Nucleosomes Bind to Cell Surface Proteoglycans*

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Material on the surface of activated T-cells was displaced following incubation with a sulfated polysaccharide, dextrin 2-sulfate (D2S), and purified by anion-exchange chromatography. This revealed a complex comprising histones H2A, H2B, H3, and H4 and DNA fragmented into 180-base pair units characteristic of mono-, di-, tri-, and polynucleosomes, a pattern of fragmentation similar to that found in apoptotic cells. An antibody raised against the purified nucleosome preparation bound to the plasma membrane of activated T-cells confirming the surface location of nucleosomes. The interaction of sulfated polysaccharides with nucleosomes was investigated using a biotinylated derivative of D2S. It was found that sulfated polysaccharides bound to nucleosomes via the N termini of histones, especially H2A and H2B. Treatment of T-cells with either heparinase or heparitinase abolished nucleosome binding to plasma membranes. This suggests that nucleosomes are anchored to the surface of T-cells by heparan sulfate proteoglycans through an ionic interaction with the basic N-terminal residues in the histones. Furthermore, nucleosomes bound to the cell surface in this manner are then able to bind other sulfated polysaccharides, such as D2S, heparin, or dextran sulfate, through unoccupied histone N termini forming a complex comprising surface heparan sulfate proteoglycans, nucleosomes, and sulfated polysaccharides.

There is increasing evidence that nuclear material, including histones, DNA, and nucleosomes, may under certain conditions become located on the surface of cells (1–4). These observations are likely to have relevance to autoimmune diseases such as systemic lupus erythematosus (SLE),1 where antibodies against nuclear material are found (5–8).

EXPERIMENTAL PROCEDURES

Cell Culture—HPB-ALL cells (15) were maintained in RPMI 1640 medium containing 20 mM HEPES buffer that was supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, 250 IU/ml penicillin, and 250 μg/ml streptomycin.

Purification of D2S-related Cell Surface Material—HPB-ALL cells were washed three times in phosphate-buffered saline (PBS) using repetitive centrifugation and then resuspended in 5–20 ml (as appropriate) of ice-cold PBS containing 0.2 mg/ml D2S (ML Laboratories, Wavertree, UK) for 1 h at 4 °C. The cells were pelleted, and the supernatant was passed through a 0.2-μm filter to remove any residual cells. Analytical anion-exchange chromatography was performed with a MonoQ column using a fast protein liquid chromatography system (Amersham Pharmacia Biotech) at a flow rate of 1 ml/min with a continuous gradient of 0–3 M NaCl in 20 mM Tris-HCl, pH 7.4, over 40 min, and the absorbance was monitored at 280 nm. Preparative anion-exchange chromatography was performed using a 5.5 × 1 cm (inner diameter) Q fast flow column (Amersham Pharmacia Biotech) at a flow rate of 2 ml/min and a step gradient of 0.15, 0.5, 0.9, and 2.5 M NaCl in 20 mM Tris-HCl, pH 7.4. Eluted fractions were either dialyzed against PBS overnight in preparation for SDS-PAGE or dialyzed overnight against 0.1 M sodium borate, pH 8.4, in preparation for DNA extraction.

SDS-PAGE—Purified cell surface material or purified calf thymus histones were separated using 15% (w/v) polyacrylamide gels as described previously by Watson et al. (14).

Quantification of DNA—The amount of DNA present in the purified

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‡ The abbreviations used are: SLE, systemic lupus erythematosus; D2S, dextrin 2-sulfate; HSPG, heparan sulfate proteoglycans; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin.

1 The abbreviations used are: SLE, systemic lupus erythematosus; HPB-ALL cells, human T-cell leukemia lymphoma cells; RPMI, Roswell Park Memorial Institute; HEPES, N-(2-hydroxyethyl)piperazine- N′-2-ethanesulfonic acid; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BSA, bovine serum albumin.
D2S-eluted material was determined fluorometrically using the fluorophore Hoechst H33258 and compared with purified calf thymus standards. Samples of the material (0.1 ml) and calf thymus DNA standards (0.1 ml of 0–50 μg/ml diluted in TNE buffer (10 mM Tris, 1 mM EDTA, 0.2 mM NaCl, pH 7.4) were mixed with 2.9 ml of 0.1 μg/ml H33258 in TNE buffer and the fluorescence was measured using excitation and emission wavelengths of 360 and 450 nm, respectively.

