Evidence for Ternary Complex Formation by Histone H1, DNA, and Liposomes[†]

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ABSTRACT: Using three different donor−acceptor pairs for resonance energy transfer, interactions in systems composed of histone H1, liposomes, and DNA were investigated. While weak attachment of H1 to phosphatidylcholine (PC) liposomes was observed, the inclusion of phosphatidylserine (PS), phosphatidylglycerol (PG), or phosphatidic acid (PA) strongly enhanced the membrane association of H1, the extent of binding increasing with the content of the acidic lipid. Increasing the content of the negatively charged lipid also made the membrane attachment of H1 less susceptible to dissociation by NaCl, thus indicating, in keeping with our previous studies, that protonation of the acidic lipid is an important factor. Whereas DNA binds to sphingosine-containing cationic liposomes, these vesicles did not bind H1. Instead, H1 effectively competed with sphingosine for binding with DNA. In systems comprising DNA, liposomes, and H1, the interactions were clearly dependent on the liposome composition. While moderately acidic liposomes (PS content <30 mol %) seemed to form ternary complexes with DNA and H1, strongly acidic liposomes (PS content >30 mol %) competed with DNA for binding H1, partly removing the histone from the nucleic acid. The tendency to form ternary complexes also seemed to depend on the type of the acidic lipid. Possible physiological consequences of the interactions detected in these simple model systems are discussed.

Histones, basic proteins, abundant in the cell nucleus, play a major role in chromatin condensation and regulation of gene expression (Grunstein, 1990; Workman & Buchman, 1993; Wolffe, 1994). The DNA in eukaryotic nuclei is organized into nucleosomes each of which consists of 146 bp of DNA wrapped about two turns around a core of histone proteins. The latter is an octamer containing two copies of each of the core histones H2A, H2B, H3, and H4 (van Holde, 1989). The linker histone H1 binds to the nucleosome across the entry and exit points of DNA, bringing the two helical strands together. H1 also interacts with the linker DNA between nucleosomes and is thought to play an important role in organizing the nucleosome chain into the 30-nm fiber of chromatin (Thoma et al., 1979; Allan et al., 1980, 1986).

Numerous recent reports have suggested the possible participation of lipids in eukaryotic transcription and replication processes. Active chromatin has been found to be vicinal to the nuclear membrane (Hutchinson & Weintraub, 1985), and both replication and transcription have been suggested to occur in association with the nuclear matrix (Berezney & Coffey, 1975; Ciejek et al., 1983; Cook, 1991). Likewise, the activity of enzymes such as DNA polymerases (Sylvia et al., 1988; Yoshida et al., 1989), RNA polymerases (Lezius & Müller-Lornsen, 1972), and topoisomerase I (Tamura et al., 1990) has been shown to be influenced by lipids. Moreover, lipids have been found in the chromatin fraction and nuclear matrix, and their amount seems to vary between active and repressed chromatin (Alessenko, 1990).

While complex formation between DNA and histones has been extensively studied under various experimental conditions, the interaction of histones with lipids and its possible influence on their attachment to DNA have been paid relatively little attention to. Recently, binding of various phospholipids to histones has been demonstrated (Hirai et al., 1992). It was also shown that two of these lipids, phosphatidylglycerol (PG)[1] and CL, were able to reverse the inhibition of transcription from DNA template in vitro caused by the histone mixture. While these results strongly suggest that some lipids can indeed alter the state of binding of histones to DNA, more detailed investigations into these processes are clearly needed.

In the present study, we have specifically dealt with the binding of the linker histone H1 to liposomes of different phospholipid composition and the influence of these interactions on the attachment of H1 to DNA. Of particular interest with liposomes containing sphingosine, as we have recently observed direct binding of this positively charged biologically active sphingolipid metabolite to DNA and suggested the feasibility of its possible influence on chromatin structure in vivo (Kinnunen et al., 1993b).

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[1] Abbreviations: bisPDPC, 1,2-bis[(pyren-1-yl)decanoyl]-*sn*-glycero-3-phosphocholine; Sph, sphingosine; PC, phosphatidylcholine; eggPC, egg yolk phosphatidylcholine; PA, phosphatidic acid; eggPA, egg yolk phosphatidic acid; PS, phosphatidylserine; brainPS, bovine brain phosphatidylserine; PG, phosphatidylglycerol; eggPG, egg yolk phosphatidylglycerol; CL, cardiolipin; Adr, adriamycin; Adr−DNA, adriamycin−DNA complex; PKC, protein kinase C; RFI, relative fluorescence intensity.

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EXPERIMENTAL PROCEDURES

Materials. Calf thymus DNA, Hepes, EDTA, fluorescein 5-isothiocyanate (FITC), spermine, spermidine, and the inorganic salts NaCl, $CaCl_2 \cdot 2H_2O$, and $MgCl_2 \cdot 6H_2O$ as well as the lipids eggPC, eggPA (sodium salt), eggPG (sodium salt), and D-sphingosine (from bovine brain sphingomyelin) were purchased from Sigma. BisPDPC was obtained from K&V Bioware Inc. (Espoo, Finland) and bovine brain PS (sodium salt) from Avanti Polar Lipids, Inc. The purity of the lipids was checked by thin-layer chromatography on silicic acid-coated plates (Merck, Germany) using a chloroform/methanol/water (65:25:4, by volume) solvent system. In each case, only one, homogeneous, spot was detected after staining with iodine vapor. Hoechst 33258 was purchased from Molecular Probes, Inc. (Eugene, OR) while adriamycin (doxorubicin) was a generous gift from Farmitalia Carlo Erba. Histone H1 was purified from calf thymus (Johns, 1976). Water used in the experiments was freshly deionized in a Milli RO/Milli Q (Millipore) filtering system.

