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DNA Binding Fluorochromes as Probes for Histone H1–Chromatin Interactions In Situ

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We have investigated using the DNA binding fluorochromes 7-aminoactinomycin (7-AAMD) and 4′,6-diamidino-2-phenylindole (DAPI) as cytochemical probes for linker histone (H1)–chromatin interactions in situ. Human lymphocytes, permeabilized with digitonin, were exposed to increasing concentrations of sodium chloride to remove ionically bound H1 from the nuclei. The cells were stained to equilibrium with 1 μM 7-AAMD or 50 nM DAPI. Lymphocytes stained with 7-AAMD showed a gradual increase from 11% to 36% of HCl treated cell fluorescence intensity when the salt concentration was increased from 0.15 to 0.7 M. The corresponding increase for DAPI was 53–68%. The 7-AAMD obviously showed higher sensitivity for H1–chromatin interactions that DAPI but had disadvantages such as high background fluorescence and an affinity that was dependent on the preparation procedure. DAPI had negligible background fluorescence, and its fluorescence intensity resembles the number of available high-affinity dye-binding sites when used at 50 nM. We conclude that both fluorochromes can be used as probes for H1–chromatin interactions in situ and that our method has a potential to provide new information on such interactions. Cytometry 28: 212–219, 1997. © 1997 Wiley-Liss, Inc.

Key terms: DNA; chromatin structure; linker histones; dye binding

The lysine-rich linker histones (H1 and H5) are involved in the formation and maintenance of chromatin structure. In vitro studies have shown that H1 coils the 10-nm fiber into a more condensed structure (27; for reviews, see 28,32). Linker histones are also regarded as general repressors of genetic activity (31) and might as well be involved in controlling the transcription of individual genes (6,20; for review, see 17). H1 is present in active chromatin regions, but it seems to be reduced in its amount and may be bound in a different manner (14; for review, see 35), which probably will contribute to a more open chromatin formation. The use of reconstituted material to investigate the linker histone interaction with chromatin (22,33) has provided valuable information on the interactions within individual fibers. However, it is difficult to create a structure in vitro that resembles the situation in the cell nucleus. Therefore, new methods to investigate H1–chromatin interactions in situ may yield additional information on chromatin structure in vivo.

Many DNA binding fluorochromes show restrictions in their binding to DNA by the chromatin structure, and extraction of basic proteins results in an increased nuclear fluorescence intensity (11,12,15,19,21). Salt extraction of H1 also results in higher fluorescence intensity (15,23). This increased intensity implies the possibility of using DNA binding fluorochromes to study the specific interaction between histone H1 and chromatin in more detail.

An obvious candidate for the study of protein–DNA interactions is the DNA binding fluorochrome 7-aminoactinomycin D (7-AAMD), which is regarded as rather sensitive to such interactions (12,21,24). Actinomycin D may have some preference for active chromatin (3,34), where the amount of linker histones may be reduced. Another DNA binding fluorochrome, 4′,6-diamidino-2-phenylindole (DAPI), may have a preference for binding sites similar to those of histone H1 (7). Although H1 has a rather low sensitivity to protein–DNA interactions (12,21,24), its binding pattern suggests that it may be used as a specific probe for H1–chromatin interactions.

Thus, the main purpose of this study was to investigate how linker histones affect the binding of 7-AAMD and DAPI in situ. Dye binding was studied in intact cells, in cells extracted with different salt concentrations, and in linker histone-depleted cells after reassociation of H1. Our results indicate that both fluorochromes can be used as probes for histone H1–chromatin interactions and may provide information on the amount of H1 and its binding strength to chromatin in situ.

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MATERIAL AND METHODS

Cell Preparation

Human peripheral lymphocytes were isolated from whole blood obtained from healthy donors. The blood was diluted 1:1 with ice-cold KRG buffer (120 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 1.7 mM KH_2PO_4, 8.3 mM NaHPO, 10 mM glucose) and separated on Ficoll (Pharmacia, Uppsala, Sweden) at 400*g* for 30 min at 4°C. The mid phase was collected and washed twice in ice-cold KRG at 200*g* for 10 min.

Unextracted (intact) cells. The lymphocytes were fixed in 4% neutral buffered paraformaldehyde (PFA; Merck, Darmstadt, Germany) for 30 min at a concentration of 5×10^5 cells/ml. Two hundred microliters of the cell suspension were placed on cover glasses pretreated with 0.005% protamine sulphate (Sigma, St Louis, MO), and the cells were allowed to adhere for 10 min on ice and then continuously fixed in 4% PFA for 2–5 d. Immediately before staining, the cells were washed in a Tris-NaCl buffer (10 mM Tris-HCl, 0.5 mM $MgCl_2$, 150 mM NaCl, pH = 7.4) for 30 min, permeabilized with 40 μg/ml digitonin (Sigma) in Tris-NaCl buffer for 10 min, and washed again for 1 min.

