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Measurement of the Binding of DNA to Liposomes by Resonance Energy Transfer

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A rapid and simple semiquantitative method for monitoring the attachment of DNA to liposomes is described. This technique is based on the measurement of resonance energy transfer between a membrane-embedded fluorescent pyrene lipid donor and DNA labeled with adriamycin as an acceptor.

KEY WORDS: DNA binding; liposomes; resonance energy transfer; pyrene; adriamycin.

INTRODUCTION

During recent years the role of lipids such as phosphatidic acid and cardiolipin as well as sphingosine and its metabolites in mitogenic signal transmission has become established [1–4]. However, their exact molecular mechanisms of action have remained poorly understood. Both replication and transcription processes have been suggested to occur associated with the nuclear matrix [5,6]. Importantly, direct influence of lipid membranes on chromatin structure has been proposed [7]. To this end we have developed a facile method to monitor the binding of DNA to liposomes.

EXPERIMENTAL PROCEDURES

Calf thymus DNA, Hepes, and EDTA as well as dimyristoylphosphatidylcholine (DMPC) and D-sphingosine (Sph) were purchased from Sigma. 1,2-Bis[(pyren-1-yl)decanoyl]-*sn*-glycero-3-phosphocholine (bisPDPC) was obtained from K&V Bioware Inc. (Finland). Adriamycin (doxorubicin) was a generous gift from Farmitalia Carlo Erba. The purity of the lipids was checked by TLC using a chloroform/methanol/water (65:25:4) solvent system. Multilamellar liposomes (MLV) in 20 m*M* Hepes/0.1 m*M* EDTA buffer, pH 7.4, were prepared as described in Ref. 8. Small unilamellar vesicles (SUV) were made from MLV by sonication at 37°C for 2 min with a Branson microtip sonifier. Alternatively, large unilamellar vesicles (LUV) were obtained by extrusion of MLV through 0.1-mm polycarbonate membranes with a LiposoFast membrane homogenizer (Avestin, Canada) [7]. Solutions of adriamycin and DNA were mixed in buffer to yield adriamycin–DNA complex (Adr-DNA). Small aliquots of this complex were added to the liposomes and the bisPDPC excimer fluorescence was recorded using a SLM-4800S spectrofluorimeter. Excitation and emission wavelengths were 344 and 478 nm, respectively. The fluorescence intensity values were corrected for dilution. The inner filter effect was reduced to negligible levels using sufficiently low reagent concentrations. The experiments were carried out at 37°C, where the lipid mixtures employed are in the liquid crystalline state.

RESULTS AND DISCUSSION

The technique described here for the detection of the binding of DNA to liposomes is based on the meas-

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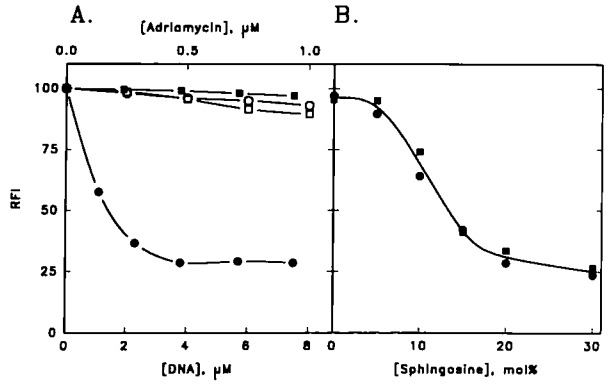

Fig. 1. Binding of DNA to liposomes. (A) Changes in the RFI due to quenching by Adr-DNA complex (nucleotide/Adr ratio, 8) for DMPC/sphingosine/bisPDPC (79:19:2) (●) and DMPC/bisPDPC (98:2) (■) SUVs. For comparison binding of free adriamycin to DMPC/sphingosine/bisPDPC (○) and DMPC/bisPDPC (□) is also depicted. (B) The dependence of the extent of quenching of bisPDPC fluorescence by Adr-DNA at 7.5 μ*M* DNA concentration (nucleotide/Adr ratio 8) on the mole percentage of sphingosine in DMPC/sphingosine/bisPDPC SUVs (●) and LUVs (■).

urement of resonance energy transfer (RET) between a membrane-embedded fluorescent donor, bisPDPC, and DNA labeled with adriamycin (Adr-DNA) as an acceptor. This anthraquinone chromophore forms a tight noncovalent complex with DNA by intercalation and hydrogen bonding [9]. BisPDPC, which contains two pyrene moieties, was chosen to obtain a high yield of excimer fluorescence. Due to the spectral overlap of pyrene excimer emission and adriamycin absorption at ≈480 nm, excitation of pyrene results in RET between these two chromophores [8]. Upon attachment of Adr-DNA to the membrane, the distance between the donor and the acceptor becomes small enough (≈20 Å) as to allow RET. Accordingly, the decrease in pyrene excimer emission at ≈480 nm allows monitoring of the membrane association of DNA. Free adriamycin also binds to lipid membranes, however, the affinity of this drug to DNA is very high and exceeds that to lipids [10]. Using excess DNA adriamycin can be safely assumed to be practically quantitatively scavenged by DNA.

Employing this method binding of DNA to sphingosine-containing liposomes was demonstrated. Figure 1A depicts the dependence of the relative fluorescence intensity [RFI; defined as (I/I_0)*100] on the concentrations of DNA and adriamycin. The addition of Adr-DNA to positively charged unilamellar lipid vesicles composed of DMPC, sphingosine, and bisPDPC (78:19:2 molar ratio) resulted in a rapid (within approx 1 min) decrease in pyrene excimer fluorescence. In contrast, changes in the fluorescence of DMPC/bisPDPC liposomes due to Adr-DNA were small. For comparison, binding of free adriamycin to liposomes of both compositions is also shown. At 1 μ*M* adriamycin 11% fluorescence from DMPC liposomes was quenched. For vesicles containing 19 mol% sphingosine the quenching was slightly reduced due to introduction of the positive surface charge. For DMPC liposomes complex formation between adriamycin and DNA further attenuates quenching of bisPDPC fluorescence by adriamycin for DMPC liposomes, while in the presence of sphingosine quenching is greatly enhanced due to the attachment of this polyanionic complex to the liposome surface. Similar quenching curves were obtained using Adr-DNA complexes with a nucleotide/Adr ratio varying between 4.75 and 10.0. This selective binding of DNA to sphingosine-containing liposomes has been verified also by differential scanning calorimetry [11].

The extent of DNA attachment to DMPC SUVs containing sphingosine was dependent on the mole percentage of the latter in the liposomes (Fig. 1B). We also examined the binding of DNA to LUVs prepared by the extrusion method. As shown in Fig. 1B, also LUVs efficiently bind DNA when they incorporate more than 5 mol% sphingosine and exhibit a dependence of DNA binding on the sphingosine content similar to that observed for SUVs.

Due to the complex dependence of the extent of donor fluorescence quenching on the acceptor concentration, this method does not allow quantitative determination of DNA–lipid binding stoichiometry yet allows for qualitative and semiquantitative studies.

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