**DNA Extraction and Agarose Gel Electrophoresis**—Samples were treated with 50 μg/ml proteinase K for 1 h at 37 °C, and DNA was extracted by addition of 1.5 volumes of 50% (v/v) phenol, 48% (v/v) chloroform, and 2% (v/v) isomyl alcohol solution. The solution was allowed to gently rotate for 5 min; then the samples were subjected to centrifugation (900 × g for 10 min), and the aqueous layer was removed. DNA in the aqueous fraction was precipitated by addition of 0.2 volumes of 3 M potassium acetate, pH 9.95, and 2.5 volumes of ice-cold 95% (v/v) ethanol. The precipitate was collected after centrifugation at 900 × g for 10 min and then dissolved in 100 μl of 45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0, and 20 μl of 0.05 mg/ml xylene cyanol containing 8% (w/v) sucrose. Samples were electrophoresed on a 1% (w/v) agarose gel in 45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0, and 0.5 μg/ml ethidium bromide at 100 V for 1 h. DNA present in the gel was visualized under long wavelength (366 nm) UV light.

**Nucleosome Preparation from HPB-ALL Nuclear Fraction**—Mononucleosomes were prepared from HPB-ALL cell nuclei using micrococcal nuclease as described by Kornberg et al. (10).

**Antibody Production**—New Zealand White rabbits were immunized with purified D2S-eluted cell surface material as described by Edwards et al. (17) except that 40 μg of purified protein was administered on each occasion.

**Enzyme-linked Immunosorbent Assay**—This was performed according to Edwards et al. (17) using wells of microtiter plates coated with either 2 μg/ml purified cell surface material, mononucleosomes from micrococcal nuclease digestion, or purified calf thymus histones. All antigens were diluted in PBS.

**Immunocytochemistry**—HPB-ALL cells were washed three times in PBS by repetitive centrifugation. The cells were counted on a hemocytometer, and approximately 1 × 10⁶ cells were fixed for 10 min in 4% (v/v) formaldehyde in PBS. The cells were then applied to a poly-L-lysine-coated microscope slide using a Shandon Cytospin 3 set at 400 rpm for 3 min. The preparation was washed briefly in PBS before endogenous peroxidase activity was quenched by incubation for 30 min in 70% (v/v) methanol containing 0.03% (v/v) hydrogen peroxide. Non-specific binding sites were blocked by the addition of 100 μl of a 1/20 dilution of normal goat serum in PBS for 20 min at room temperature. The slides were then air-dried and incubated with 100 μl of a 1/2000 dilution of antiserum for 1 h at room temperature. The slides were washed three times in PBS and then incubated with 100 μl of a 1/100 dilution of goat anti-rabbit horseradish peroxidase in 0.1% (v/v) bovine serum albumin (BSA) in PBS for 30 min at room temperature. After three washes in PBS, the slides were incubated with 100 μl of a 1/300 dilution of peroxidase anti-peroxidase antibody in 0.1% (v/v) BSA in PBS for 30 min at room temperature. Finally, the slides were washed three times in PBS, and peroxidase activity was developed by incubation at room temperature with 100 μl of 0.025% (w/v) diaminobenzidine in 0.033% (v/v) hydrogen peroxide in PBS for 5 min or until a clear dark brown stain was noted. The cells were then dehydrated in graduated alcohol (70–100%) and xylene and fixed under coverslips.

**Chemical Modification of D2S with Biotin Hydrazide and [1-H]Ethanol-1-ol-2-amine**—The incorporation of biotin hydrazide (Pierce, Warrington, UK) into D2S was undertaken using the conditions described by Kornberg et al. (10). For the production of radiolabeled D2S, 5 μmol of ethanolamine containing 20 mmol of [1-H]ethanol-1-ol-2-amine (specific activity 29 Ci/mmol) (Amersham Pharmacia Biotech) was substituted for biotin hydrazide. The specific activity of the radiolabeled D2S product was 1.14 × 10⁶ dpm/mg D2S.