Preparation of Liposomes. Appropriate amounts of the lipid stock solutions in chloroform were first mixed to yield the desired molar ratios. These mixtures were then evaporated to dryness under a stream of nitrogen and traces of solvent subsequently removed by evacuating under reduced pressure for at least 2 h. The dry residues were hydrated above the main transition temperature of the lipid composite with 20 mM Hepes buffer, containing 0.1 mM EDTA (pH 7.4), so as to yield multilamellar liposomes. Unilamellar vesicles were then obtained by extrusion through 100 nm polycarbonate membranes (Nuclepore, Pleasanton, PA) with a LiposoFast membrane homogenizer (Avestin, Ottawa, Canada). The total lipid concentration was 26 μM if not indicated otherwise. When indicated, 1 mol % of the pyrene-labeled lipid bisPDPC was included in the liposomes. The pyrene moieties of this lipid reside in the central, hydrocarbon region of the bilayer. Under the experimental conditions used by us, the lipids were in the liquid-crystalline state, where their acyl chains have a comparatively high degree of motional freedom, particularly in the bilayer core. We can thus expect the pyrene moieties to be easily accommodated within the bilayer and cause negligible perturbation either of the vesicle structure of or the electrostatic interactions taking place on the liposome surface.

Labeling of Histone H1 with FITC. Histone H1 was labeled with fluorescein 5-isothiocyanate (FITC) essentially as described by Favazza et al. (1990). In brief, 15 mg of H1 was dissolved in 3 mL of sodium phosphate buffer adjusted to pH 9.5. A concentrated ethanolic solution of FITC was added to this to yield a [FITC]/[histone] molar ratio of 4.0. The mixture was incubated for 3 h in the dark at room temperature with occasional shaking. The reaction was stopped by adjusting the pH of the reaction mixture to 6.0. In order to separate the unreacted FITC from the FITC–histone complex and to remove electrostatically adsorbed dye from H1, the reaction mixture was extensively dialyzed against a high ionic strength buffer containing amino groups, i.e., 0.6 M NaCl/10 mM Tris-HCl, pH 7.0. The protein solution was then desalted by dialysis against 20 mM Hepes buffer, containing 0.1 mM EDTA (pH 7.4). The amount of FITC bound to histone was determined by light absorption at 500 nm using a molar extinction coefficient $\epsilon = 46\,600$ L/(mol·cm) (Favazza et al., 1990). Protein concentration was determined by the method of Bradford (1976). The labeling ratio was found to be approximately 1.1 mol of FITC per mole of H1. At this labeling ratio, the change in the surface charge of H1 can be considered minimal.

Fluorescence Measurements. Förster resonance energy transfer provides a convenient means whereby to assess the proximity of appropriate fluorophore pairs (Stryer, 1978). In the present study, three different donor–acceptor couples were utilized to monitor interactions between histone H1, DNA, and liposomes (Table 1). More specifically, quenching of bisPDPC emission by fluorescein-labeled H1 can be used to monitor the binding of the histone to liposomes. Likewise, the spectral overlap of the emission of the strongly DNA-interacting dye Hoechst 33258 with the absorption of fluorescein-labeled H1 allows observation of the binding of the histone to DNA. Finally, we have previously described how the quenching of pyrene excimer fluorescence from bisPDPC by Adr, an intercalating dye, can be used to monitor the attachment of the Adr–DNA complex to liposomes containing the fluorescent lipid (Kinnunen et al., 1993b; Kõiv et al., 1994). The dye intercalated between the bases should have little influence on the primarily electrostatic interactions of DNA with either cationic liposomes or the strongly basic histone H1. This notion is supported by the similar results from our previous studies on DNA–sphingosine association obtained with differential scanning calorimetry employing no fluorescent probes (Kõiv et al., 1994).

Fluorescence intensities were measured using an SLM-4800S spectrofluorometer equipped with a magnetically-stirred thermostated cuvette compartment. The excitation and emission wavelengths used are summarized in Table 1. Excitation and emission bandwidths were 1 and 16 nm, respectively. The fluorescence intensity values were corrected for dilution. The concentrations of the reactants were kept low (in the micromolar range) so as to obtain negligible interference due to inner filter effects (Mustonen & Kinnunen, 1993). The advantages as well as limitations of this type of resonance energy transfer measurements have been discussed elsewhere (Drake et al., 1991; Kaihovaara et al., 1991; Mustonen et al., 1993). Importantly, while this technique yields useful qualitative data, quantitation is ambiguous. This is due to complications arising from the fact that fluorescence from multiple donors embedded in membranes or in DNA is being quenched. Emission spectra were recorded with a Perkin Elmer LS 50B spectrofluorometer connected to a Compaq ProLinea computer. The instrument was operated and the collected data were analyzed using the dedicated software from Perkin Elmer. The excitation wavelengths were 344 and 360 nm for bisPDPC and for Hoechst 33258, respectively, with excitation and emission bandwidths of 2.5 and 4 nm. Absorption spectra were obtained using a Shimadzu Graphicord spectrophotometer. All experiments were carried out at 25 °C where the lipid membranes used are in a fluid, liquid-crystalline state.

Analytical Methods. Phospholipid concentrations were determined by phosphorus assay (Bartlett, 1959). The concentration of sphingosine was determined by dry weight. DNA concentration was determined by the absorption at 260 nm [$\epsilon = 6600$ L/(mol·cm)] and by the phosphorus assay and is given in millimolar nucleotide. Within error limits, the two methods gave identical results. The concentration of Adr was determined by the absorption at 480 nm [$\epsilon = 11\,500$