Salt-extracted cells. Unfixed lymphocytes (5×10^5 cells/ml in KRG) were placed on cover glasses and permeabilized with 40 μg/ml digitonin in Tris-NaCl buffer for 10 min. The cells were either fixed directly with 4% PFA for 2–5 d or extracted with different salt concentrations of 0.15–0.75 M NaCl in Tris-NaCl buffer for 5 min followed by fixation in 4% PFA for 2–5 d. In some experiments, the extraction buffer was supplemented with the protease inhibitors AEBSF 69 μg/ml (Calbiochem), pepstatin 2 μg/ml (Boehringer, Mannheim, Germany), and leupeptin 5 μg/ml (Boehringer). The salt-extraction buffer also contained 1 M sucrose to prevent the cells from disrupting.

Acid-extracted cells. Unfixed lymphocytes (5×10^5 cells/ml in KRG) were placed on cover glasses and permeabilized, extracted with 0.1 M HCl for 10 min, and fixed in 4% PFA for 2–5 d.

Reassociation of H1. The lymphocytes were placed on cover glasses, permeabilized, and extracted with 0.75 M NaCl. After extraction, the lymphocytes were incubated with 30 μg/ml H1 (Boehringer) in Tris-NaCl buffer supplemented with protease inhibitors for 5, 10, 20, 40, or 80 min. Alternatively, the cells were incubated with 2, 5, 10, or 20 μg/ml H1 in the same buffer for 40 min. After treatment with H1, the cells were fixed in 4% PFA for 2–5 d.

All preparations were performed on ice.

Staining

Stock solutions of 7-AAMD (0.2 mM) and DAPI (0.5 mM; Sigma) were prepared by dissolving the dye in sterile millipore water. Solutions were stored at 4°C and used within 1 month. The fixed lymphocytes were washed in Tris-NaCl buffer for 30 min at room temperature and stained with either 1 μM 7-AAMD or 50 nM DAPI until equilibrium was reached. The staining time was at least 2 h for 7-AAMD and 1 h for DAPI, and the staining volume was large enough to keep the dye concentration constant at the indicated level. Immediately before measurements, the cover glasses were mounted on a slide provided with printed rings (Erie Scientific, Portsmouth, NH), thus giving a thin chamber filled with dye solution.

Cytofluorometry

The fluorescence intensities from 25 individual cells on each cover glass were measured by image cytofluorometry by using a Nikon Microphot FXA fluorescence microscope equipped with a Nikon P102 photometer interfaced to an Apple Macintosh SE computer. The instrument was equipped with two light sources, a tungsten bulb for phase contrast illumination to select and focus the cells and a stabilized xenon arc lamp (Osram XBO 100 W) for incident fluorescence excitation. The filter set for 7-AAMD (Nikon G-2A) contained an excitation filter, 510–560 nm, and a dichromatic beam splitter with 50% reflection at 580 nm, but the standard barrier filter in this set was replaced by a bandpass interference filter with its center wavelength at 605 nm and a half-width of 55 nm (Omega Optical Inc., Brattleboro, VT). For DAPI, a standard filter set (Nikon UV-2A) was used. This filter set included an excitation filter, 330–380 nm, a dichromatic beam splitter with 50% reflection at 400 nm, and a barrier filter with its edge at 420 nm. A Nikon phase contrast objective (Fluor 40×, N.A. = 0.85) was used.

Before measuring the cellular fluorescence, 5 measurements on each slide were made in areas without cells to correct for background fluorescence from free dye. All measurements were related to a uranyl glass standard and are expressed as arbitrary uranyl units (A.U.).

Analysis of Salt-Extraction Curves

The salt-induced dissociation of linker histones from chromatin has been described in detail by Kumar and Walker (18). Their binding equation (18),

$$K = \frac{[DNH'][H1]^m}{[DNH][NaCl]^n}$$

with the assumption that m = 1, was used to formulate the expression:

$$y = \frac{\sqrt{k^2x^{2n} + 4kx^n} - kx^n}{2}$$

where y is the fraction of linker histones that is dissociated, k is a constant dependent on the apparent equilibrium constant and the total histone concentration, x is the NaCl concentration, and n is the number of Na^+ ions required to dissociate one linker histone molecule from chromatin. This expression was then used in a least-squares fit to our fluorescence data with the fluorescence intensity at 0.15 M NaCl as y = 0 and the highest

fluorescence intensity for the salt-extracted cells as y = 1. The NaCl concentration required to induce 50% H1 release (y = 0.5) was then calculated from the fitted equation.