**Measurement of the Binding of Biotinylated D2S to Macromolecules**—The binding of D2S to either purified protein, mononucleosomes, or membrane preparations was determined as described in Watson et al. (18). From the curves produced, an IC₅₀ value (inhibitory concentration resulting in a 50% decrease in biotinylated D2S binding) for each compound studied was determined.

**Trypsinization of D2S-eluted Material**—Nucleosomes (10 μg/ml) prepared from the cell surface were dialyzed against PBS to remove the residual salt and then warmed to 37 °C. Trypsin digestion was undertaken for 30 min at 37 °C with 50 μg/ml porcine pancreas trypsin (EC 3.4.21.4) (Sigma, Poole, UK). The trypsinized nucleosomes were separated from other components by gel filtration using a Sephadex G-25 column (2 cm inner diameter × 20 cm) at a flow rate of 2 ml/min equilibrated in 0.2 M sodium phosphate, pH 7.0.

**Membrane Preparation by Ultracentrifugation**—Between 5 and 10 × 10⁶ HPB-ALL cells were cultured in 10 ml of PBS and washed three times with PBS by repetitive resuspension and centrifugation at 200 × g for 5 min. A cell membrane fraction was then prepared by the method described by Blair and MacDermot (19) except that the homogenization buffers also contained 0.2 mg/ml D2S to remove cell surface nucleosomes. Typically this yielded between 1 and 3 mg of protein, as determined by the method of Lowry et al. (20). In some experiments cells were treated with 1 unit/ml heparitinase I or 5 units/ml heparinase I (Sigma, Poole, UK) for 1 h at 37 °C prior to homogenization.

**RESULTS**

**Purification and Analysis of D2S-eluted Cell Surface Material**—Preliminary analysis using analytical anion-exchange chromatography with a continuous salt gradient showed that material eluted from the surface of HPB-ALL cells by incubation with 0.2 mg/ml D2S contained only one major UV-absorbing peak that was eluted at 0.9 M NaCl (Fig. 1a). This material was purified using a preparative anion-exchange column, employing a step gradient of 0.15, 0.5, 0.9, and 2.5 M NaCl. The major UV-absorbing peak was eluted with 0.9 M NaCl (Fig. 2a). D2S was eluted at 2.5 M NaCl (Fig. 2a). Analysis of the 0.9 M NaCl fraction by SDS-PAGE revealed the presence of histones H2A, H2B, H3, and H4 in the preparation (Fig. 2b). The DNA content of the 0.9 M NaCl fraction was measured using Hoechst stain H33258 and was shown to be 0.56 mg of DNA/mg of protein, and following extraction and analysis by agarose gel electrophoresis, it was found that the DNA was fragmented into a regular pattern of 180-base pair units typical of mononucleosomes (Fig. 2c). Analysis of a mononucleosome preparation by analytical anion-exchange chromatography (Fig. 1b) showed an identical elution to the major UV-absorbing peak in the D2S-eluted cell surface material (Fig. 1a), whereas all of the individual histones H2A, H2B, H3, and H4 were eluted with a very short retention time (Fig. 1c). An antibody raised against purified D2S-eluted cell surface material bound equally to a mononucleosome preparation as it did to the purified D2S-eluted cell surface material (Fig. 3). The antibody also bound to histones H2A and H2B, albeit less strongly than to mononucleosomes and more weakly to histones H3 and H4 (Fig. 3). All together these results strongly suggest that the purified D2S-eluted cell surface material is comprised of nucleosome particles.

**Localization of Nucleosomes on HPB-ALL Cells**—The antibody was used to determine the location of nucleosomes on HPB-ALL cells that had been cultured for 5 days. Immunocytochemistry showed that the antibody bound strongly to the plasma membrane of most of these cells (Fig. 4). However, cells that had been incubated with preimmune serum had no detectable staining (Fig. 4).

