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Interaction of avidin with spin-labelled *N*-biotinyl phosphatidylethanolamine in a lipid membrane

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N-Biotinyl phosphatidylethanolamine spin labelled at the C-14 position of the *sn*-2 chain has been incorporated at a level of 1 mol% in bilayers of dimyristoyl phosphatidylcholine, and the effects on the chain mobility of binding avidin to the biotin lipid headgroup have been studied by electron spin resonance spectroscopy. In the fluid phase, avidin causes a large and selective restriction in the chain motion of the biotin lipids to which it is attached, without perturbing appreciably the mobility of the bulk lipid chains. This specific type of lipid–protein interaction is different in kind from that observed both with integral and peripheral membrane proteins and may be involved in transmembrane communication on ligand binding to lipid headgroups, as well as lateral communication (at high packing densities) between proteins with covalent lipid anchors.

Biotin-lipid; Avidin; Spin label; ESR; Lipid anchor

1. INTRODUCTION

A variety of proteins appear to be linked to cellular membranes by covalently attached fatty acyl chains, among the most notable being those involving glycosyl phosphatidylinositol (GPI) anchors [1]. Compared to the lipid interactions with integral [2] and peripheral [3] membrane proteins, relatively little is known, however, about the details of the interaction of the covalently linked chains with the membrane. It has been suggested, for example, that lipid interactions with GPI-anchored proteins may be involved in the sorting and targetting of membrane components in polar epithelial cells [4]. Because of the extraordinarily high affinity of avidin for the vitamin biotin [5], the non-covalent binding of avidin to biotinylated phospholipids serves as a model system for investigating the nature of association of covalently attached lipid chains with the membrane and their interaction with the other membrane lipids. Additionally, the association of avidin with biotin lipids bears a strong resemblance to the binding of toxins, such as cholera toxin, to lipid membrane receptors and therefore also is capable of giving information on this type of lipid–protein interaction.

In the present work, *N*-biotinyl phosphatidylethanolamine has been spin labelled in the *sn*-2 chain and ESR spectroscopy used to investigate the effects of binding avidin on the lipid chain mobility. The biotin lipid is present at only 1 mol% in bilayers of the zwitterionic lipid phosphatidylcholine and is tightly bound at the headgroup by the water-soluble avidin protein. Therefore the situation approximates that of peripheral proteins with covalently attached fatty acid chains. Binding of the protein is found to have a direct and specific effect on the lipid chain mobility and causes a large motional restriction at the C-14 chain segments of the lipid to which it is attached.

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Abbreviations. biotin-PE, *N*-biotinyl phosphatidylethanolamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; 14-PESL, 1-acyl-2-[14-(4,4-dimethyloxazolidine-*N*-oxyl)]stearoyl-*sn*-glycero-3-phosphoethanolamine; 14-BPESL, 1-acyl-2-[14-(4,4-dimethyloxazolidine-*N*-oxyl)]-stearoyl-*sn*-glycero-3-(*N*-biotinyl)phosphoethanolamine, GPI, glycosyl phosphatidylinositol, EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulphonic acid; ESR, electron spin resonance.

2. MATERIALS AND METHODS

Avidin was obtained from Sigma Chemical Co. (St. Louis, MO) and dimyristoyl phosphatidylcholine from Fluka (Buchs, Switzerland). Phosphatidylethanolamine spin-labelled on the C-14 atom of the *sn*-2 chain, 14-PESL, was synthesized as described in [6] and spin-labelled biotinyl phosphatidylethanolamine, 14-BPESL (see Scheme 1), was synthesized from this as described in [7].

Samples were prepared by co-dissolving 1 mg of DMPC and 1 mol% 14-BPESL in dichloromethane and the solvent was evaporated under a stream of dry nitrogen gas. After removal of the residual solvent by vacuum drying overnight, the lipid film was hydrated with a 45 mg/ml solution of avidin in 2 mM HEPES, 1 mM EDTA, pH 7.4. After mild vortex mixing, the sample was incubated at 35–40°C for 1 h and then pelleted in a 1-mm i.d. glass capillary. The excess supernatant was removed and the capillary was flame-sealed before ESR measurements.

ESR spectra were recorded on a Varian Century Line 9 GHz spectrometer equipped with nitrogen gas flow temperature regulation. Samples in 1-mm i.d. 100 μl glass capillaries were accommodated in standard 4-mm diameter quartz ESR tubes containing light silicon oil for thermal stability.