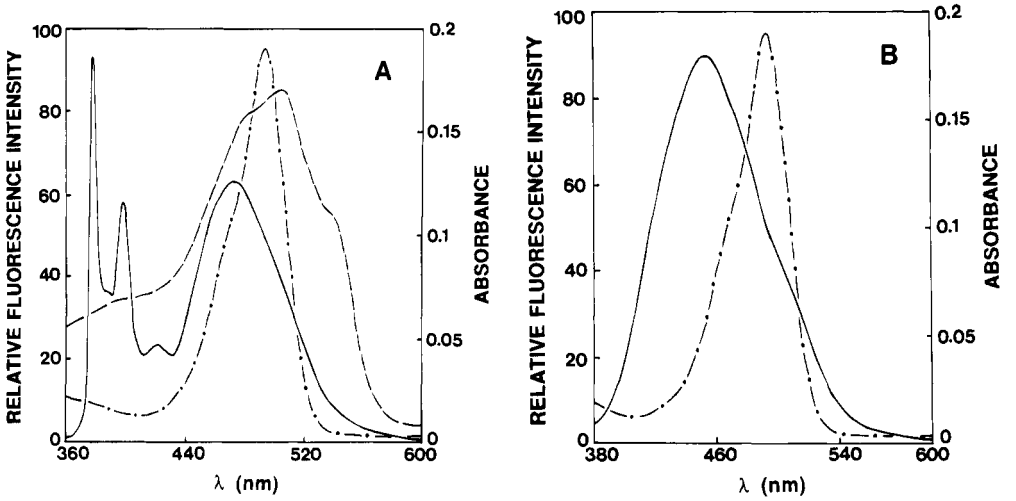

FIGURE 1: Fluorescence and absorption spectra of the donor–acceptor pairs used for detecting interactions between H1, DNA, and liposomes. Panel A shows the emission spectrum of 1 mol % bisPDPC in eggPC (excitation wavelength 344 nm) (—) and the absorption spectra of the adriamycin–DNA ([nucleotide]/[Adr] = 10) complex (– –) and FITC–H1 (– • –) employed as quenchers of bisPDPC fluorescence. Panel B depicts the emission spectrum of the Hoechst–DNA complex ([nucleotide]/[Hoechst] = 25; excitation wavelength 360 nm) (—) and the absorption spectrum of FITC–H1 (– • –) used as a quencher of Hoechst fluorescence.

L/(mol•cm)], and that of Hoechst 33258 at 345 nm [ϵ = 48 000 L/(mol•cm)].

RESULTS

Binding of H1 to Liposomes. The binding of histone H1 to liposomes of different compositions was investigated by labeling H1 with fluorescein isothiocyanate so as to allow its use as a quencher for the fluorescence of the strongly excimer-forming phospholipid analog bisPDPC. Efficient dipole–dipole coupling necessitates extensive spectral overlap of the relevant donor and acceptor spectra which is readily evident (Figure 1). Accordingly, membrane association of H1 can easily be monitored using this pair for resonance energy transfer. While previously acidic phospholipids have been shown to bind histones (Hirai et al., 1992), eggPC liposomes also associate with H1, as can be seen from the moderate decrease in RFI upon addition of the histone [Figure 2, panel A (top panel)]. This interaction was readily reversible on increasing the ionic strength by addition of NaCl. Inclusion of the acidic phospholipid PS into the liposome considerably improved the binding capacity for H1, and increasing the mole percent of PS in the liposome resulted in progressively augmented fluorescence quenching by H1 until saturation was reached at about 30 mol % PS. Interestingly, increasing the mole percent of PS also made the histone attachment less susceptible to reversal by salt. At 30 mol % PS, addition of NaCl up to 200 mM was practically ineffective in detaching the histone from the liposome surface (Figure 2, panel A). The above findings were not specific for PS, and nearly identical results were obtained with liposomes containing PG (data not shown). Liposomes containing PA also effectively bound H1. For this acidic lipid, which has two ionizable hydroxyls in the phosphate group, saturation of H1 binding was achieved already at 10 mol % PA (Figure 2, panel B, middel panel). Notably, inclusion of 20 mol % sphingosine into the liposomes totally abolished the membrane association of the histone (Figure 2, panel C, bottom panel). The binding of H1 to liposomes varied depending on their content of cationic and anionic lipids (Figure 2, panel C).

Inclusion of DNA in the liposome solution prior to addition of the histone shifted the H1 binding curve to the right in keeping with competition between liposomes and DNA for the histone (Figure 3). At the same time, the presence of DNA enhanced the fluorescence quenching due to the labeled histone.

Reversal of Binding of DNA to Sphingosine-Containing Liposomes by Histone H1. Similar to the quenching of bisPDPC fluorescence by FITC-labeled H1 described above, we have previously utilized resonance energy transfer from bisPDPC to Adr-labeled DNA to study the membrane association of the nucleic acid. In keeping with our previous results (Kinnunen et al., 1993b; Kõiv & Kinnunen, 1994), DNA efficiently bound to cationic liposomes composed of eggPC/sphingosine/bisPDPC at a 79:20:1 molar ratio (Figure 4). Interestingly, addition of increasing concentrations of histone H1 led to the detachment of DNA from the liposomes as reflected by a rapid increase in the relative fluorescence intensity due to the loss of pyrene fluorescence quenching by the Adr–DNA complex in the presence of H1 (Figure 4). Under these conditions, complete reversal of the binding of DNA was achieved at about 0.2 μM H1, corresponding to [H1]/[Sph] and [H1]/[nucleotide] molar ratios of 0.04.

The effect of H1 on the DNA–sphingosine interaction was also compared with those due to the polyamines spermine and spermidine, as well as the divalent cations Mg^{2+} and Ca^{2+}. Interestingly, only spermine was capable of detaching DNA from sphingosine-containing liposomes, but to a lesser degree than H1. As shown in Figure 4, the initial drop in RFI due to the binding of DNA to the liposomes was reversed by 70% by spermine. Maximal detachment was achieved at 300 μM spermine, corresponding to a [spermine]/[Sph] ratio of 60. In contrast, the influence of spermidine at concentrations up to 200 μM was negligible, and at 560 μM, only 30% of the initial decrease in RFI was reversed. Addition of up to 5.5 mM of either Ca^{2+} or Mg^{2+} to the DNA–liposome aggregates did not detach DNA from the lipid.

Interactions in Systems Containing H1, Liposomes, and DNA. By comparing the three different fluorescent donor–