**D2S Binds to the N-Terminal Regions of Histones**—A biotinylated derivative of D2S was synthesized and used to measure the binding of D2S to cell surface nucleosomes and purified histones. D2S bound strongly to purified calf thymus nucleosomes (EC₅₀ = 0.12 μg/ml n = 5) although binding to histones H2B and H2A was only slightly lower (Fig. 5). D2S also bound to histones H3 and H4, although both the maximum binding and the binding affinities were reduced (Fig. 5). However, D2S was unable to bind to trypsin-treated purified cell surface nucleosomes (Fig. 5). In competition studies, various sulfated...
polysaccharides were also able to bind to purified nucleosomes, although dextrin and glucose 6-sulfate did not (Table I).

Effect of Heparinase or Heparitinase Treatment—D2S bound only very poorly to an HPB-ALL cell membrane preparation from which cell surface nucleosomes had been removed. However, after addition of purified cell surface nucleosomes to the membrane preparation, D2S bound extensively. In contrast, when the membrane preparation was treated with either heparitinase I or heparinase I, D2S binding was poor, even after the addition of purified cell surface nucleosomes (Fig. 6a).

Similarly, the anti-nucleosome antibody bound poorly to the nucleosome-depleted membrane preparation, although when purified cell surface nucleosomes were added to the membrane preparation extensive antibody binding was found. However, after treatment of the membrane preparation with either heparitinase I or heparinase I, little antibody binding was detected even when purified cell surface nucleosomes were added to the membrane preparation (Fig. 6b).

DISCUSSION

The results from this study show that histones, previously identified to be present on the surface of activated T-cells (14), are in the form of nucleosomes, comprising histones H2A, H2B, H3, H4, and DNA fragmented into 180-base pair units. It is also shown that cell surface nucleosomes are attached to the plasma membrane through an interaction with cell surface proteoglycans.

In a previous study it was found that histones could be eluted from the cell surface by incubation of cells with the sulfated polysaccharide, D2S (14). Here, purification of material eluted from the surface of activated T-cells by anion-exchange chromatography performed at pH 7.4 revealed that it comprised a single major UV-absorbing peak that was eluted with 0.9 M NaCl. The relatively high salt concentration needed to elute the material indicated that it carried a predominantly negative charge at pH 7.4; however, analysis of the composition of the
proteins in this material by SDS-PAGE revealed that it comprised histones H2A, H2B, H3, and H4, which are all basic proteins. The negative charge of the material was not due to D2S, as this was clearly separated during anion-exchange chromatography. However, reactivity of the purified preparation to the cell surface-derived material, in contrast to the anion-exchange chromatography column. Furthermore, antisera raised against the cell surface-eluted material bound as strongly to mononucleosomes as it did to the cell surface material. Thus, the cell surface-eluted material is indistinguishable from nucleosomes. The surface location of nucleosomes in activated cells was confirmed by immunocytochemistry, as the antibody bound strongly to the cell surface of 5-day-old HPB-ALL cells.

The pattern of fragmentation of DNA obtained from the purified nucleosome sample was similar to that observed in the nuclei of cells undergoing apoptosis. Apoptosis is a mode of cell death that is defined morphologically; chromatin undergoes condensation, the cytoplasmic organelles become compacted and the cell surface undergoes blebbing (21, 22). Furthermore, apoptotic cell death is accompanied by the activation of a nuclear endonuclease that cleaves chromatin at sites of nucleosome attachment. Furthermore, an agarose gel electrophoresis revealed that the DNA was fragmented into regular 180-base pair units. These results strongly suggested that the material eluted from the cell surface was a mixture of mono-, di-, tri-, and polynucleosomes. This assertion was supported by analytical anion-exchange chromatography of mononucleosomes, which produced an identical elution profile to the cell surface-derived material, in contrast to the elution of each of the individual histones, which did not bind to the anion-exchange chromatography column. Furthermore, an antibody raised against the cell surface-eluted material bound as strongly to mononucleosomes as it did to the cell surface material. Thus, the cell surface-eluted material is indistinguishable from nucleosomes. The surface location of nucleosomes in activated cells was confirmed by immunocytochemistry, as the antibody bound strongly to the cell surface of 5-day-old HPB-ALL cells.

