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Biochemistry

A chemically cleavable biotinylated nucleotide: Usefulness in the recovery of protein–DNA complexes from avidin affinity columns

(disulfide bond/nick-translation/nucleosome reconstitution/velocity sedimentation)

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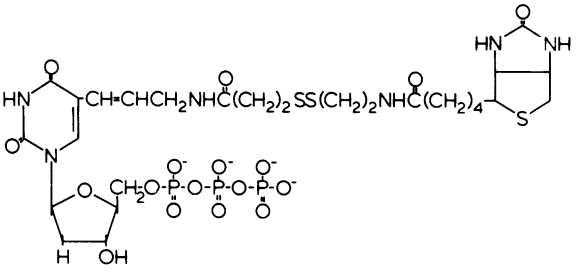
ABSTRACT **A biotinylated nucleotide analog containing a disulfide bond in the 12-atom linker joining biotin to the C-5 of the pyrimidine ring has been synthesized. This analog, Bio-SS-dUTP, is an efficient substrate for *Escherichia coli* DNA polymerase I. Bio-SS-dUTP supported DNA synthesis in a standard nick-translation reaction at 35%–40% the rate of an equal concentration of the normal nucleotide, TTP. DNA containing this analog was bound to an avidin-agarose affinity column and subsequently eluted after reduction of the disulfide bond by dithiothreitol. The ability to recover biotinylated DNA from an avidin affinity column under nondenaturing conditions should prove useful in the isolation of specific protein–DNA complexes. As a demonstration of this approach, Bio-SS-DNA was reconstituted with histones to form 11S monomer nucleosomes. Bio-SS-nucleosomes were shown to selectively bind to avidin-agarose. Ninety percent of the bound Bio-SS-nucleosomes were recovered from the affinity column by elution with buffer containing 50–500 mM dithiothreitol. The recovered nucleosomes were shown to be intact 11S particles as judged by velocity sedimentation in a sucrose gradient. This approach may prove to be generally useful in the isolation of protein–DNA complexes in a form suitable for further analysis of their native unperturbed structure.**

The high affinity of biotin for the glycoprotein avidin (K_d = 10^{-15} M) provides the basis for many established procedures for the detection and isolation of biotin-associated proteins (1–3). With the introduction of biotinylated nucleotide analogs by Langer *et al.* (4), it has recently become possible to apply this affinity system to the detection of specific DNA sequences by using biotinylated DNA as hybridization probes (5–7). Biotinylated DNA probes have several advantages over traditional radiolabeled probes. Unlike ^{32}P-labeled DNA, biotinylated DNA is chemically stable. In addition, after hybridization of a biotinylated-DNA probe to immobilized DNA, a sensitive detection system based on the use of avidin and biotinylated polymers of alkaline phosphatase can be used to detect target DNA sequences in the 1–10 pg range after enzyme incubations for 1 hr or less (8).

Another area in which biotinylated DNA would appear to be useful is in the isolation of specific protein–DNA complexes. However, because the avidin–biotin interaction is essentially irreversible (9), it has not been possible to recover biotinylated proteins or DNA from the affinity column without the use of harsh denaturing conditions. Previous approaches to solve this problem have involved two different attempts to decrease the affinity of the system so that the interaction can be reversed. Monomeric avidin binds biotin with significantly less affinity than native tetrameric avidin (9). Therefore, affinity columns constructed with monomeric avidin bind biotin in a reversible manner, but with low affinity. Propionyl CoA carboxylase, a biotin-containing enzyme, has been purified from a crude extract by chromatography on a monomeric avidin-Sepharose affinity column (2). In a second approach, an analog of biotin, iminobiotin, has been used. Unlike biotin, iminobiotin does not bind to avidin at pH <4 (10). However, the initial binding of iminobiotin to avidin must occur at a pH of 9.5 or above. Even at this high pH, the affinity of iminobiotin for avidin is not as great as that of biotin. Therefore, both of these approaches have sacrificed the high inherent sensitivity of the system, and, in the case of iminobiotin, still require the use of denaturing conditions to recover the biotinylated material from the column.

As an alternative solution to the problem of recovering biotinylated-DNA–protein complexes from an avidin agarose column under gentle nondenaturing conditions, we have synthesized a biotinylated-nucleotide analog that contains a chemically cleavable disulfide bond in the linker arm between biotin and dUTP (Bio-SS-dUTP). The use of this chemically cleavable biotinylated nucleotide to bind reconstituted nucleosomes to an avidin-agarose column and to subsequently recover them as intact 11S nucleosomes is described.