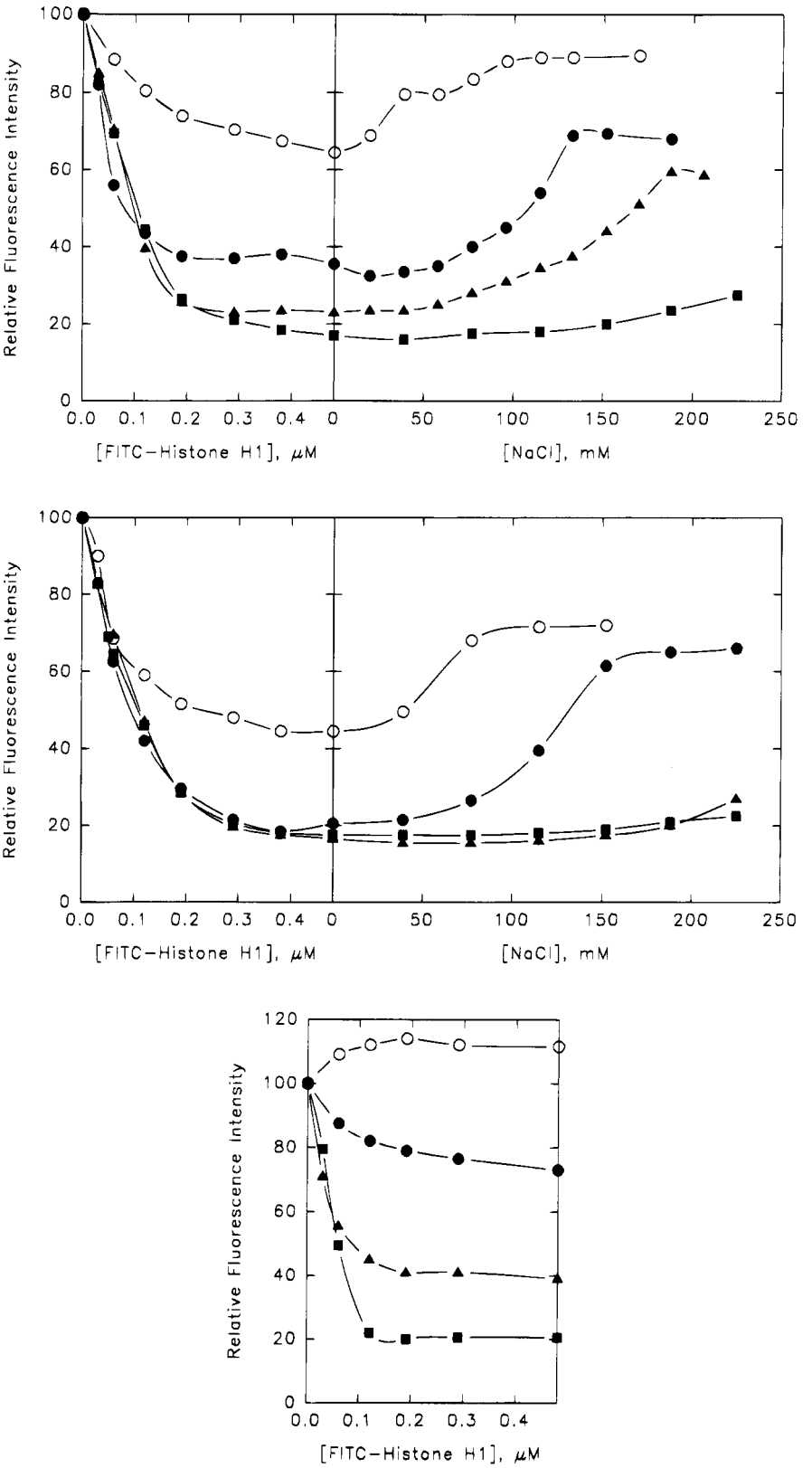

FIGURE 2: Binding of histone H1 to liposomes of different composition and its reversal by salt. The decrease in RFI due to quenching by FITC-labeled H1 upon attachment of the latter to the liposome surface is shown for neat eggPC (○) vesicles and for vesicles containing either 10 (●), 20 (▲), or 30 (■) mol % PS (panel A, top) or 5 (○), 10 (●), 20 (▲), or 30 (■) mol % PA (panel B, middle). Addition of NaCl restored the RFI to an extent dependent on the liposome composition. Panel C (bottom) depicts binding of FITC−H1 to eggPC vesicles containing 20 mol % sphingosine together with 10 (●), 20 (▲), and 30 (■) mol % PS. The RFI for vesicles containing only 20 mol % sphingosine in eggPC (○) is also shown. The total lipid concentration was 26 μM in all cases.

acceptor pairs (Table 1) under identical experimental conditions, the interactions between DNA, histone H1, and liposomes can be separated. Attachment of H1 to DNA in the absence and presence of liposomes is shown in Figure 5. At 26 μM lipid concentration, liposomes composed to 10 mol % PS in eggPC or 20 mol % sphingosine in eggPC

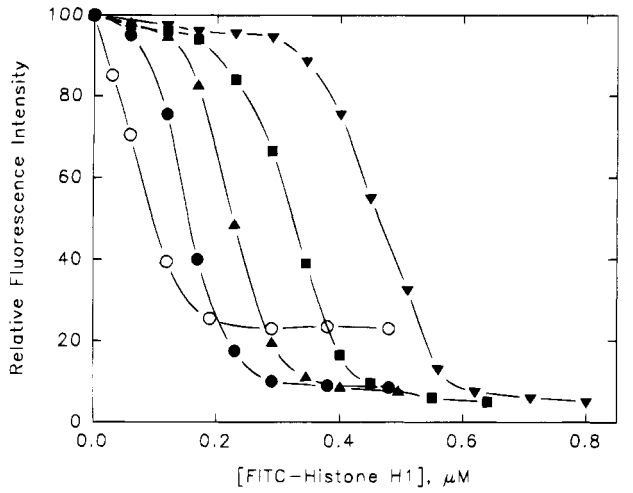

FIGURE 3: Binding of FITC-labeled histone H1 to liposomes containing 20 mol % PS in eggPC in the absence (○) and presence of 2.5 (●), 5 (▲), 7.4 (■), and 12.2 (▼) μM DNA. The lipid concentration was 26 μM.

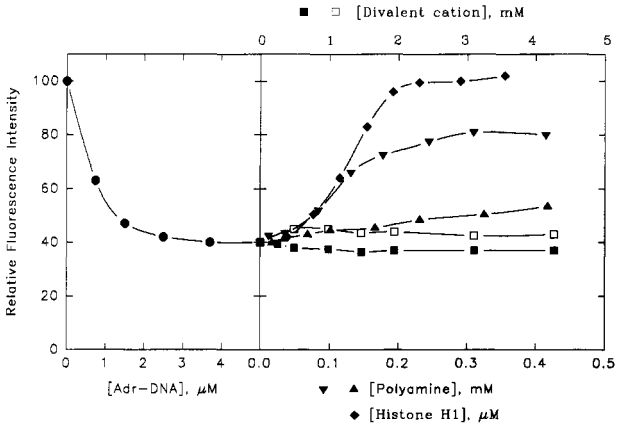

FIGURE 4: Reversal of the interaction between sphingosine-containing liposomes and DNA by some cations. Binding of DNA to eggPC vesicles containing 20 mol % sphingosine (●) was monitored by the decrease in RFI from bisPDPC incorporated into the liposomes due to quenching by Adr−DNA. This interaction was fully reversed by H1 (♦) and partly by the polyamines spermine (▼) and spermidine (▲). The divalent cations Ca^{2+} (■) and Mg^{2+} (□) were ineffective.