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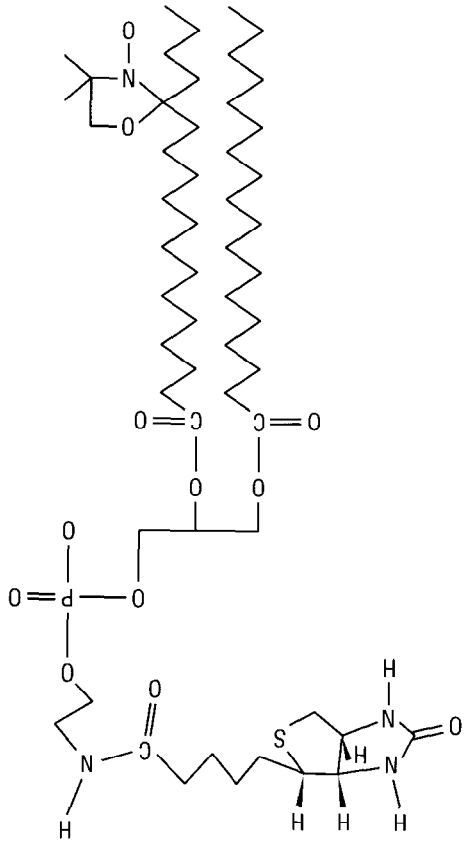

Scheme 1. Structure of the 14-BPESL spin-labelled biotin-PE.

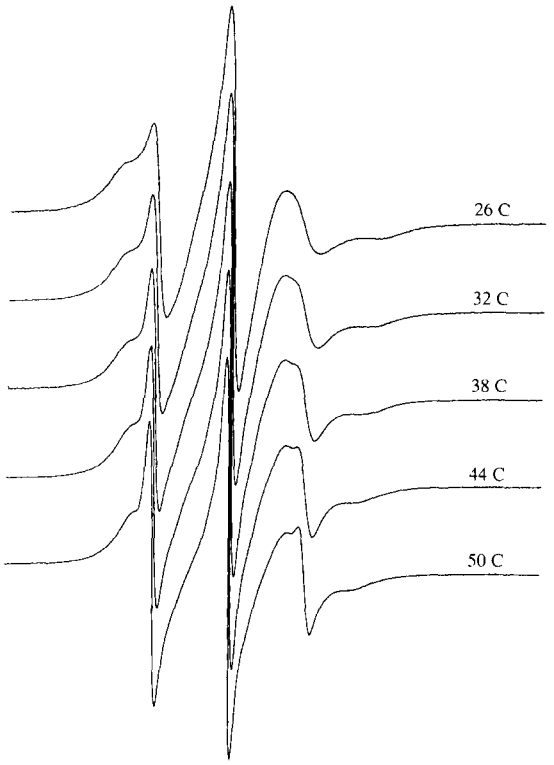

Fig. 1. ESP spectra of the 14-BPESL biotin-PE spin label (1 mol%) in DMPC bilayers in the presence of avidin. Buffer: 2 mM HEPES, 1 mM EDTA, pH 7.4. The spectra were recorded at the temperatures indicated. Total scan width: 100 gauss.

3. RESULTS AND DISCUSSION

The ESR spectra of the spin-labelled biotin-PE, 14-BPESL, at a relative concentration of 1 mol% in DMPC bilayer dispersions in the presence of an excess of avidin are given in Fig. 1. The temperatures at which the spectra were recorded are above the chain-melting transition of DMPC bilayers and therefore correspond to the fluid lipid phase. The spectra all consist of two components corresponding to environments in which the mobility of the spin-labelled chains is different. From Fig. 2, it can be seen that the narrower component corresponds to biotin-PE spin labels to which avidin in not bound, since it is demonstrated by spectral subtraction (Fig. 2C) that it is identical to the spectrum of the spin label in the absence of avidin (Fig. 2A). This is a relatively minor component, since spectral subtraction yields an intensity of only ca. 17% relative to the total spin label for this component. Most probably this represents those biotin-PE spin labels that are inaccessible, possibly for steric reasons, to binding of avidin. The identity in spectral line-shape of this component with the spectrum of the spin label in the absence of avidin therefore indicates that there are little or no long-range perturbations of the lipid chain mobility by specific binding of the avidin to the low concentration of biotin-PE lipids. From the