Bio-SS-dUTP

MATERIALS AND METHODS

Materials. Deoxyuridine 5′-triphosphate (dUTP) was purchased from Sigma. Allylamine was obtained from Aldrich. Radiolabeled nucleotides were purchased from Amersham ([^{3}H]dTTP) and New England Nuclear ([^{32}P]dCTP). Sulfosuccinimidyl-2-(biotinamido)ethyl-1,3′-dithiopropionate (sulfo-NHS-SS-biotin) was generously provided to us by Pierce. The TTP analog Bio-11-dUTP (analog that contains a biotin molecule linked to the C-5 position of the pyrimidine ring through a linker arm 11 atoms long) was purchased from Enzo Biochemicals (New York). *Escherichia coli* DNA polymerase I was obtained from New England Biolabs.

Synthesis of Bio-SS-dUTP. Bio-SS-dUTP was synthesized and purified by a modification of the procedure described by

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Abbreviations: Bio-SS-dUTP, a biotinylated nucleotide analog containing a disulfide bond in the 12-atom linker joining biotin to the C-5 of the pyrimidine ring; Bio-4-dUTP and Bio-11-dUTP, analogs of TTP that contain a biotin molecule linked to the C-5 position of the pyrimidine ring through linker arms that are 4 and 11 atoms long; AA-dUTP, 5-(3-amino)allyldeoxyuridine 5′-triphosphate; sulfo-NHS-SS-biotin, sulfosuccinimidyl-2-(biotinamido)ethyl-1,3′-dithiopropionate; bp, base pair(s).

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Langer *et al.* (4). Briefly, beginning with 50 mg of dUTP, 5-(3-amino)allyldeoxyuridine 5′-triphosphate (AA-dUTP) was synthesized and purified by chromatography on DEAE-Sephadex A-25 as described (4). Column fractions containing AA-dUTP, identifiable by its unique absorbance spectrum (λ_{max} at 289 nm and 240 nm; λ_{min} at 262 nm), were pooled and AA-dUTP was precipitated by the addition of 3 vol of cold, absolute ethanol. From 90 μmol of dUTP, ≈18 μmol of AA-dUTP can be obtained.

We have not found it necessary to further purify the AA-dUTP by HPLC at this stage in the synthesis. Instead, 2.0 μmol of AA-dUTP in 200 μl of 0.1 M sodium borate (pH 8.5) was added directly to 2.0 μmol of sulfo-NHS-SS-biotin. The reaction was allowed to proceed at room temperature for 1 to 2 hr. The resulting Bio-SS-dUTP was purified by ion-pair reversed-phase HPLC using a Bio-Sil ODS-5S column (250 × 4 mm; Bio-Rad). Aliquots of the reaction (100 μl) were adjusted to 50 mM Et_3NHCO_3 (pH 7.5) and 10% acetonitrile, diluted to 500 μl with the same buffer, and applied to the column at a flow rate of 0.5 ml/min (1600 psi). Bio-SS-dUTP was eluted isocratically with 50 mM Et_3NHCO_3 (pH 7.5) and 10% acetonitrile. Column fractions containing Bio-SS-dUTP were pooled, rotary evaporated, resuspended in 10 mM Tris·HCl (pH 7.5), and stored at −80°C. From 2.0 μmol of AA-dUTP, 1.0–1.6 μmol of Bio-SS-dUTP can be obtained by this procedure.

A more detailed description of the synthesis and characterization of Bio-SS-dUTP and other biotinylated nucleotide analogs will be published elsewhere.

Nick-Translation Reactions. Nucleosome length DNA fragments [145 base pairs (bp)] were purified from monomer nucleosomes prepared by micrococcal nuclease digestion of chicken erythrocyte nuclei followed by fractionation on an agarose A-5m column (11). Four-microgram aliquots of 145-bp DNA fragments in 0.1 ml of 50 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$/50 μg of bovine serum albumin per ml were nick-translated (12) in the presence of 20 μM dATP and dGTP, 20 μM [^{32}P]dCTP (2–5 μCi/nmol; 1 Ci = 37 GBq), and either dTTP, Bio-4-dUTP, or Bio-SS-dUTP as indicated. 2-Mercaptoethanol was eliminated from the nick-translation buffer to preserve the Bio-SS-dUTP. DNase I (0.01 ng per 0.1 ml of reaction mixture) was added to the reaction, which was then incubated for 5 min at 15°C prior to the addition of *E. coli* DNA polymerase I (4 units per 0.1 ml of reaction mixture).