Table 1: Summary of Fluorescence Donor−Acceptor Pairs for Resonance Energy Transfer Monitoring of Interactions between DNA, H1, and Liposomes

donor	λ (nm) ex	λ (nm) em	acceptor (quencher)	interaction probed
bisPDPC[a]	344	478	adriamycin in DNA	liposome−DNA
bisPDPC	344	478	FITC-H1	liposome−H1
Hoechst 33258 in DNA	360	478	FITC-H1	DNA−H1

[a] BisPDPC yields strong pyrene excimer fluorescence (Sunamoto et al., 1980) and has been widely used as a fluorescent probe for lipid membranes (Kinnunen et al., 1993a).

interfered very little with the binding of histone to DNA. On the other hand, liposomes containing 30 mol % PS shifted the binding curve toward higher histone concentrations, most probably reflecting competition of liposomes and DNA for the histone. At increasing lipid concentrations, all three types of liposomes interfered with the H1−DNA interaction,

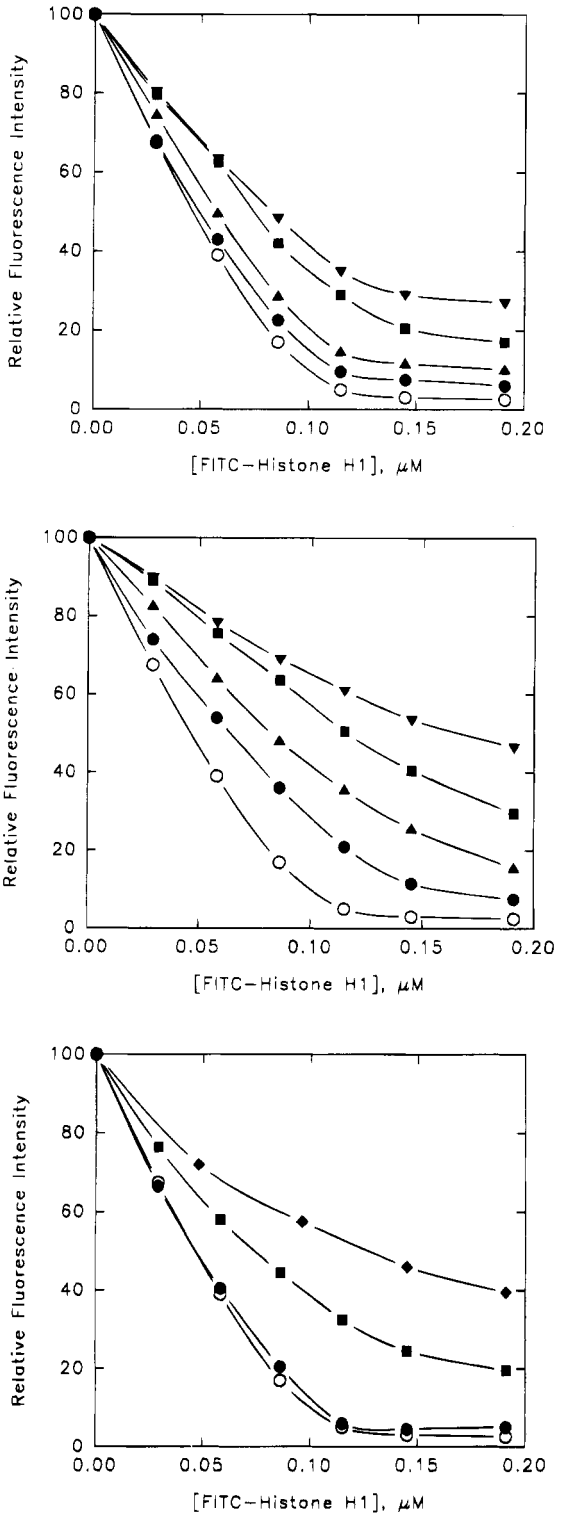

FIGURE 5: Influence of liposomes on the interaction between histone H1 and DNA as measured by a decrease in RFI from Hoechst-labeled DNA due to quenching by FITC−H1. The binding is shown in the presence of eggPC liposomes containing 10 (panel A, top) or 30 (panel B, middle) mol % PS at total lipid concentrations of 26 (●), 52 (▲), 130 (■), and 260 (▼) μM. Panel C (bottom) depicts the attachment of H1 to DNA in the presence of eggPC membranes containing 20 mol % sphingosine at 26 (●), 130 (■), and 235 (♦) μM total lipid. For comparison, H1−DNA interaction in the absence of liposomes (○) is included in each panel. The binding curve in the presence of 26 μM of pure eggPC liposomes coincided with the latter.

liposomes containing 10 mol % PS being the least effective and those containing 30 mol % PS the most.

Interactions between liposomes and DNA in the absence and presence of histone H1 are illustrated in Figure 6. Contrasting with the results for vesicles containing 20 mol % of the positively charged sphingosine, no attachment of DNA occurred either to neutral eggPC liposomes or to negatively-charged liposomes containing varying amounts of PS. However, upon the addition of histone H1, a decrease in RFI was evident, revealing an interaction between DNA and the negatively-charged liposomes. The decrease in RFI was dependent on the liposome composition. EggPC vesicles showed a small decrease up to 20% in fluorescence, whereas for liposomes containing 10 and 20 mol % PS, quenching in the presence of H1 was significantly more extensive, about 40 and 60%, respectively. Further increase in the PS content of the vesicles again led to a decrease in the extent of quenching in the presence of H1 (Figure 6, panel A). Liposomes containing PG as the acidic component behaved analogously to those containing PS (Figure 6, panel B). However, when PA was used, only minor quenching (by 10−20%) was observed upon the addition of H1 at all tested PA contents (Figure 6, panel C).