Sodium Dodecyl Sulfate (SDS)–Polyacrylamide Gel Electrophoresis and Western Blotting

Lymphocytes were separated and washed, as above. About 5×10^7 cells were split into three equal parts. The cells were permeabilized with digitonin (40 µg/ml in Tris-NaCl buffer) for 10 min. The permeabilization was finalized by the addition of ice-cold Tris-NaCl buffer to the double volume, and the cell suspension was centrifuged at 200*g* for 10 min. The cells were then resuspended in an extraction buffer (Tris-NaCl and protease inhibitors) containing 0.35, 0.4, or 0.75 M NaCl. The extraction was performed for 5 min at 4°C, whereafter the cell suspension was centrifuged at 15,000*g* for 15 min. Supernatants were also collected from unextracted cells after the permeabilization step. Proteins were precipitated by the addition of cold acetone (−20°C) to a final concentration of 80%. The precipitation continued at −20°C overnight. The precipitated proteins were centrifuged at 2,000*g* for 15 min and washed three times in −20°C acetone, 2,000*g* for 15 min, and dried at room temperature for 30–60 min.

In a second experiment, permeabilized lymphocytes were extracted with 0.3, 0.4, or 0.7 M NaCl. The supernatants were collected and treated as above. The pellet remaining after 0.7 M salt extraction was washed three times in KRG buffer and resuspended in 1 ml 0.1 M HCl. After 30 min, precipitation was performed as above.

The dried proteins were resuspended in 50–100 µl sample buffer (0.5 M Tris-HCl, 20% glycerol, 10% SDS, 0.1M dithiothreitol, 0.05% bromophenol blue), boiled for 3 min, and centrifuged for 3 min in an Eppendorf centrifuge. Four microliters of the protein solution were loaded on a 20% SDS gel and separated by using a Phast system (Pharmacia). From the first experiment, one gel was silver stained and the other was blotted onto a nitrocellulose filter according to standard procedures (Pharmacia). The filter was blocked with rinsing buffer (0.14 M NaCl, 1.5 mM KH_2PO_4, 8 mM $Na_2HPO_4 \times 12H_2O$, 2.7 mM KCl, 0.05% Tween 20), supplemented with 5% fat-free milk powder (Semper, Stockholm, Sweden) overnight, and washed three times in rinsing buffer. The filter was then exposed to a mouse monoclonal anti-histone pan antibody (1 µg/ml; cat. no. 1492519, Boehringer Mannheim) for 1 h at room temperature, washed three times in rinsing buffer, exposed to a sheep anti-mouse antibody conjugated with horseradish peroxidase (1:5,000; Amersham, Buckinghamshire, England) for 1 h, washed, and developed according to standard ECL procedures (Amersham).

The second experiment was performed in a similar way, but the time for the exposure to the primary antibody was 48 h at 4°C and the exposure to the secondary antibody (1:2,000) was prolonged to 2 h.

RESULTS

Lymphocytes fixed in PFA and permeabilized with digitonin after fixation (referred to as intact cells) showed distinct nuclei with a heterogenous fluorescence after staining with 50 nM DAPI (Figure 1a). Permeabilization with digitonin before fixation resulted in slightly larger, more homogeneously stained nuclei (Figure 1b). Gradual salt extraction of nuclear proteins resulted in increasingly more fragile nuclei that often showed some DNA that was expelled outside the nuclear boundaries (Figure 1c).

Staining with 1 µM 7-AAMD resulted in much lower fluorescence intensities than staining with 50 nM DAPI. Unextracted cells or cells extracted with low salt concentrations showed fluorescence intensities that were barely visible in the fluorescence microscope. Background fluorescence from cell-free areas sometimes reached about 50% of cellular fluorescence for such preparations. DAPI showed negligible background fluorescence intensities for all preparation procedures.

HCl-treated cells, which are expected to contain nucleosome-free DNA (12), showed the highest fluorescence intensities for both dyes and were chosen to represent 100% dye binding. Intact cells generally showed the lowest fluorescence intensities of all preparations. After staining with 7-AAMD, intact cells showed about 5% of the fluorescence intensity in HCl-treated cells. The corresponding fluorescence intensity after staining with DAPI was about 53% of that of HCl-treated cells. Permeabilization before fixation resulted in a doubling of the fluorescence intensity for 7-AAMD but almost no increase for DAPI. The fluorescence intensity remained at this level after treatment with 0.15 M NaCl, and this level was chosen to represent zero H1-release in the binding equation (y = 0). A small increase in fluorescence intensity for both dyes was then observed at 0.35 M sodium chloride (Figure 2). Continued salt extraction resulted in increased fluorescence intensities. At 0.7 M NaCl, 7-AAMD fluorescence reached about 36% of the level of the HCl-extracted cells. The corresponding result for DAPI was about 68%. The relative increase in fluorescence intensity between 0.35 M and 0.7 M salt extraction, which is the interval that is related to the release of linker histones, was thus substantially larger for 7-AAMD than for DAPI. However, the increased 7-AAMD fluorescence could be explained in part by an increased dye affinity (data not shown). The pooled fluorescence data showed a close fit to the binding equation, and the salt concentration that induced a 50% increase in fluorescence intensity was about 0.48 M NaCl for both dyes (Figure 2). Individual salt extraction curves for four experiments with duplicate glasses showed a mean ± S.D. value of 0.480 ± 0.005 M NaCl for DAPI and 0.486 ± 0.007 M NaCl for 7-AAMD.