When the nick-translated DNA was to be used in either affinity chromatography studies or in nucleosome reconstitution reactions, it was separated from unincorporated nucleotides by chromatography through spin columns (13) of Sephadex G-50 in 10 mM Tris·HCl, pH 7.5/1 mM EDTA/50 mM NaCl.

Avidin-Agarose Chromatography. Affinity columns were constructed in 200-μl pipette tips (Gilson) plugged with glass wool. One hundred microliters of a 50% slurry of avidin-D-agarose (Vector Laboratories, Burlingame, CA) was added to each column and washed 3 times with 200-μl aliquots of 10 mM Tris·HCl, pH 7.5/1 mM EDTA/50 mM NaCl. Each 200-μl aliquot was gently forced through the column in ≈15 sec by air pressure. DNA samples containing 0.1–0.2 μg of nick-translated DNA in 200 μl of the same buffer were applied and forced through the column as described for the washing step. The 200-μl flow-through aliquot was reapplied to the column one time. After the last wash, the column was inverted in a scintillation vial, which was then centrifuged to remove the resin from the column. Scintillation fluid was added directly to the resin and radioactivity was measured.

It should be noted that nonbiotinylated DNA binds nonspecifically to avidin-agarose in the presence of 50 mM NaCl. However, increasing the NaCl concentration to 200 mM effectively eliminates this low-affinity binding with no effect on the binding of biotinylated DNA to the column (see Fig. 3).

To determine whether biotinylated nucleosomes could be selectively bound to and eluted from avidin-agarose, aliquots of sucrose gradient fractions containing 1–2 μg of reconstituted 11S nucleosomes were applied directly to avidin-agarose columns and washed as described for DNA samples. When the Bio-SS-nucleosomes recovered from avidin-agarose were to be reanalyzed by sedimentation in a second sucrose gradient, 50 μg of unlabeled monomer nucleosomes was added to each dithiothreitol-containing wash immediately after its elution from the avidin-agarose column. This addition of excess unlabeled nucleosomes was necessary to stabilize the recovered Bio-SS-nucleosomes that would have otherwise dissociated because of their low concentration (14).

Reconstitution of Biotinylated Nucleosomes. Reconstitution of nucleosomes containing biotinylated DNA was done by the step-dialysis procedure described by Tatchel and Van Holde (15). Two micrograms of nick-translated 145-bp DNA fragments was mixed with 20 μg of unlabeled monomer nucleosomes (11) in a total volume of 0.4 ml of 10 mM Tris·HCl, pH 7.5/1 mM EDTA/2.0 M NaCl. After dialysis, the reconstitution mixture was then layered on a 5%–20% neutral sucrose gradient in 10 mM Tris·HCl, pH 7.5/1 mM EDTA/0.05 M NaCl and centrifuged at 5°C for 15 hr at 35,000 rpm in an SW41 rotor. The radioactivity present in aliquots of each fraction was measured to establish the sedimentation profile of each reconstitution mixture. As a control, authentic 11S monomer nucleosomes (11) were sedimented in a parallel gradient and were detected by measuring the absorbance (260 nm) of each fraction.

RESULTS

Synthesis of Bio-SS-dUTP. Bio-SS-dUTP, a chemically cleavable biotinylated nucleotide by virtue of the disulfide bond contained in the linker arm that joins biotin to the C-5 of the pyrimidine ring, has been synthesized. Its synthesis followed closely the procedure originally described by Langer *et al.* (4) for the synthesis of Bio-4-dUTP. The reaction between sulfo-NHS-SS-biotin and AA-dUTP proceeded quickly with 50%–80% of the AA-dUTP being converted to Bio-SS-dUTP. Bio-SS-dUTP was purified from the reaction by ion-pair reversed-phase HPLC (Fig. 1*A*). Under the conditions described, Bio-SS-dUTP eluted from the column with a retention time of 22 min (flow rate, 0.5 ml/min), immediately after two minor (<1%) reaction products.