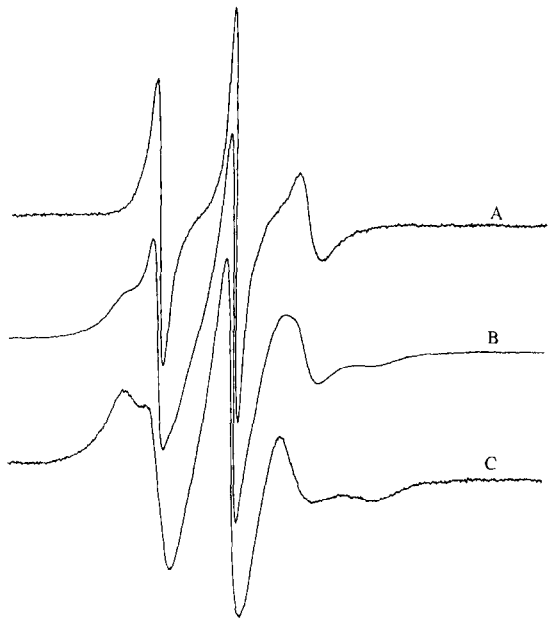

Fig. 2. ESP spectra at 30°C of the 14-BPESL biotin-PE spin label (1 mol%) in DMPC bilayers: (A) in the absence of avidin, and (B) in the presence of avidin. (C) Difference spectrum obtained by subtracting 17.5% of the integrated intensity of spectrum (A) from spectrum (B). Spectra are normalized to the same maximum line height. Total scan width: 100 gauss.

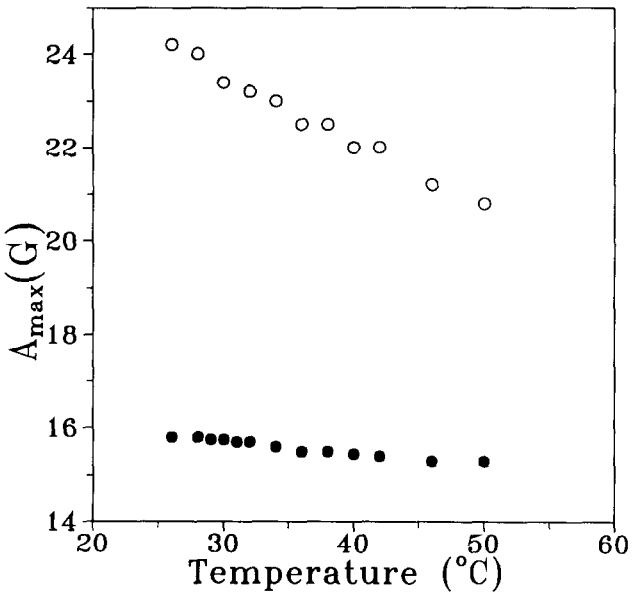

Fig. 3. Temperature dependence of the outer hyperfine splitting constants, A_{max}, in the ESR spectra of 14-BPESL (1 mol%) in DMPC bilayers. (○) motionally restricted spectral component (cf. Fig. 2C) induced in the presence of avidin, (●) fluid component obtained in the absence of avidin.

dimensions of the avidin molecule, deduced from the crystal structure of the homologous streptavidin [8], it can be estimated that approximately 20% of the lipid surface is covered by the protein at this level of binding.

The temperature dependences of the outer hyperfine splitting constants, A_{max}, for both the free and the avidin-bound biotin-PE spin labels are given in Fig. 3. Clearly, the ESR spectra of the 14-BPESL biotin-PE spin label to which avidin is bound (cf. Fig. 2C) are much broader than those of the unbound label (cf. Fig. 2A), evidencing a large degree of restriction in the lipid chain mobility caused by the specific association of avidin. The values of A_{max} obtained from the difference spectra for the avidin-bound biotin-PE spin label (cf. Fig. 2C) display an appreciable temperature dependence, registering an increased chain mobility at the higher temperatures (see Fig. 3). This indicates that the spectra lie in the intermediate motional regime of conventional spin label ESR spectroscopy (cf. [9]), and although the extent of motional restriction is less than that of lipid chains interacting directly with the intramembranous surface of integral membrane proteins (see e.g. [2]), it is much greater than that observed on the electrostatic surface association of peripheral membrane proteins with negatively charged lipid bilayers (see e.g. [10]).

Thus, the experiments with spin-labelled biotin-PE indicate a specific effect on the lipid mobility of the avidin protein bound to the lipid headgroup. These results could have implications for the lateral interactions between, for instance, GPI-anchored proteins and their lipid environment. The specific immobilization of the protein-attached chains could enhance interactions with less mobile lipid domains, such as has been suggested for coupling of the sorting of GPI-anchored proteins with that of glycolipids in the Golgi of polar cells [4]. Alternatively, the resultant mismatch in packing with fluid lipid chains might contribute, for instance, to the mechanism of import into the cell of the cholera toxin α-subunit via binding of the β-subunit to the ganglioside G_{M1} (see e.g. [11]).

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