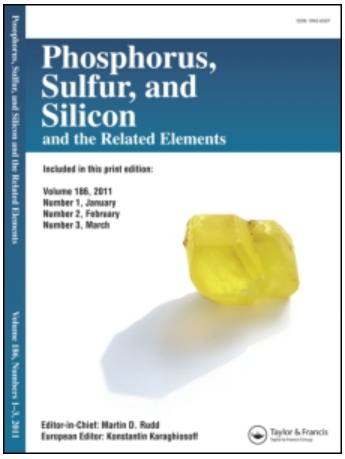

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Fluorinated Phospholipids Versus Natural Phospholipids in Oxygen and Drug Delivery Systems

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FLUORINATED PHOSPHOLIPIDS VERSUS NATURAL PHOSPHOLIPIDS IN OXYGEN AND DRUG DELIVERY SYSTEMS

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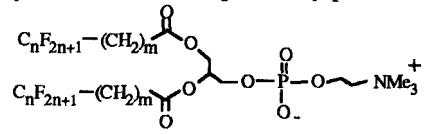
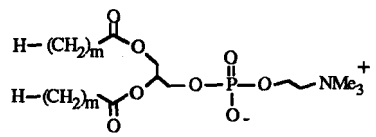
Abstract The physicochemical characteristics and biological behavior of phosphatidylcholines and of the membranes they form (e.g. membrane stability, fluidity/rigidity and lipophilicity, surface activity and fluorocarbon emulsifying properties) are significantly modified by the replacement of the hydrocarbon fatty chains by perfluoroalkylated ones.

INTRODUCTION

Vesicles formed from phospholipids (liposomes) provide chemical models for the study of biological membrane functions as well as challenging drug carrier and delivery systems.[1] Vesicles made from pure phospholipids have low stability. The development of more stable membranes usually requires multicomponent systems and elaborate formulations resulting in increased complexity. Phospholipids, when used as emulsifiers, have also allowed the development of improved fluorocarbon emulsions to serve as artificial oxygen carrier and delivery systems.[2] Although they are well accepted and routinely used in injectable preparations, phospholipids have their limitations and leave little leeway for varying the vesicles' and emulsions' properties. Future progress will aim at obviating these limitations and therefore at developping new lines of biocompatible membrane- and vesicle-forming or modifying amphiphiles ; these should also be able to emulsify fluorocarbons.

Accordingly, we have synthesized a series of perfluoroalkylated analogs of phosphatidylcholines - the main component of natural phospholipids - in which the natural fatty acid chains are replaced by perfluoroalkylated ones (compounds **1** to **6**).[3]





The *F*-alkyl tails were expected to increase the hydrophobic and amphiphilic character of the membrane lipids, which usually leads to improved stability of the vesicle shell, and to combine fluorophilicity hence efficient emulsifying properties.

The replacement of the hydrocarbon fatty chains by *F*-alkylated ones was indeed found to induce strong modifications of the physicochemical characteristics and biological behavior of the phospholipids, and of the membranes they form (e.g. membrane stability, fluidity/rigidity and lipophilicity, surface activity and fluorocarbon emulsifying properties). These changes will be discussed here with respect to the potential applications of these amphiphiles in the field of drug delivery systems.

MEMBRANE PROPERTIES

The ability of the *F*-alkyl phosphatidylcholines (*F*-alkyl-PCs) to form uni- and/or multilamellar vesicles was evidenced by electron microscopy after freeze fracture.[4] The total chain length (n + m) required to form vesicles is of 8 to 14 carbon atoms while, in the hydrocarbon series, m is from 11 to 21. These "perfluoroalkylated" vesicles are remarkably stable since they may be heat sterilized without destruction of their structure nor modification of the average particle size.[4] Compared to their hydrocarbon analogs, the sterilized vesicles demonstrate significantly superior shelf life (over one year). This is consistent with the general concept that raising the hydrophobic interactions, can increase membrane stability.

The polymorphic phase behavior of the *F*-alkyl-PCs **1** to **5**, which all form a lamellar phase, was investigated using DSC, ESR, and ^{19}F NMR. A crystal to liquid-crystal phase transition ($L\beta'$ or $P\beta'$ to $L\alpha$) was observed only for **3** to **5** and the critical phase transition temperature (T_C) was measured. Our data show that T_C is related to the total length of the hydrophobic chain and, more markedly, to the length of the perfluoroalkylated segment. We established that Tc rises with the increase of the length of the lipidic chain as well as with the replacement of a part of the hydrocarbon chain by a fluorinated one. Thus, we found that, introducing a C_8F_{17} fragment, causes a significant increase in Tc : for example, the T_C of **3** (69°C for a 12 carbon atom chain) is significantly higher than that of DMPC (23°C, 13 carbons) and even higher than that of DSPC (55°C, 17 carbons). More surprisingly, and in contradiction with the established model, we found that the introduction of a short *F*-alkyl tail (C_4F_9) as in **4**, in spite of an overall chain lengthening to 14 carbon atoms, results in a decrease in T_C (18°C). This apparent discrepancy is accounted for by recognizing that the introduction of a *F*-alkylated chain can modulate the membrane fluidity in two opposite directions : (i) enhancement of fluidity, which is evidenced by a lowering in Tc, due to weaker intermolecular van der Waals interactions and increased steric repulsions between the