The presence of a chemically cleavable disulfide bond in the Bio-SS-dUTP is demonstrated in Fig. 1*B*. An identical aliquot of the Bio-SS-dUTP reaction as is shown in Fig. 1*A* was supplemented with 2-mercaptoethanol to a final concentration of 5 mM immediately prior to its application and chromatography on HPLC. As expected, no UV-absorbing material eluted from the column with the retention time expected for intact Bio-SS-dUTP. Instead, there was a corresponding increase in the material eluting from the column with the solvent breakthrough (3.3 min). Since AA-dUTP elutes from this column with the breakthrough, and Bio-4-dUTP has a retention time of 5.7 min, the nucleotide-containing product of disulfide cleavage of Bio-SS-dUTP would be expected to elute with or near the breakthrough volume.

Bio-SS-dUTP Is a Substrate for *E. coli* DNA Polymerase. To determine whether Bio-SS-dUTP could serve as a substrate for *E. coli* DNA polymerase I, its incorporation into DNA in a nick-translation reaction was measured. The incorporation of [^{32}P]dCTP into purified nucleosome-length DNA fragments (145 bp) in the presence of 20 μM dATP, dGTP, dCTP, and either the normal nucleotide, TTP, or one of

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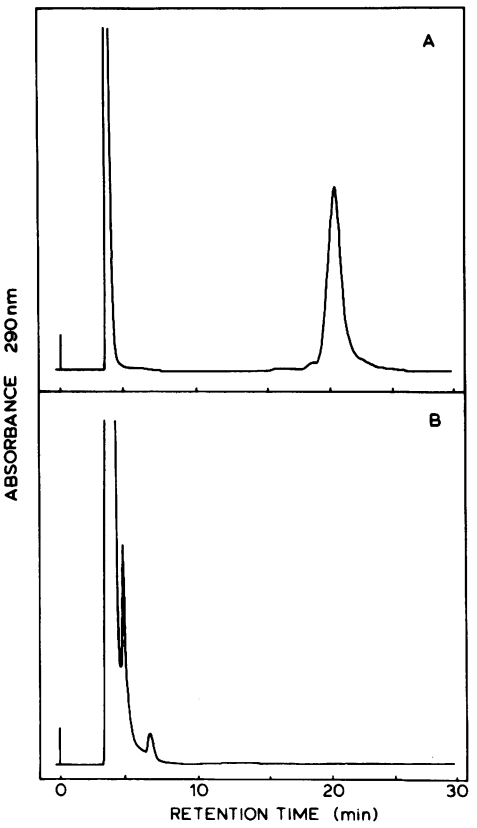

FIG. 1. Reversed-phase HPLC of Bio-SS-dUTP reaction. (*A*) A 2-μl aliquot of the Bio-SS-dUTP reaction mixture was diluted to 250 μl with 50 mM Et_3NHCO_3, pH 7.5/10% acetonitrile, and was chromatographed as described. Reaction products were detected by absorbance at 290 nm, 0.2 AUFS. (*B*) A reaction aliquot was chromatographed as in *A*, except that 2-mercaptoethanol was added to a final concentration of 5 mM immediately prior to its application to the column.

three biotinylated nucleotide analogs was measured (Fig. 2). Bio-SS-dUTP was capable of supporting the nick-translation reaction at 35%–40% the rate observed with an equal concentration of TTP. Bio-11-dUTP was similar to Bio-SS-dUTP, while the smaller biotinylated nucleotide analog, Bio-4-dUTP, supported the reaction at a rate ≈60% that observed for TTP. Therefore, just as was reported earlier for Bio-4-dUTP and Bio-11-dUTP (4, 7) Bio-SS-dUTP is a good substrate for *E. coli* DNA polymerase. If no TTP is present with the biotinylated nucleotides in the nick-translation reaction, the newly synthesized DNA is very heavily biotinylated. To reduce this level of modification, the nick-translation reactions can be performed in the presence of 10 μM TTP/10 μM Bio-SS-dUTP. Under these conditions, the reaction proceeds at a rate only slightly slower than in the presence of 20 μM TTP alone, and the resulting DNA contains sufficient biotinylated nucleotides to bind to an avidin-agarose affinity column (see Fig. 5).

