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Review

Structure, phase behaviour and membrane interactions of *N*-acylethanolamines and *N*-acylphosphatidylethanolamines

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ABSTRACT

N-Acylethanolamines (NAEs) and *N*-acylphosphatidylethanolamines (NAPEs) are naturally occurring membrane lipids, whose content increases dramatically in a variety of organisms when subjected to stress, suggesting that they may play a role in the stress-combating mechanisms of organisms. In the light of this, it is of great interest to characterize the structure, physical properties, phase transitions and membrane interactions of these two classes of lipids. This review will present the current status of our understanding of the structure and phase behaviour of NAEs and NAPEs and their interaction with major membrane lipids, namely phosphatidylcholine, phosphatidylethanolamine and cholesterol. The relevance of such interactions to the putative stress-combating and membrane stabilizing properties of these lipids will also be discussed.

Contents

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1. Introduction

Ethanolamine is a key building block of several important membrane phospholipids and amphiphiles. Among these, diacylphosphatidylethanolamine, dialkylphosphatidylethanolamine and phosphatidylethanolamine plasmalogen, which contain ethanolamine and phosphatidylcholine, sphingomyelin and platelet activating factor (PAF), which contain choline, derived from ethanolamine, are all well characterized. Additionally, ethanolamine is also a structural component of *N*-acylphosphatidylethanolamines (*N*-acyl PEs, NAPEs) and *N*-acylethanolamines (NAEs) (see Fig. 1 for structures) that are present in a wide variety of organisms. The content of NAPEs and NAEs increases dramatically in the parent organisms when subjected to various kinds of stress, suggesting that they may take part in stress-combating responses of the parent organisms (Schmid et al., 1990, 1996; Chapman, 2004). In addition to the putative stress-combating role, NAEs also exhibit interesting biological and medicinal properties, which may be of considerable application potential. In view of the above, during the last two decades considerable efforts have been focused on investigating the structure, phase properties and interaction of these two classes of lipids with major membrane lipids, namely phosphatidylcholine, phosphatidylethanolamine and cholesterol. The early work carried out in this direction until the end of 1999 was reviewed by Marsh and Swamy (2000). Results of the subsequent studies will be reviewed here.

2. *N*-Acylphosphatidylethanolamines

N-Acyl PEs are negatively charged lipids that are obtained by acylating the amino group of diacylphosphatidylethanolamine with a long-chain carboxylic acid via an amide link. NAPEs have been found to occur under normal conditions in a variety of species including bacteria, fungi, viruses, plants and animals including fish. A number of reports indicate that the content of these phospholipids increases quite dramatically when the parent organism or tissue is subjected to stress such as wounding in animals or a degenerate change such as dehydration in plant seeds (reviewed in Schmid et al., 1990).

Besides the interest generated in them due to the above interesting biological properties, NAPEs may also find use in biotechnological/biomedical applications as *N*-acyl egg PE and *N*-palmitoyl DPPE have been reported to stabilize liposomes against leakage (Domingo et al., 1993; Mercadal et al., 1995). Since the latter lipid was shown to stabilize liposomes even in the presence of human serum (Mercadal et al., 1995), these results suggest that NAPEs may find use in developing lipid-based drug delivery agents.

2.1. *N*-Acyl chain location and mobility

In natural membranes, the *N*-acyl chain of NAPEs is usually long and the most predominant chains are palmitoyl and stearoyl, which are saturated (Epps et al., 1979, 1980). Biophysical studies employing differential scanning calorimetry (DSC), ^{31}P NMR and FTIR spectroscopy have shown that in NAPEs with long *N*-acyl chains, the *N*-acyl chain folds back into the hydrophobic interior of the membrane (Akoka et al., 1988; LaFrance et al., 1990, 1997; Domingo et al., 1995). ATR-FTIR studies have shown that the *N*-palmitoyl chain of *N*-palmitoyl DPPE is oriented parallel to the *O*-acyl chains whereas the *N*-hexanoyl chain of *N*-hexanoyl DPPE is disordered or randomly oriented.

While thermodynamic considerations suggest that in the case of NAPEs with long *N*-acyl chains, the *N*-acyl chains should fold back and interact with the *O*-acyl chains in the hydrophobic interior of the membrane, as was inferred from the spectroscopic and calorimetric studies mentioned above, the exact vertical location of the *N*-acyl chain vis-à-vis the *O*-acyl chains and their mobility was not readily obvious. Because the *N*-acyl chains of NAPEs are attached to the phospholipid head group, and not to the glycerol backbone, it is possible that their vertical position and mobility differ from that of the *O*-acyl chains in the fluid membranes. This issue has been investigated by using ESR spectroscopy, employing synthetic *N*-acyl PE derivatives that have been labeled with the stable nitroxide spin probe at different sites on the *N*-acyl chain (*n*-NAPESL) and comparing their spectral characteristics with those of phosphatidylcholine derivatives bearing the spin label at different positions on the *sn*-2 acyl chain (*n*-PCSL). ESR spectra of the two spin labeled lipid series recorded in three different host lipids, namely dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylethanolamine (DMPE) and *N*-myristoyldimyristoylphosphatidylethanolamine (*N*-14 DMPE) were found to exhibit a close match (Fig. 2), indicating that the *N*-acyl chain in NAPE and the *sn*-2 acyl chain of PC are in nearly identical environment and have comparable segmental motion. This suggests the *N*-acyl chain of NAPE and *sn*-2 (*O*-) acyl chain of PC are located at approximately the same depth from the membrane interface. However, in most cases the outer peaks in

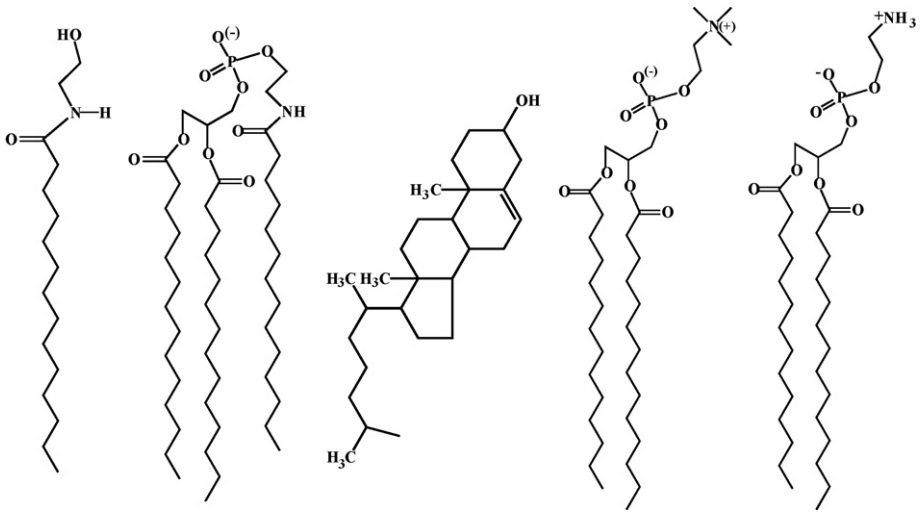

Fig. 1. Structures of *N*-acylethanolamine and *N*-acylphosphatidylethanolamine. The structures of cholesterol, diacylphosphatidylcholine and diacylphosphatidylethanolamine are also given since their interaction is investigated in the studies reviewed here. The acyl chains are all shown with 14 C-atoms.