Electrophoresis and Western Blots

Separation of proteins obtained from cells permeabilized with digitonin on an SDS gel showed a number of low-molecular-weight protein bands in silver staining. Many of these bands were also detectable in the other lanes, a result most likely explained by the fact that it was impossible to include enough washing between permeabilization and salt extraction. However, none of these bands appeared at the same level as pure linker histones (Figure

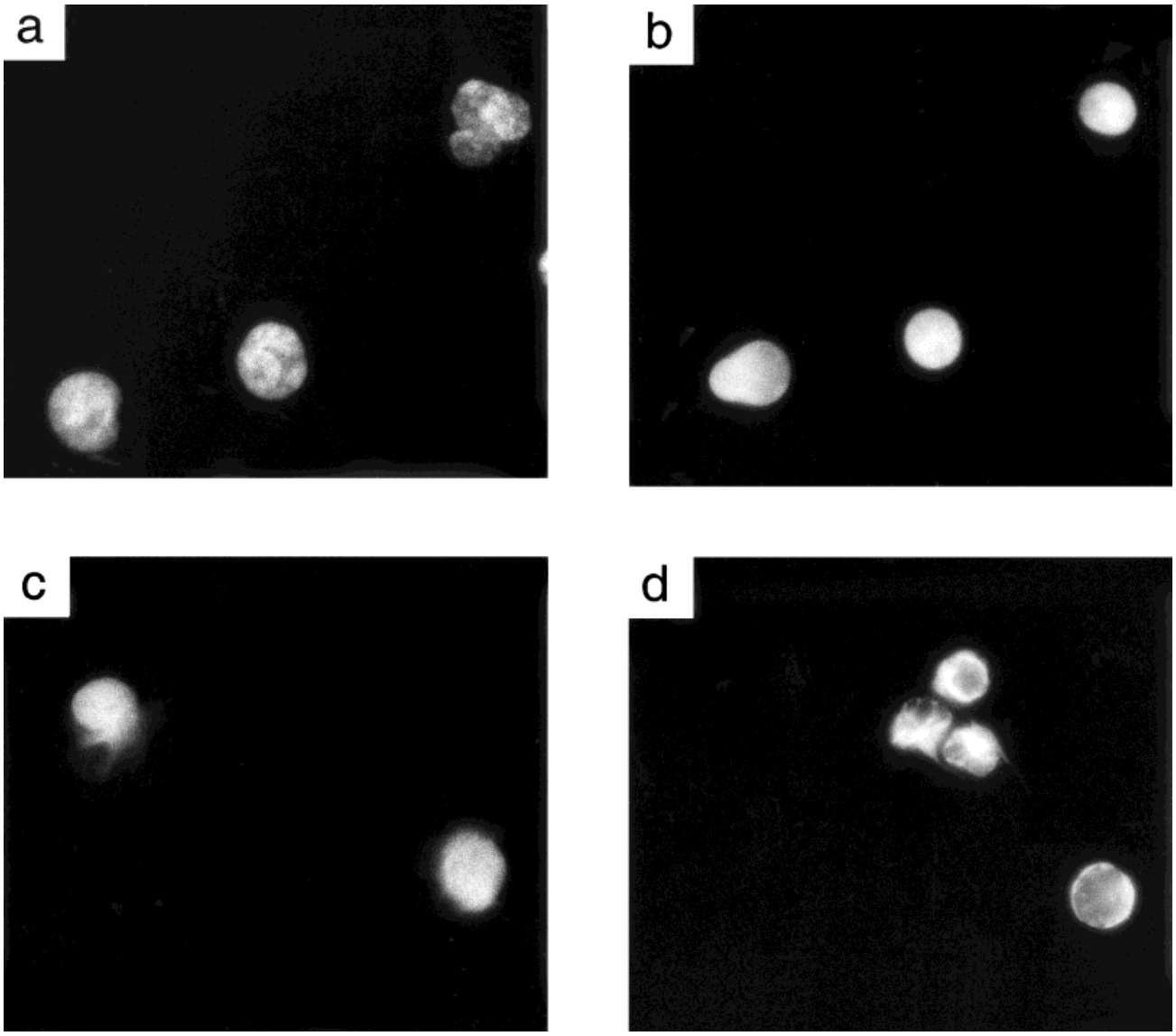

FIG. 1. Fluorescence photomicrographs of lymphocytes fixed in PFA and permeabilized with digitonin (**a**), lymphocytes permeabilized with digitonin before PFA fixation (**b**), lymphocytes permeabilized with digitonin and extracted with 0.75 M NaCl and PFA fixed (**c**), lymphocytes permeabilized with digitonin, extracted with 0.75 M NaCl, and treated with 30 μg/ml histone H1 for 40 min before PFA fixation (**d**). All cells were stained with 50 nM DAPI.

3a). The lane with proteins extracted with 0.35 and 0.4 M NaCl showed an additional band at the same level as pure H1 (Figure 3a). The lane containing proteins extracted with 0.75 M sodium chloride showed two additional bands, one of which was at the same level as pure H1 (Figure 3a).

The corresponding Western blot showed detectable amounts of H1 in the lanes with pure H1 and in the lanes loaded with proteins extracted with 0.35 M, 0.4 M, and 0.75 M NaCl (Figure 3b). After extraction with 0.75 M NaCl, a second band was sometimes detectable at a level corresponding to core histones (data not shown).

The Western blot from the second experiment showed detectable amounts of H1 in the lanes with pure H1 and in the lanes loaded with proteins extracted with 0.4 and 0.7 M NaCl (Figure 4). No H1 was found among the proteins extracted with 0.3 M NaCl (Figure 4, lane 1). HCl extraction of the pellet remaining after extraction with 0.7 M NaCl showed only core histones (Figure 4, lane 4). A small amount of core histones was also detected after 0.7 M salt extraction in this experiment (Figure 4, lane 3).

Reassociation of H1 to H1-Depleted Nuclei

Cells extracted with 0.75 M NaCl and then exposed to pure H1 showed an artificial condensation of chromatin as visualized by DAPI staining (Figure 1d). Treatment with increasing concentrations of H1 also resulted in substantially decreased fluorescence intensities for both dyes

