) spectroscopy was employed to monitor protein B23 induced perturbation of nucleic acid structure. Addition of protein B23 to poly(A) and poly(C) suppressed the CD signal at 264 nm and caused a shift to higher wavelengths (Figure 5A,B). This is indicative of disruption of the base stacking of the polymers by protein B23 (Karpel & Burchard, 1980). Lesser effects were seen with poly(U) (Figure 5C) as expected since this polymer exists predominantly as a random coil (Nowak et al., 1980). Filter binding assays indicated poly(U) was an effective competitor (data not shown), indicating that protein B23 binds to poly(U) and that the small effects were not due to the inability of B23 to bind the polymer. Addition of B23 to poly(dA·dT) had little or no effect on the CD spectrum (Figure 5D), probably because of the low affinity of protein B23 for double-stranded nucleic acids (see above). A plot of the change in ellipticity of poly(rA) versus protein concentration (Figure 5D) indicated

that the helix to coil transition varied sigmoidally with protein concentration, confirming the cooperativity of binding seen with fluorescence studies. These results indicated that protein B23 not only binds single-stranded nucleic acids but also disrupts their ordered structures.

Discussion

In this study, we show that protein B23 has three distinguishing characteristics in its interactions with nucleic acids: (1) preferential binding affinity for single-stranded nucleic acids; (2) cooperativity in that binding; and (3) the ability to disrupt ordered nucleic acid structure. These results were dependent on the development of a simple, rapid, and reliable method for purification using native conditions to obtain material suitable for binding studies. The purification strategy utilized a nucleolar extract (rather than a whole cell extract) which is enriched in protein B23 and nucleolin (Herrera & Olson, 1986). Essentially in one step, small quantities of highly purified native protein B23 were obtained via chromatography on heparin–Sepharose (Figure 1A). However, since the protein tended to elute over a broad range of ammonium sulfate concentrations, a significant amount of it coeluted with nucleolin. This portion of B23 could be recovered by rechromatoraphy of fractions containing essentially only B23 and nucleolin on DNA–cellulose columns, exploiting different affinities of B23 and nucleolin for DNA. This approach conveniently provided nearly homogeneous protein samples for the nucleic acid binding studies.

Protein B23 qualifies as a single-stranded nucleic acid binding protein (SSB) by several criteria [see reviews by Chase and Williams (1986) and Lohman et al. (1988)]. First, it binds to single-stranded DNA–cellulose and elutes at relatively high ionic strength. Second, in competition binding assays, double-stranded nucleic acids are very poor competitors compared to single-stranded nucleic acids. This was confirmed by experiments employing CD, where changes in ellipticity were seen only when B23 was added to single-stranded nucleic acids and no effect was seen with double-stranded nucleic acids, i.e., poly(dA·dT). Finally, protein B23 does not appear to be able to distinguish between single-stranded RNA and DNA, which is also a general characteristic of SSBs. It should be noted that the SSBs appear to fall into two major categories: proteins which are involved in DNA replication, repair, or recombination and may be considered as DNA binding proteins (Chase & Williams, 1986; Lohman et al., 1988) and proteins associated with ribonucleoprotein particles and which are predominantly RNA bound (Dreyfuss et al., 1988). Although protein B23 has some of the characteristics of the former group, it is unlikely to be involved in DNA replication. Because of its association with nucleolar RNA (see introduction) and its proposed role as a ribosome assembly factor, it seems reasonable to classify it as a RNA binding protein. How the protein becomes associated with nucleolar RNA as opposed to other RNAs in the nucleus remains to be determined. This specificity may be dictated by interactions with other proteins.

Use of the fluorescence properties of poly(ethenoadenylate) allowed us to estimate the nucleic acid binding parameters of protein B23, i.e., the nucleic acid binding site size (n), the intrinsic binding constant (K), and the cooperativity parameter (ω). It was found that each monomer of protein B23 covers approximately 11 nucleotide residues. This is slightly larger than the binding site size of the *Escherichia coli* SSB protein or the T4 gene 32 protein, which are 7 and 8 nucleotides per monomer, respectively, at low ionic strength (Kowalczykowski et al., 1981), but slightly smaller than the site size of the hnRNP A1 protein, which is 12 (Cobianchi et al., 1988). Thus, protein B23 appears to be typical in its coverage of bound nucleic acids.

The most striking result of the binding studies was the high level of cooperativity exhibited by the B23–poly(ethenoadenylate) interaction, with an ω value of approximately 800. This is less than the range of cooperativity seen with the gene 32 protein (ω = 1000–5000) but lies within the values seen with the *E. coli* SSB protein (ω = 400–1000; Lohman et al., 1988). On the other hand, the only eukaryotic RNA binding protein for which these binding parameters have been investigated, the hnRNP A1 protein, exhibits a low level of cooperativity (ω = 50; Cobianchi et al., 1988). We interpret the high level of cooperativity as an indication that the protein can efficiently cover relatively long stretches of single-stranded RNA at low protein concentrations, possibly for protection from nucleases (Olson, 1989) or for exchange with ribosomal proteins (Borer et al., 1989). Cooperativity normally involves protein–protein interactions in multisubunit proteins; in this case, there is evidence that protein B23 exists as a hexamer (Yung & Chan, 1987). Thus, it is conceivable that the cooperativity is mediated by interaction of monomers within the hexamer or by communication between hexamer units.

Not only does protein B23 bind single-stranded nucleic acids, but it also has the ability to alter their conformation. In CD studies, the decrease in ellipticity at 275 nm as well as the shift of the maximum signal to the higher wavelengths seen with poly(rA) is believed to be indicative of tilting and rotation of bases when bound by proteins (Van Amerongen et al., 1986). This is a typical effect of SSBs which are often called helix destabilizing proteins (Chase & Williams, 1986). The precise biological role of helix destabilizing by protein B23 is not known; however, it is conceivable that the change in nucleic acid structure could facilitate attachment of ribosomal proteins to ribosomal RNA. The cooperative binding would tend to keep relatively long stretches of RNA in an altered conformation which, in turn, may aid in the exchange of ribosomal proteins for B23. Thus, the protein may have a "shoehorn" effect in the ribosome assembly process. This possibility will have to await the development of in vitro ribosome assembly systems.

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Registry No. Poly(rA), 24937-83-5; poly(rC), 30811-80-4; poly(rU), 27416-86-0; poly(dA), 25191-20-2; poly(dC), 25609-92-1; poly(ethenoadenylic acid), 41911-88-0.

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