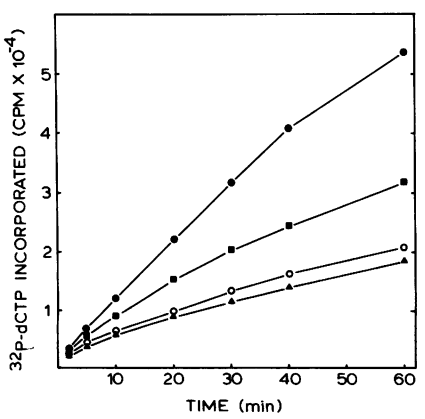

FIG. 2. Bio-SS-dUTP is a substrate for *E. coli* DNA polymerase I. Four-microgram aliquots of purified nucleosome-length DNA fragments (145 bp) were nick-translated in the presence of 20 μM dATP, dGTP, [^{32}P]dCTP (2 μCi/nmol) and either 20 μM TTP (●), 20 μM Bio-4-dUTP (■), 20 μM Bio-11-dUTP (▲), or 20 μM Bio-SS-dUTP (○). Five-microliter aliquots of the reactions were removed at the indicated times and the amount of [^{32}P]dCTP incorporated into trichloroacetic acid-insoluble DNA was determined.

Bio-SS-DNA Can Be Recovered from an Avidin-Agarose Affinity Column. Having established that Bio-SS-dUTP could be incorporated into DNA by nick-translation, we next asked whether (*i*) the biotinylated DNA could be bound to an avidin-agarose affinity column and (*ii*) the bound DNA could be recovered from the column after reduction of the disulfide bond in the linker arm by dithiothreitol. Two controls were included. First, nonbiotinylated DNA, nick-translated in the presence of [^{3}H]TTP (no biotinylated nucleotide), was mixed with ^{32}P-labeled DNA to demonstrate the initial selective binding of the biotinylated DNA to the affinity column. Second, the recovery of ^{32}P-labeled Bio-SS-DNA from the column was compared with that of DNA containing a noncleavable biotinylated nucleotide, Bio-4-dUTP.

As shown in Fig. 3, both ^{32}P-labeled Bio-4-DNA and ^{32}P-labeled Bio-SS-DNA were selectively bound to the avidin-agarose columns. Whereas 98% of the ^{3}H-labeled DNA was recovered from the columns after washing with 200 mM NaCl, 95% of the biotinylated DNA (both ^{32}P-labeled Bio-4-DNA and ^{32}P-labeled Bio-SS-DNA) remained bound under these conditions. The addition of 50 mM dithiothreitol to the

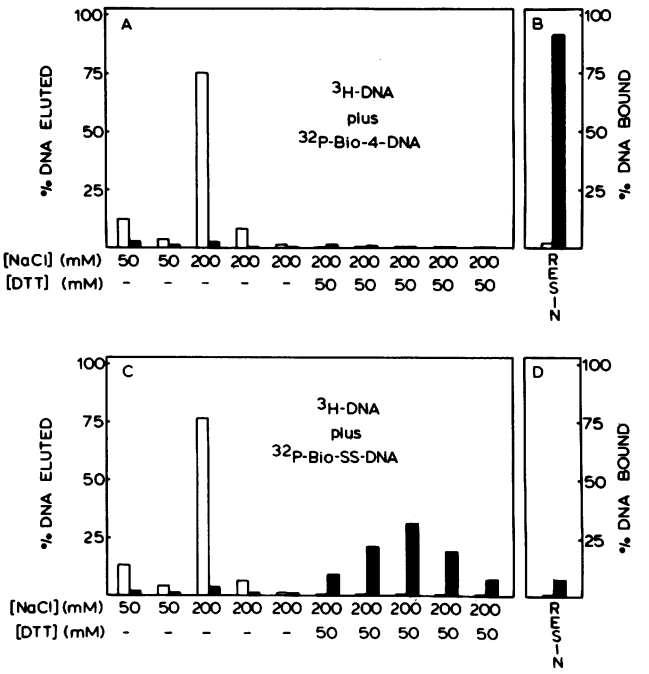

FIG. 3. Bio-SS-DNA can be selectively bound to avidin-agarose and subsequently recovered by dithiothreitol (DTT) treatment. A mixture of nonbiotinylated ^{3}H-labeled DNA and either ^{32}P-labeled Bio-4-DNA (*A*) or ^{32}P-labeled Bio-SS-DNA (*C*) was chromatographed on avidin-agarose columns as described. Each column was washed with 10 mM Tris·HCl, pH 7.5/1 mM EDTA and NaCl and dithiothreitol as indicated. The percent of each DNA eluting from the column in each wash is plotted in *A* and *C*; open bars, ^{3}H-labeled DNA; solid bars, ^{32}P-labeled Bio-4-DNA (*A* and *B*) or ^{32}P-labeled Bio-SS-DNA (*C* and *D*). The percent of each DNA remaining bound to the avidin-agarose after the indicated washes is shown in *B* and *D*.