DISCUSSION

The linker histone H1 has been suggested to play a dual role in the maintenance of chromatin structure. First, H1 seals the nucleosome by binding via its globular domain to DNA at the entry and exit points, bringing the two ends together (Allan et al., 1980). H1 also stabilizes the higher-order 30-nm chromatin fiber by interacting via its basic C-terminal region with the linker DNA between nucleosomes (Thoma et al., 1979; Allan et al., 1986) locating H1 in the interior of the fiber (Graziano et al., 1994). In addition, cell-cycle-dependent hyperphosphorylation of H1 is thought to be the driving force for the condensation of chromatin into chromosomes (Bradbury et al., 1973; Th'ng et al., 1994).

Experimental evidence connects histone H1 binding to DNA with effective repression of transcription [Wolffe, 1989; Croston et al., 1991; Laybourn & Kadonaga, 1991; reviewed by Zlatanova (1990)]. The depletion of H1 as well as its phosphorylation is expected to unfold the chromatin fiber and have been correlated with transcriptional activity (Wolffe, 1992). It has also been suggested that, besides acting as a general repressor of transcription for large regions of chromatin, H1 might be involved in controlling the expression of individual genes by competing with transcription factors for the same binding sites on DNA (Zlatanova, 1990).

The mechanism of the binding of H1 to DNA is not fully understood. While it seems to rely in the first place on the high concentration of positive charge on H1, facilitating strong electrostatic interaction with the polyanionic DNA, it appears that the hydrophobic effect and steric hindrance are also involved. Accordingly, H1 has been reported to prefer AT-rich over GC-rich sequences (Sponar & Sormova, 1972; Renz & Day, 1976) and to recognize a narrow minor groove (Turnell et al., 1988; Churchill & Suzuki, 1989). In addition, H1 attaches to supercoiled rather than relaxed linear DNA (Vogel & Singer, 1975; Singer & Singer, 1978). While any kind of DNA apparently binds histone H1 to some extent, both increased (Sevall, 1988; Izaurralde et al., 1989; Yaneva & Zlatanova, 1992) as well as decreased (Hendrickson & Cole, 1994) affinities for certain sequences have been documented. The latter case is particularly intriguing, demonstrating diminished affinity of H1 toward a part of the regulatory region near the replication origin and transcription start sites of the SV40 genome. This phenomenon could not be mimicked by the strongly cationic polylysine (Hendrickson & Cole, 1994).

The key position of H1 in allowing/preventing transcription initiation events in certain chromatin areas potentially renders its phospholipid interactions particularly interesting. Positively charged histones have been widely used as PKC substrates, and the histone−membrane interaction has been claimed to be essential for phosphorylation by PKC (Bazzi & Nelsestuen, 1987). However, the binding mechanism has not been studied in detail. There are also some discrepancies as to the binding specificity. While a mixture of histones has been reported to effectively attach to PG and CL as well as to PA, PS, and PI by Hirai et al. (1992), other authors have reported attachment to PS only, and no binding to PG or PI (Bazzi & Nelsestuen, 1987). Our present resonance energy transfer measurements with the linker histone H1 revealed this histone to bind avidly to liposomes containing PS, PG, or PA in eggPC, in agreement with the expected strong electrostatic attraction. The extent of H1 attachment was dependent on the content of the acidic component in the liposome. Binding to liposomes containing PS (carrying one negative charge at the pH of our experiments) was saturated at about 30 mol % PS, whereas for liposomes containing PA (with two ionizable hydroxyls) saturation was reached already at about 10 mol %. However, electrostatics is clearly not the only driving force for the histone−lipid complex formation. The attachment of H1 to the electroneutral eggPC liposomes, although weaker than binding to liposomes containing acidic phospholipids, suggests the involvement of hydrophobicity. This is also supported by the observation that, while positively charged vesicles containing sphingosine did not bind H1, liposomes containing both anionic and cationic lipids systematically showed a larger extent of histone binding than could be expected on the basis of an electrostatic interaction only.

Binding of H1 to liposomes could be reversed by salt. Interestingly, upon increasing the content of the acidic lipid, the [NaCl] needed for reversal of H1 binding was also considerably increased, while the maximal extent of reversal was decreased. Analogous phenomena have previously been observed in our laboratory for the membrane attachment of cytochrome *c* (Rytömaa & Kinnunen, 1994). This protein is known to bind to lipid membranes containing a critical minimal concentration of acidic lipid (Mustonen et al., 1987). In addition to simple electrostatic attraction, interaction of the protonated form of the acidic lipid with cytochrome *c* via hydrogen bonding was suggested (Rytömaa et al., 1992). Similar mechanisms might also be involved in the binding of H1 to membranes containing acidic phospholipids. At high contents of acidic lipid, protonation would be favored to overcome electrostatic repulsion, and the binding of H1 via the protonated form of the lipid would dominate, thus leading to the formation of a hydrogen-bonded lipid−H1 complex resistant to salt. This possibility is also supported by our observation that the attachment of H1 to the membrane could not be reversed by incubating for 100 min in the presence of a 10-fold excess of liposomes not labeled with bisPDPC (Kõiv and Kinnunen, unpublished results).

H1 did not interact with liposomes containing the positively charged sphingosine. Instead, H1 effectively prevented