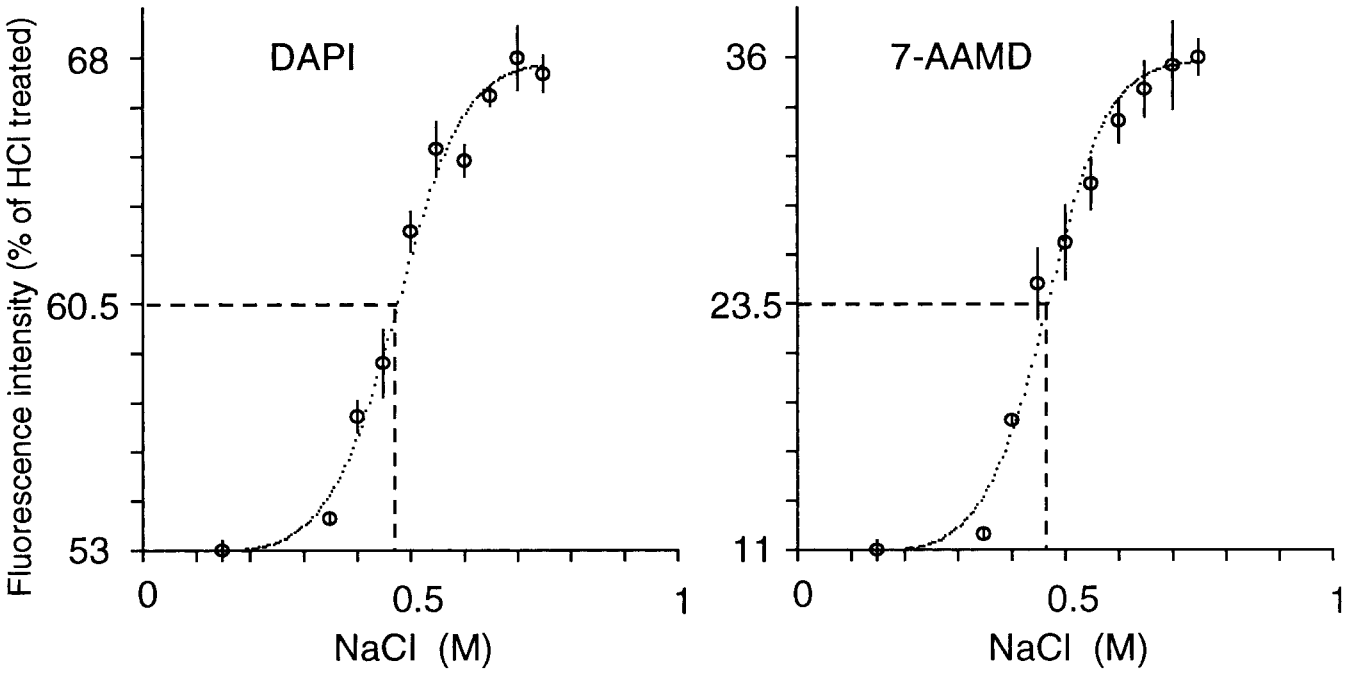

FIG. 2. Relative fluorescence intensity of lymphocytes stained with 50 nM DAPI or 1 μM 7-AAMD after permeabilization with digitonin and extraction with different concentrations of sodium chloride before fixation with PFA. The bar through each data point indicates its standard error of the mean. Most experiments were repeated four times with duplicate glasses.

(Figure 5a). Incubation with 30 μg/ml H1 also resulted in a time-dependent decrease in fluorescence intensities (Figure 5b). The fluorescence intensities after maximal H1 reassociation were lower compared with intact cells for both dyes.

DISCUSSION

Restrictions in the accessibility of DNA binding fluorochromes have been used to investigate differences in chromatin structure, both between cell types (21,26) and between cells of different maturity status (12). In the present study, we have shown that such fluorochromes can be used to obtain information about linker histone–chromatin interactions. Unfortunately, the tendency of the cells to aggregate after salt extraction makes it extremely difficult to use flow cytometry for such applications (12). However, careful handling of the cover glasses during the preparation steps resulted in cells that were quite feasible to analyse in a microscope-based system.