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elution buffer failed to remove any of the bound ^{32}P-labeled Bio-4-DNA (Fig. 3*A*). As expected, 95% of the ^{32}P-labeled Bio-4-DNA was found still bound to the avidin-agarose resin at the end of the experiment (Fig. 3*B*). It should be noted that previous attempts to remove the ^{32}P-labeled Bio-4-DNA from avidin-agarose columns in our laboratory as well as others (4) by washing with buffers containing 1.0 M NaCl, 8 M urea, or 2 mM biotin have been unsuccessful. Therefore, ^{32}P-labeled Bio-4-DNA is irreversibly bound to avidin-agarose. In contrast, five consecutive washes of the column containing bound ^{32}P-labeled Bio-SS-DNA with buffer containing 50 mM dithiothreitol resulted in the recovery of a total of 87% of the DNA from the affinity column (Fig. 3*C*). Only 7.3% of the ^{32}P-labeled Bio-SS-DNA remained bound to the resin (Fig. 3*D*). Thus, Bio-SS-dUTP functions as a chemically cleavable nucleotide analog that can be used to initially bind biotinylated DNA to an avidin-agarose affinity column and subsequently release that DNA when its linker arm is broken by a reducing agent.

Biotinylated Nucleosomes Can Be Selectively Bound to and Recovered from Avidin-Agarose. Our initial interest in biotinylated nucleotides resulted from their potential usefulness in affinity labeling newly replicated DNA. If the newly biotinylated DNA was assembled into nucleosomes, we reasoned that it might be possible to isolate the newly formed DNA–protein complexes by avidin-agarose chromatography and then recover the intact complex for further analysis. To test the feasibility of such an approach, we first asked whether biotinylated DNA could be assembled into the characteristic 11S nucleosome in an *in vitro* nucleosome reconstitution reaction. Nucleosome-length fragments of DNA were nick-translated in the presence of [^{32}P]dCTP and either 20 μM TTP, a mixture of 10 μM TTP and 10 μM Bio-4-dUTP, or a mixture of 10 μM TTP and 10 μM Bio-SS-dUTP. The labeled DNA was then added to a 10-fold excess of nonlabeled monomer nucleosomes. NaCl was added to the mixture to a final concentration of 2.0 M to dissociate the nucleosomes into their DNA and histone components (15). The mixture was then dialyzed in a step-wise fashion into a buffer containing 50 mM NaCl. To assess how effectively the biotinylated DNA fragments had competed for the available histone octamers during the dialysis, the reconstitution mixtures were sedimented in neutral sucrose gradients (Fig. 4). Both biotinylated DNAs were capable of interacting with histone octamers to form an 11S nucleosome in yields comparable to that observed for the nonbiotinylated nick-translated DNA. Between 40% and 60% of the biotinylated DNA has been routinely found assembled into 11S nucleosomes in this type of experiment. The reconstituted material sedimenting ahead of the 11S monomer nucleosomes in Fig. 4 can be attributed to the presence of histone H1 and DNA fragments >145 bp in the preparation of nucleosomes used in this experiment.

To determine whether the biotinylated nucleosomes could be bound to and recovered from avidin-agarose as was previously shown for biotinylated DNA, aliquots of the sucrose gradient fractions containing reconstituted 11S nucleosomes were applied directly to avidin-agarose columns (Fig. 5). The nonbiotinylated and biotinylated nucleosomes behaved in the same way as their DNA counterparts, although a somewhat higher level of nonbiotinylated nucleosomes remained bound to the avidin-agarose after extensive washing (Fig. 5 *B* and *D*). It should be possible to eliminate the nonspecifically bound nucleosomes by use of a streptavidin affinity column (7). Note also that the concentration of dithiothreitol used to elute the Bio-SS-nucleosomes was increased to 500 mM in the experiment shown in Fig. 5. However, other experiments have shown that 50 mM dithiothreitol results in the recovery of the same percent of Bio-SS-nucleosomes.