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![Fig. 3. Relative binding of antibody to cell surface.](image)

**Fig. 3. Relative binding of antibody.** The wells of microtiter plates were coated with purified cell surface material (●), mononucleosomes (○), purified calf thymus histones H2A (□), H2B (●), H3 (■), H4 (▲), or BSA (○), and antibody binding was determined by enzyme-linked immunosorbent assay as described under "Experimental Procedures." Each value is the mean of duplicate determinations, and the data shown are representative of two experiments with similar results.

![Fig. 4. Localization of nucleosomes on the cell surface.](image)

**Fig. 4. Localization of nucleosomes on the cell surface.** HPB-ALL cells were cultured for 5 days and then fixed in 4% (v/v) formaldehyde, cytospun onto slides, and then incubated with either (a) anti-nucleosome antibody or (b) preimmune serum as described under "Experimental Procedures." Strong surface staining was found on almost all cells. There was no surface staining to any of the cells incubated with preimmune serum (magnification × 400).

![Fig. 5. Binding of D2S to purified histones.](image)

**Fig. 5. Binding of D2S to purified histones.** The wells of microtiter plates were coated with purified nucleosomes (▲), trypsin-treated nucleosomes (●), purified histones H1 (■), H2A (□), H2B (○), H3 (■), H4 (▲), or BSA (●) in PBS, blocked by the addition of 2% (w/v) BSA, and then incubated with 0.03–30 μg/ml biotinylated D2S (B-D2S). Binding of biotinylated-D2S to histones, represented as the absorbance at 490 nm, was determined as described under "Experimental Procedures." Each point is the mean of duplicate determinations, and the data shown are representative of either 5 experiments for purified nucleosomes or 2 experiments for the remaining samples tested, with similar results.

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<th>Compound</th>
<th>Mass (kDa)</th>
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<th>IC₅₀ (µg/ml)</th>
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</table>

**Table I**

Comparison of IC₅₀ values of sulfated polysaccharides determined from competition with biotinylated D2S for binding to nucleosomes

Nucleosomes were coated onto microtiter plate wells and incubated with various concentrations of each sulfated polysaccharide containing 0.2 µg/ml biotinylated D2S. Competition for binding to nucleosomes was determined, and IC₅₀ values were calculated as described under "Experimental Procedures." Each value is the mean ± S.E. determined from three separate experiments.
The DNA superhelix to allow contact with other macromolecules, normally other nucleosome particles. Here, we suggest that negatively charged D2S binds to the positively charged N-terminal regions of the histones that protrude from the nucleosome core (Fig. 7a). It has been shown previously that as a result of their accessibility the N-terminal regions of histones are better competitors. For example, high molecular weight dextran sulfate and fucoidan, the two largest compounds, are better competitors. For example, high molecular weight dextran sulfate and fucoidan, the two largest compounds, are better competitors.

FIG. 6. Heparinase and heparitinase treatment of HPB-ALL cells. a, HPB-ALL cells were grown for 5 days, and cell surface nucleosomes were removed with 0.2 mg/ml D2S. The cells were washed liberally with PBS and then incubated with either 1 unit/ml of heparitinase or 5 units/ml of heparinase for 30 min at 37 °C. Cell membrane fractions were prepared as described under “Experimental Procedures.” Membrane protein was coated on to the wells of microtiter plates and incubated with 10 μg/ml exogenous purified nucleosomes in PBS (hatched bars) or PBS alone (open bars), followed by incubation with 10 μg/ml biotinylated D2S. Each value is the mean of quadruplicate determinations. b, cell membrane fractions described above were coated onto the wells of microtiter plates and incubated with a 1/300 dilution of anti-nucleosome antibody in the presence of 10 μg/ml exogenous purified nucleosomes in PBS (hatched bars) or PBS alone (open bars). Bound antibody was detected as described under “Experimental Procedures.” Each value is the mean of duplicate determinations.