The binding of lysine-rich histones to DNA is mainly electrostatic, with only a small contribution of hydrophobic forces (2; for review, see 36), which makes it possible to extract linker histones by increasing ionic strength (10). Previous results have clearly shown that salt extraction of linker histones is a continuous and selective process that occurs within a defined range of salt concentrations, i.e., 0.3–0.7 M NaCl or KCl (1,2,4,5,18), whereas the core histones require at least 0.7 M salt to appear in solution (2,8). Our results show that detectable amounts of H1 were extracted with ≥0.35 M NaCl (Figure 3), in accordance with those reports. This extraction also resulted in a

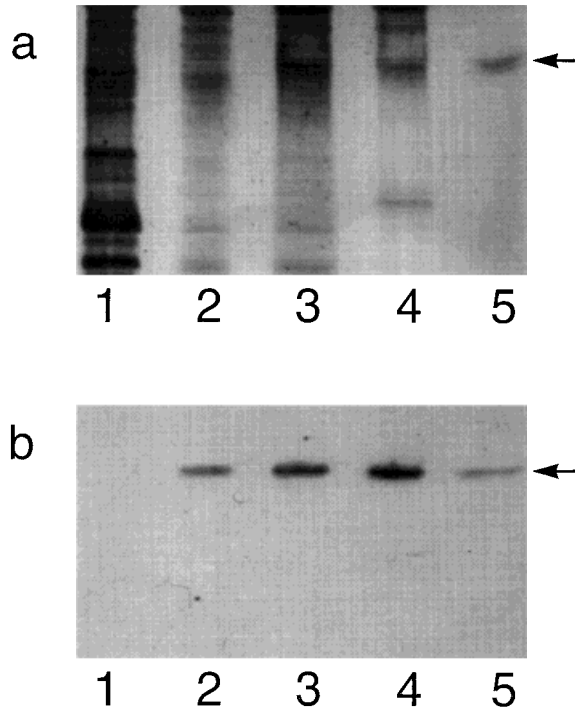

FIG. 3. Silver-stained SDS-PAGE (**a**) and Western blot (**b**) of proteins released from lymphocytes by permeabilization with digitonin (**lane 1**), proteins extracted with 0.35 M NaCl (**lane 2**), 0.4 M NaCl (**lane 3**), 0.75 M NaCl (**lane 4**), and pure linker histone (**lane 5**). The arrows indicate linker histones.