Finally, to demonstrate that the recovered Bio-SS-nucleosomes were intact 11S particles, they were sedimented a second time in a sucrose gradient (Fig. 6). Eighty percent of the recovered ^{32}P-labeled Bio-SS-nucleosomes cosedimented with authentic 11S monomeric nucleosomes. The small

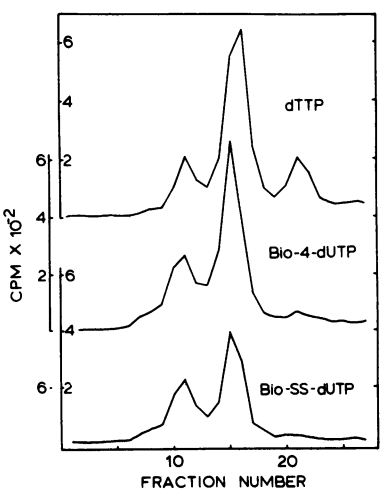

FIG. 4. Biotinylated DNA can be assembled into nucleosomes. Nucleosome-length DNA fragments were nick-translated with [^{32}P]dCTP and either TTP (20 μM), Bio-4-dUTP/TTP (10 μM each), or Bio-SS-dUTP/TTP (10 μM each), and then added to a 10-fold excess of unlabeled monomer nucleosomes. After reconstitution, the mixture was sedimented in a 5%–20% sucrose gradient as described. Authentic 11S nucleosomes run on a parallel gradient sedimented to fraction 15. Sedimentation was from right to left.

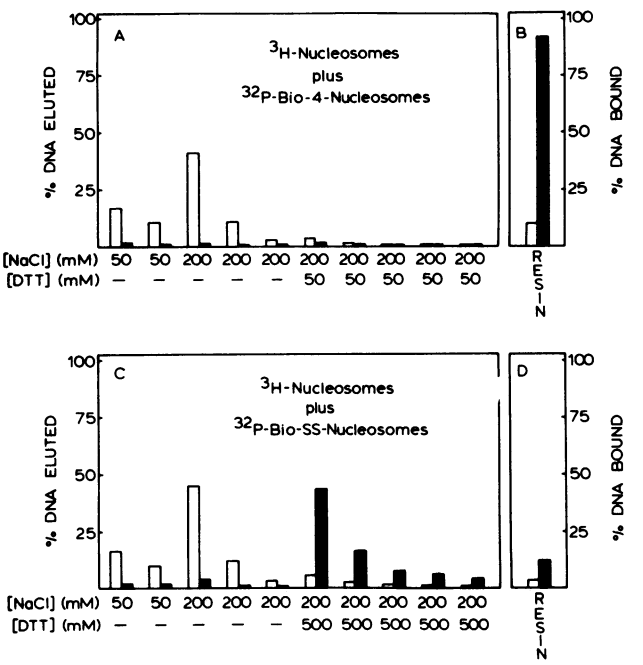

FIG. 5. Bio-SS-nucleosomes can be selectively bound to avidin-agarose and subsequently recovered by dithiothreitol (DTT) treatment. Aliquots of neutral sucrose gradient fractions containing 2 μg of reconstituted 11S nucleosomes were applied directly to avidin-agarose columns and washed as described. A mixture of nonbiotinylated ^{3}H-labeled nucleosomes and either ^{32}P-labeled Bio-4-nucleosomes (*A*) or ^{32}P-labeled Bio-SS-nucleosomes (*C*) were chromatographed. Each column was washed with 10 mM Tris·HCl, pH 7.5/1 mM EDTA and NaCl and dithiothreitol as indicated. The percent of reconstituted nucleosomes eluting from the column in each wash is plotted in *A* and *C*; open bars, ^{3}H-labeled nucleosomes; solid bars, ^{32}P-labeled Bio-4-nucleosomes (*A* and *B*) or ^{32}P-labeled Bio-SS-nucleosomes (*C* and *D*). The percent of reconstituted nucleosomes remaining bound to the avidin-agarose after the indicated washes is shown in *B* and *D*.