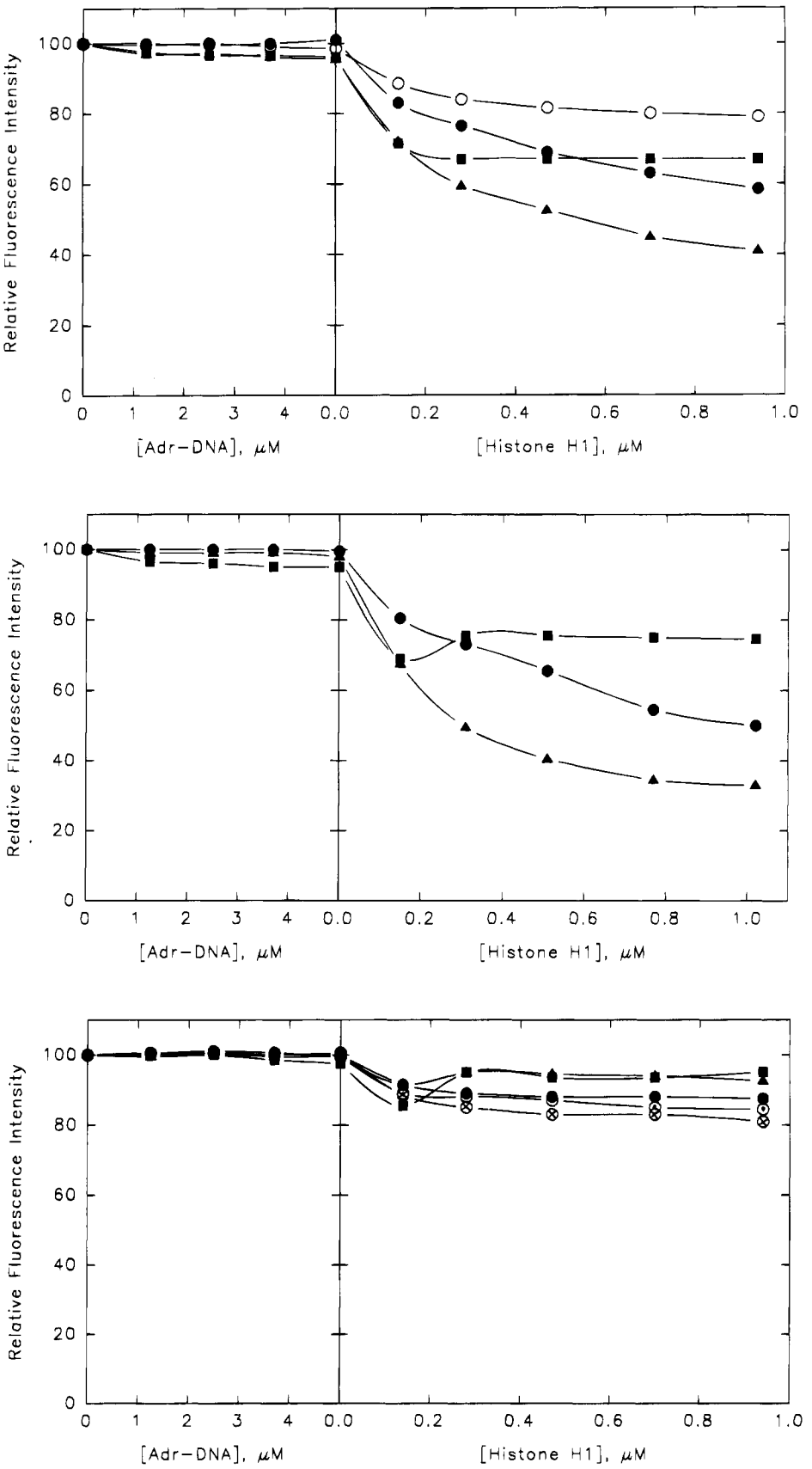

FIGURE 6: Binding of DNA to acidic liposomes of different composition in the presence of H1. Adriamycin-labeled DNA was first mixed with the liposomes to check for direct interaction, whereafter unlabeled H1 was added. Attachment of DNA to the vesicles was monitored by the decrease in RFI from bisPDPC due to quenching by Adr–DNA. The binding curves are shown for neat eggPC liposomes (○) and eggPC liposomes containing 10 (●), 20 (▲), or 30 (■) mol % PS (panel A, top), PG (panel B, middle), or PA (panel C, bottom). For PA, also vesicles containing 5 (⊙) and 7.5 (⊗) mol % of the acidic lipid were tested (panel C). The total lipid concentration was 26 μM in all cases.

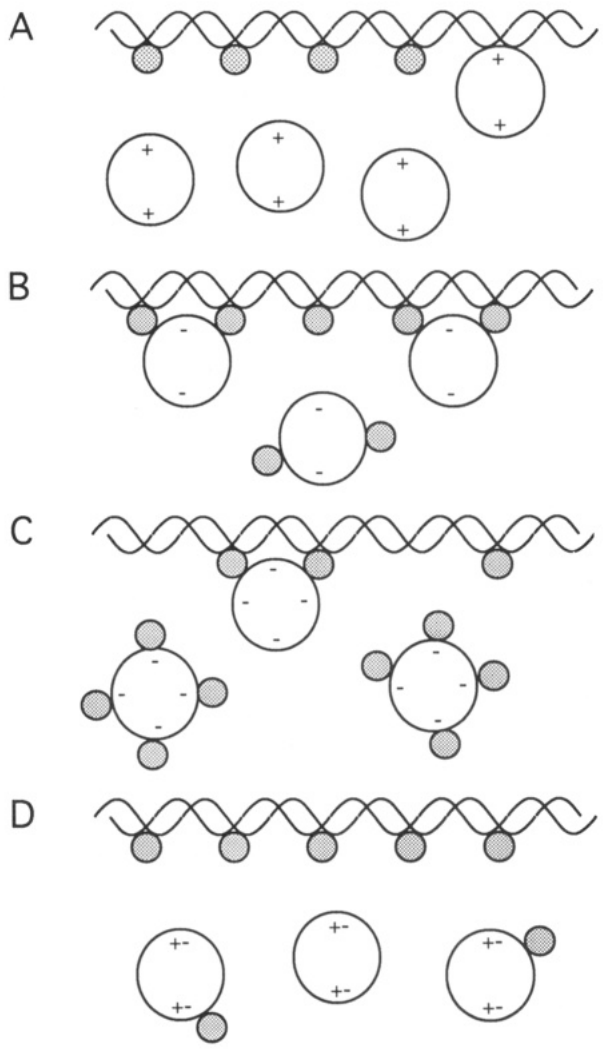

FIGURE 7: Tentative summary of interactions between DNA, H1, and liposomes of different composition. Small shaded circles denote H1 while large circles represent liposomes. Model situations are shown for cationic liposomes containing sphingosine (A), moderately acidic liposomes (<30 mol % PS) (B), strongly acidic liposomes (>30 mol % PS) (C), and liposomes of neat zwitterionic PC (D). See Discussion for more details.

the binding of DNA to these cationic vesicles and displaced it from previously-formed complexes. The displacement was obviously not dependent on simple electrostatics only, as the polyamine spermine was significantly less effective in displacing DNA from the liposomes than was H1. Spermidine, carrying three positive charges, was even more inefficient, and the divalent cations Ca^{2+} and Mg^{2+} did not cause any reversal of Sph−DNA binding in the concentration ranges tested.