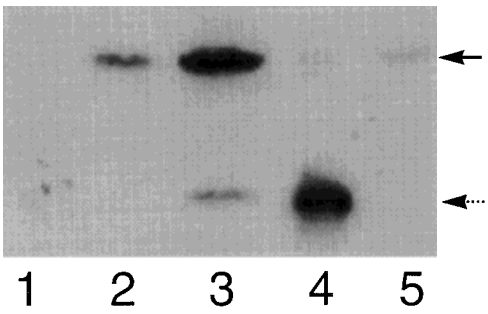

FIG. 4. Western blot of proteins released from permeabilized lymphocytes extracted with 0.3 M NaCl (**lane 1**), 0.4 M NaCl (**lane 2**), 0.7 M NaCl (**lane 3**), 0.1 M HCl after 0.7 M NaCl (**lane 4**), and pure linker histone (**lane 5**). The solid arrow indicates linker histones and the dotted arrow indicates core histones.

gradual increase in fluorescence intensity for both DAPI and 7-AAMD, indicating that linker histone extraction can be followed by cytofluorometry. This result was also supported by the good agreement between our fluorescence data and the H1 binding equation (18) and by the H1 reassociation experiment, which resulted in a substantially reduced fluorescence (Figure 5). Although other lysine/arginine-rich proteins, like core histones, would most probably be able to cause a reduction in fluorescence intensity, our result clearly demonstrates the relation between H1 binding to chromatin and 7-AAMD or DAPI fluorescence intensity.

The amount of extracted linker histones at each salt concentration will most likely depend on both H1 subtype composition and their degree of phosphorylation (16). Other posttranslational modifications of nuclear proteins or changes in DNA, such as methylation, may also affect the binding strength of H1 to chromatin. Because other abundant nonhistone nuclear proteins bound to chromatin, such as HMG proteins, can be extracted before 0.35 M NaCl (for reviews, see 9,32), our results also suggest that such proteins do not substantially reduce the accessibility of 7-AAMD or DAPI to chromatin (Figure 2).

Cellular proteins were obviously lost during cell permeabilization (Figure 3a). This step, when performed before fixation, resulted in a doubling of the fluorescence intensity for 7-AAMD without affecting DAPI fluorescence. Digitonin is considered to be a mild detergent, and the concentration used (40 μg/ml) is supposed to extract only the cholesterol fraction of the plasma membrane. Other membranes surrounding cytoplasmic organelles or the cell nucleus should not be affected (25). Therefore, a possible explanation for the change in 7-AAMD fluorescence caused by digitonin permeabilization is that the integrity of the plasma membrane is vital for the chromatin structure. Similar results, showing chromatin decondensation and increased accessibility of olivomycin and propidium iodide caused by Triton permeabilization, have been reported (29). These observations suggest that cell permeabilization can cause a reorganization of chromatin that also may alter the salt-induced extraction of linker histones. Although our effort has been to preserve the native chromatin structure as far as possible before this extraction, data on linker histone binding strength in situ must be interpreted with some precaution.

Electrophoretic separation of the proteins, obtained after permeabilization with digitonin, showed a number of bands after silver staining. These proteins are probably soluble and leak out when the membrane opens up at permeabilization. We could also detect most of these leaks in the lanes containing proteins from cells extracted with different salt concentrations (Figure 3), which was expected because it was impossible to insert washing steps in the preparation procedure. After permeabilization, the cells became extremely adhesive, and centrifugation resulted in a cell pellet that was difficult to resuspend and reextract with higher salt concentrations.

Our results show that most H1 was released after treatment with 0.7 M sodium chloride (Figure 4, lane 4), which is in accordance with previous results (1,2,4,5,13,18,30), and occasionally we detected small amounts of core histones released at 0.7 M NaCl (Figure 4, lane 3). The form of the extraction curves (Figure 2) indicates that the increase in fluorescence intensity in the interval between 0.3 and 0.7 M NaCl could be proportional to the amount of linker histones. Useful information on H1 affinity may be provided by the salt concentration that induces 50% increase in fluorescence intensity, which should be proportional to the average of an apparent H1–chromatin binding strength in situ. The results show that such data may be obtained with reasonable precision by using a microscope-based cytofluorometric method.

Although our fluorescence data fit well to the H1 binding equation (18), it is important to point out that there may not be a linear relationship between the increase in fluorescence intensity and the appearance of H1 in solution. Different parts of the H1 molecule may require slightly different salt concentrations to loose their attachment to chromatin. These parts may also have different capacities to affect fluorochrome binding, and it is therefore possible that the fluorescence intensity may increase somewhat before the protein appears in solution.

Both fluorochromes displayed an additional increase in fluorescence intensity from the level obtained after extraction with 0.7 M NaCl to the level obtained after HCl extraction. This increase was probably caused by the interaction between core histones and DNA (12). Thus, the effect of core histones to prevent accessibility of both fluorochromes to DNA was about twice the effect of linker histones. Because the core histones outnumber the linker histones by approximately a factor of eight, our results suggest that H1 has a larger effect on dye binding than core histones have. This conclusion was also supported by the large decrease in fluorescence intensity after reassociation of H1 to H1-depleted lymphocytes.