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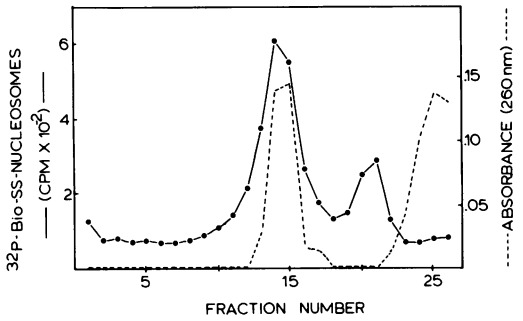

FIG. 6. Intact 11S Bio-SS-nucleosomes are recovered from avidin-agarose. ^{32}P-labeled Bio-SS-nucleosomes were eluted from an avidin-agarose column in 50 mM dithiothreitol. Unlabeled monomer nucleosomes were added to the recovered Bio-SS-nucleosomes to a final concentration of 40 μg/ml. This mixture was sedimented in a 5%–20% sucrose gradient as described. The absorbance at 260 nm (broken line) of each fraction was measured to determine the location of the unlabeled monomer nucleosomes. The absorbance seen at the top of the gradient is due to dithiothreitol. The radioactivity in each fraction was measured to locate the recovered Bio-SS-nucleosomes. Sedimentation is from right to left.

amount of dissociation that was observed ($\approx$20%) can be attributed to the normal dissociation of purified monomer nucleosomes in solution at concentrations <20 μg/ml (14).

DISCUSSION

We have described the synthesis and one specific application of Bio-SS-dUTP, a biotinylated nucleotide analog containing a chemically cleavable disulfide bond in the linker arm joining biotin to dUTP. The properties of Bio-SS-dUTP are similar to those of Bio-4-dUTP and Bio-11-dUTP in terms of its incorporation into DNA by nick-translation and the subsequent binding of the biotinylated DNA to an avidin-agarose affinity column. However, the presence of the disulfide group in the linker of Bio-SS-dUTP provides a means to recover the Bio-SS-DNA from an avidin-agarose column. Approximately 90% of the bound Bio-SS-DNA was recovered by washing the column with buffer containing 50 mM dithiothreitol. This approach to recovering biotinylated DNA from an avidin affinity column is superior to other approaches, such as the use of low affinity monomer avidin columns or of iminobiotin-labeled DNA. In our procedure, the high affinity of avidin for biotin can be exploited fully while allowing for recovery of DNA by a mild nondenaturing treatment.

The ability to cleave the linker between biotin and dUTP under mild nondenaturing conditions should make it possible to isolate intact protein–Bio-SS-DNA complexes by avidin-agarose chromatography. As a demonstration of such an application, we have shown that reconstituted nucleosomes containing Bio-SS-DNA can be specifically bound to and subsequently recovered from an avidin-agarose column. The Bio-SS-DNA used for nucleosome reconstitution was nick-translated in the presence of equal concentrations of TTP and Bio-SS-dUTP. In this way, the incorporation of Bio-SS-dUTP into DNA was limited to 8–16 residues per nucleosome-length fragment (unpublished results). Bio-SS-DNA prepared in this way was reconstituted into nucleosomes with the same efficiency as nick-translated nonbiotinylated DNA. At the same time, the binding of the Bio-SS-nucleosomes to avidin-agarose was both highly selective and sensitive. We have observed that biotinylated nucleosomes are quantitatively bound to avidin-agarose even when mixed with a 10,000-fold excess of nonbiotinylated nucleosomes (data not shown). Most importantly, Bio-SS-nucleosomes were recovered from the affinity column as intact 11S nucleosomes as judged by velocity sedimentation in neutral sucrose gradients.

Many areas of research in molecular biology are concerned with the identification and isolation of proteins or protein complexes that bind in a sequence-specific manner to DNA. It should be possible to isolate these proteins by affinity chromatography after their binding to specific Bio-SS-labeled DNA fragments. Most importantly, in addition to the isolation and subsequent identification of these proteins, this approach may provide a means to recover the complexes in their native form. These complexes could then be further analyzed with respect to both the specific protein–protein and protein–DNA interactions that may be present. Specific examples of areas in which this approach might prove useful include the deposition of preexisting or new histones at DNA replication forks, the identification of specific transcription factors, and the isolation of hormone–receptor–DNA complexes.

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