FIG. 7. Proposed interaction between nucleosomes, the cell surface, and sulfated polysaccharides. a, the positively charged N termini of histones protrude out from the core of the nucleosome particles and bind to the negatively charged heparan sulfate regions of cell surface proteoglycans. Although nucleosomes also have strongly, negatively charged regions, due to phosphate groups of DNA, this does not interfere with this interaction. Nucleosomes bound to the cell surface are then able to bind to exogenous negatively charged sulfated polysaccharides with the protruding N termini of histones that are not involved in the binding with heparan sulfate. The number of nucleosome particles that the sulfated polysaccharides bind is dependent on the length of the carbohydrate backbone. Here, for simplicity, an interaction of just one nucleosome particle with each chain of heparan sulfate and three nucleosome particles with one molecule of an exogenous sulfated polysaccharide is shown for illustration, although it is likely that several nucleosome particles will bind to each chain of sulfated polysaccharide. b, the interaction between nucleosomes and sulfated polysaccharides are in equilibrium, and in the presence of excess exogenous sulfated polysaccharide bound nucleosomes are displaced from the cell surface. c, in the absence of cell surface nucleosomes exogenous sulfated polysaccharides cannot bind to the cell surface.

The explanation for this lies in the structure of the nucleosome core particle. Luger et al. (25) have shown using x-ray crystallography that although the histone octomer, comprising 2 molecules of histones H2A, H2B, H3 and H4, is surrounded by negatively charged DNA, the positively charged N termini of the histones extend out of core particle and past internucleosomal sites (23) and the release of intact mono-, di- and polynucleosomes (24). Since the vast majority of cells were morphologically normal, it appears that the appearance of nucleosomes on the cell surface of activated T-cells is the result of a small proportion of apoptotic cells releasing their contents into the extracellular space and the consequent binding of this nuclear material on the surface of viable cells.

It was demonstrated, using a solid-phase binding assay, that D2S binds strongly to nucleosomes, and although binding to purified histones H2A and H2B was only slightly lower, binding to histones H3 and H4 was relatively poor. However, whereas the binding of D2S (and other sulfated polysaccharides) to histones is readily explained by an ionic interaction between oppositely charged molecules, the interaction between D2S and nucleosomes, which are both negatively charged, is less obvious. The explanation for this lies in the structure of the nucleosome core particle. Luger et al. (25) have shown using x-ray crystallography that although the histone octomer, comprising 2 molecules of histones H2A, H2B, H3 and H4, is surrounded by negatively charged DNA, the positively charged N termini of the histones extend out of core particle and past the DNA superhelix to allow contact with other macromolecules, normally other nucleosome particles. Here, we suggest that negatively charged D2S binds to the positively charged N-terminal regions of the histones that protrude from the nucleosome core (Fig. 7a). It has been shown previously that as a result of their accessibility the N-terminal regions of histones in a nucleosome particle can be digested with trypsin, leaving the rest of the complex intact (26). Under these conditions D2S binding to nucleosomes was completely attenuated, thus supporting the notion that binding is through these regions.

A similar interaction with nucleosomes has been described previously for heparin (27). Indeed, competition binding studies performed here indicate that this interaction is common to a number of sulfated polysaccharides. Analysis of the competition binding data suggests that larger sulfated polysaccharides are better competitors. For example, high molecular weight dextran sulfate and fucoidan, the two largest compounds, are
much better competitors than the smaller compounds such as D2S, heparin, and low molecular weight dextran sulfate, suggesting that the interaction between sulfated polysaccharides and nucleosomes is multimeric, with each molecule of sulfated polysaccharide binding to several nucleosome particles (Fig. 7). The data suggest that the binding of D2S to activated T-cells observed previously (13) is due to an interaction with nucleosomes that have adhered to the cell surface.

The binding of nucleosomes to the cell surface also appears to be due to an interaction with sulfated polysaccharides. Heparin sulfate proteoglycans (HSPG) are large macromolecules that are present in the extracellular matrix and have been implicated in various biological roles such as presentation and localization of growth factors (28–31). In competition binding studies, heparan sulfate, the sugar component of these large macromolecules, competed with D2S for binding to nucleosomes. Our data would suggest that HSPG are important for anchoring nucleosomes to the cell surface, since untreated cell membranes bound nucleosomes, whereas incubation of membranes with either heparitinase I or heparinase I abolished this binding. Heparitinase I cleaves heparan sulfate in areas of low sulfation, where N-acetylated disaccharides (GlcN-ac-α,1,4-GlcNAc (N-acetylgalactosaminyl-α,1,4-galacturonic acid) are the predominant structural unit (32). Heparinase I cleaves highly sulfated disaccharides of structure GlcNSO3 (−/−6S)−α,1,4IdceA(2S) (N-sulfated galactosamine (6-sulfate)-α,1,4-iduronic acid 2-sulfate) (32). Recently, van Bruggen et al. (33) showed that HSPG in the glomerular basement membrane can bind circulating nucleosomes through anionic interaction between HSPG and histones, although they suggested that for this to occur in vivo it would be necessary for autoimmune antibodies to bind to DNA in order to mask its negative charge. However, our work suggests that the negative charge of DNA does not interfere with the binding of sulfated polysaccharides for the reason discussed above.