A change in fluorescence intensity may be caused by a change in dye affinity and/or in the number of available dye binding sites. We previously showed that both 7-AAMD and DAPI have more than one class of binding sites to chromatin in situ, which suggests that low dye concentrations should be used to minimize the influence of less

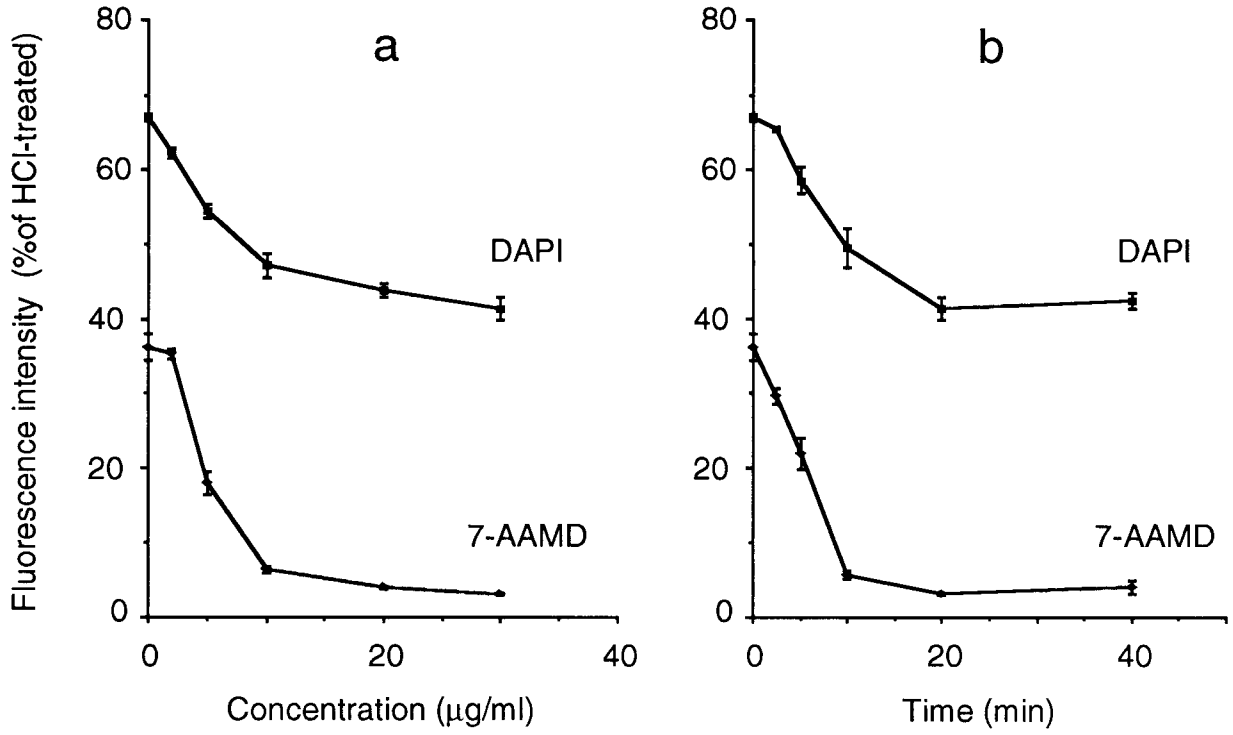

FIG. 5. The relative fluorescence intensity of lymphocytes stained with 50 nM DAPI or 1 µM 7-AAMD after reassociation of H1 to H1-depleted lymphocyte nuclei. Reassociation for 40 min using increasing concentrations of H1 (**a**) and reassociation of 30 µg/ml H1 for 0–40 min (**b**). The bar through each data point indicates standard errors of the mean (n = 4).

specific binding sites (21,24). The affinity of DAPI for chromatin in intact lymphocytes was about 100 times larger than that of 7-AAMD. Moreover, the affinity of 7-AAMD to DNA in HCl-extracted lymphocytes was about four times larger than its affinity to DNA in intact cells (21). No such change in affinity was found for DAPI (21). We also reported that DAPI has a particular advantage because its high affinity binding sites were found to be saturated at a dye concentration of 50 nM, where there was still almost no contribution from binding sites with lower affinity (21). For 7-AAMD, no such dye concentration could be found. Consequently, DAPI has a certain advantage over 7-AAMD because the number of available dye binding sites may be more relevant than dye affinity as a marker for protein–DNA interactions.

We conclude that both fluorochromes can be used as probes for H1–chromatin interactions in situ. Although DAPI has a lower sensitivity to changes in chromatin structure than 7-AAMD, it has several advantages, such as high and constant affinity, negligible background fluorescence, and a fluorescence intensity that resembles the number of available high-affinity dye binding sites when used at 50 nM. Its relatively high sensitivity to linker histone–chromatin interactions may render it useful for detailed analyses of such interactions to obtain new information about the role of linker histones in the formation and maintenance of chromatin structure.

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