When all three components, liposomes, DNA, and H1, were present simultaneously, the observed interactions between them depended on the composition of the liposomes. As summarized in Figure 7, four different possibilities featured:

(i) In a system composed of DNA, H1, and lipid membranes containing sphingosine, the liposomes and histones compete for binding to DNA. No interaction occurs between the positively charged liposomes and positively charged histones. The histones are thus mainly tightly bound to DNA. In the presence of significant amounts of sphingosine, some sphingosine−DNA interaction may also occur (Figure 7A).

(ii) When the liposomes contain either moderate amounts of acidic lipid (<30 mol % PS) or both acidic lipid and sphingosine, relatively strong interactions between all the components were present (i.e., DNA−histone, histone−lipid, and lipid−DNA interactions could all be detected). The latter contact appeared only upon inclusion of H1 without any significant decrease in the extent of the other two interactions. The existence of significant amounts of ternary complexes with H1 serving as a link between the negatively charged DNA and negatively charged liposomes may thus be expected (Figure 7B). Notably, we cannot exclude the possibility that the observed interactions would merely reflect the formation of separate DNA−lipid, DNA−H1, and H1−lipid complexes. However, in this case, rather specific properties, e.g., sequence-specific binding of H1 and sphingosine to DNA, would be required. Although we consider this to be unlikely, it should be recognized that in the strictest sense the present data do not provide direct proof for the formation of ternary complexes.

(iii) In the presence of liposomes containing at least 30 mol % of the acidic lipid, the apparent affinity of H1 toward DNA was decreased. Likewise, less interaction between lipid and DNA was detected than in case II above. On the other hand, formation of stable complexes of H1 and liposomes resistant to elevated ionic strength was observed. Thus, in this case, competition between lipids and DNA for H1 occurs, and the strongly acidic liposomes could be expected partly to remove the histones from DNA. The rest of the histones would then form ternary complexes with DNA and lipids (Figure 7C).

(iv) When the liposomes were composed of PC only, considerably less binding of H1 was observed than for vesicles containing acidic phospholipids. No detectable changes were evident in the DNA−histone interaction in the presence of eggPC liposomes. Accordingly, under these conditions, H1 could be expected to be attached solely to DNA (Figure 7D). Formation of minor amounts of ternary complex is possible, but the influence of lipid on the chromatin structure can be anticipated to be insignificant.

We may thus conclude that the binding of H1 to DNA can be modulated by membranes containing acidic phospholipids or sphingosine. However, it is important to point out that Figure 7 merely summarizes the detected interactions between liposomes, DNA, and H1 in a very schematic manner, without any attempt to picture the possible structures or stoichiometries of the complexes.

Low concentrations of H1 could reverse the binding of sphingosine-containing membranes to DNA. However, as evidenced by the lack of effect of polyamines or divalent cations, the affinity of sphingosine toward DNA is quite high. Taking into account that, in the nuclei of different cell types, the amount of H1 is generally less than one per nucleosome (Bates & Thomas, 1981), membranes locally enriched in sphingosine could compete with H1 for DNA binding sites. Accordingly, the presence of sphingosine may alter the chromatin structure so as to influence its recognition by replication or transcription machinery. While the intracellular concentration of sphingosine is generally low (Wilson et al., 1988), it can be raised by hydrolysis of sphingolipids (Kolesnick, 1987; Wilson et al., 1988). Interestingly, one of the main precursors of sphingosine, sphingomyelin, is the most abundant phospholipids present in the nuclear matrix (Alessenko, 1990). The presence of neutral sphingomyeli-

nase in the nucleus has also been demonstrated (Tamiya-Koizumi et al., 1989). The obvious possibility of generating free sphingosine in the nucleus suggests that, in addition to the well-documented inhibition of PKC (Hannun et al., 1986), modulation of receptor function (Faucher et al., 1988), and activation/inhibition of a number of enzymes involved in phospholipid metabolism (Kiss & Anderson, 1990; Lavie et al., 1990; Hashizume et al., 1992), some mechanisms of sphingosine action might be intranuclear. In fact, it has recently been demonstrated that sphingosine inhibits the synthesis of RNA primers by primase via blocking the association of primase with DNA (Simbulan et al., 1994). DNA polymerases δ, γ, and ϵ were also slightly inhibited. It is interesting to note that, while the primase inhibition was claimed to be highly specific via direct interaction with the enzyme, in the cases of the nonselective inhibition of the polymerases by two sphingoid bases, the modification of the DNA template by sphingosine binding might be one possible underlying mechanism.

The slightly acidic liposomes (<30 mol % PS or PG) where were not capable of directly binding DNA turned out to form ternary complexes attaching to the nucleic acid via H1. This result is not surprising as Hirai and co-workers have reported that binding of PG did not significantly detach the mixture of five histones from DNA (Hirai et al., 1992). On the other hand, displacement of H1 from chromatin by PS liposomes and its enhancing effect on transcription in vitro have been observed (Cocco et al., 1985, 1988). Our results suggest that the capacity to form DNA−H1−liposome ternary complexes should be dependent on the liposome charge density as well as the type of acidic phospholipid. More specifically, upon increasing the mole percent of the anionic phospholipid, the mechanism of bindig of the histone to the PS or PG liposomes seemed to change gradually to a tighter attachment and at the same time decrease the tendency for ternary complex formation. Competition between liposomes containing at least 30 mol % acidic lipid and DNA for the binding of H1 was also obvious from the measurements of H1−DNA interaction in the presence of lipids. On the other hand, the capability of PA-containing liposomes to yield ternary complexes was low, irrespective of the mole percent of PA. The differences in the tendencies demonstrated by the different lipids to form complexes with histone and DNA, as well as the structures of these complexes, might be important for regulatory purposes.

Although the simple model systems described here are structurally far from chromatin, the interactions detected in these systems may also have some significance in vivo. It is tempting to speculate that the nuclear membrane might serve as a very general modulator of DNA−histone structures. More specifically, according to the composition of local domains in the nuclear membrane, the membrane could either facilitate or prevent the binding of other, more specific transcription factors to chromatin in those locations.

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