Various workers (1, 34, 35) have tried to characterize a nucleosome receptor using SDS-PAGE and ligand blotting in an attempt to elucidate the molecular weight of the receptor, and several putative receptors with markedly different molecular weights have been reported. A 94-kDa protein that is present on the surface of Raji cells, fibroblasts, and a cell line derived from an islet tumor has been described as a nucleosome receptor by Jacob et al. (34). Hefeneider et al. (1) reported that DNA and nucleosomes bound to 29- and 68-kDa proteins present on a murine T-cell line, S49. Gasparro et al. (35) also reported multiple DNA-binding proteins expressed on human lymphocytes that had molecular masses of 28, 59, and 79 kDa. Interestingly, none of the putative receptors outlined above have been sequenced, and in most of the studies the protein bands identified were quite broad and diffuse, suggesting that the proteins are highly glycosylated. It is possible that these putative receptors are in fact all various HSPG, and in each case nucleosomes bind to the heparan sulfate component of the glycoproteins.

The function of cell surface nucleosomes is unclear at present, but several authors have suggested that they have some immunomodulatory role (1, 36–39). Bell et al. (36) showed that nucleosomes released from murine T-cells stimulate immunoglobulin synthesis in normal lymphocytes and, in a later publication (37), showed that nucleosomes from human tonsil lymphoid cells stimulate cell growth of both murine and human T-cells. Subsequently, Hefeneider et al. (1) reported that the binding of nucleosomes to the surface of murine T-cells results in antibody production and the release of interleukin-1α, an important mediator of B-cell differentiation (38). Furthermore, Emlen et al. (39) have shown that interleukin-1β and lipopolysaccharide stimulate cell surface binding of nucleosomes on monocytes, and this in turn leads to further secretion of interleukin-1β.

Additionally, there is increasing evidence that cell surface nucleosomes may be involved in the pathogenesis of autoimmune diseases such as SLE. Burlingame et al. (40) and Amoura et al. (41) have shown that the onset of the autoimmune response in murine models of SLE is characterized by the early emergence of antibodies that recognize conformational epitopes of the nucleosome particle but not its individual components, i.e. double-stranded DNA or histones. In addition, Mohan et al. (6) have demonstrated that nucleosomes are the major immunogen for pathogenic autoantibody-inducing T-helper cells in lupus mice, and accordingly, the (H2A-H2B)-DNA subnucleosome complex appears to be a potent immunogenic stimulus for autoimmune responses to histones and DNA in human SLE (40). There is also evidence that nucleosomes play a role in the pathogenicity of anti-double-stranded DNA autoantibodies. Nucleosomes perfused into mice bind to the glomerulus (33, 42), and Termaat et al. (43) reported that nucleosomes mediate the binding of anti-double-stranded DNA antibodies in the glomerulus.

This study has revealed that nucleosomes are anchored to the cell surface of activated T-cells through an interaction with HSPG. It appears that this interaction is mediated through the sulfate regions found in the heparan sulfate component of the proteoglycan and the lysine and arginine residues present in the N-terminal region of histones. D2S, like other sulfated polysaccharides, is able to bind to cell surface nucleosomes (Fig. 7a) and, at high concentrations, displace them from the HSPG (Fig. 7b). In the absence of nucleosomes, as found in inactivated T-cells, sulfated polysaccharides are unable to bind to the cell surface (Fig. 7c). Thus, the binding of sulfated polysaccharides, including D2S, to the surface of activated T-cells is explained by the presence of cell surface nucleosomes.

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Nucleosomes Bind to Cell Surface Proteoglycans

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