fluorinated chains as compared to hydrocarbon chains, (ii) increase of the rigidity of the membrane, which leads to an augmentation in Tc, as a consequence of an increase in the hydrophobic interactions. Thus, in the case of a short (C_4F_9) *F*-alkylated tail, the hydrophobic interactions appear to be insufficient to counterbalance the opposite effects which induce the fluidification of the membrane.

It is also noticeable that the melting of the chains of the *F*-alkyl-PCs is very progressive making it possible to monitor the changes in molecular motion of each individual CF_2 group and of the hydrocarbon spacer as temperature increases. Thus, the melting of the C_8F_{17} chain of **3** occurs over a temperature range of almost 20°C and is complete at nearly 50°C, a temperature which is still significantly below the Tc of the main phase transition (69°C). Such a behavior is in marked contrast with that of the hydrocarbon phosphatidylcholines for which the chain melting phenomenum, due to high cooperativity, occurs at their Tc and within a very short temperature range (1°C). In **4**, complete motion of the shorter C_4F_9 tail occurs within only 2-4°C and at a temperature close to Tc indicating that chain melting occurs with greater cooperativity than in **3**. While replacing a short hydrocarbon portion by the corresponding *F*-alkyl segment maintains the cooperative effects which characterize the phase transition of hydrocarbon phospholipids, longer *F*-alkyl segments behave as if the different fragments along the chain's skeleton enter molecular motion "individually".

Information on the lipophilicity of the *F*-alkylated vesicles' membrane was retrieved from the temperature dependant partitioning of the lipophilic/hydrophilic paramagnetic probe "Tempo" between the aqueous and lipidic phases present in the *F*-alkyl-PC dispersions. We found that an increase in fluorophilicity results in a dramatic decrease of the membrane's lipophilicity : thus, **4**, the least fluorophilic (shortest *F*-alkyl tail) but also the most lipophilic (lowest n/m ratio) of the *F*-alkyl-PCs investigated, exhibits a distribution of the Tempo probe comparable to that of DMPC ; this partitioning is, as expected, enhanced at the compound's gel to fluid phase transition temperature (18°C). By contrast, the spin probe was located only in the aqueous phase in the presence of vesicles based on the *F*-alkyl-PCs which have longer fluorinated chains or higher n/m ratio. This is particularly the case of vesicles based on **1** or **2** whose main phase transitions occur at lower temperatures than for **4**. This low solubility of a lipophilic probe in the fluorophilic/lipophilic core of these membranes most likely arises from an increase of the membrane's fluorophilic character at the expense of its lipophilic character. As a major consequence, the high stability and low lipophilicity of the vesicles formed by the new *F*-alkyl-PCs are expected to modify their interactions with hydrophobic proteins, peptides, enzymes, cells and may have potential in drug delivery systems.

SURFACE ACTIVITY AND FLUOROCARBON EMULSIFYING PROPERTIES

The *F*-alkyl-PCs display much higher surface activity than their saturated hydrocarbon analogs : low concentrations (10^{-3} M) of **1** to **4** in water are sufficient to lower the surface tension γ_s, from 73 to 19-35 mNm^{-1}, and the water/FDC interfacial tension γ_i from 53.5 to 0.9-2 mNm^{-1} for compounds **1**, **2**, **4**, and to 9 mNm^{-1} for **3**. Comparatively, their hydrocarbon analogs are significantly less effective with γ_s in the range of 40 to 60 mNm^{-1} and γ_i in the range of 27-41 mNm^{-1}. The least efficient surface active *F*-alkyl compound of the series, i. e. **4**, is also the least fluorophilic one (shortest *F*-alkyl tail for the longest hydrocarbon spacer).[3]

The increased fluorophilicity and hydrophobicity of the *F*-alkyl-PCs allowed the formation of stable emulsions of perfluorodecalin (a fluorocarbon rather difficult to emulsify), with various *F*-alkyl-PC when used either as the sole surfactant (where DPPC and even a mixture of natural but hydrogenated phospholipids failed) or as co-surfactant with natural egg yolk phospholipids (EYP).[5] The stability of these emulsions is comparable to or better than for those prepared with EYP alone. When used with EYP, the *F*-alkyl-PCs act on particle sizes as a result of interactions of the amphiphile's perfluoroalkylated tail with the fluorocarbon phase (stabilizing effect) or with the hydrocarbon chains of the EYP in the interfacial film (destabilizing effect). The former become predominant only when the amphiphile's total chain length is comparable to the EYP monolayer's thickness, which is the case for **4**.

BIOCOMPATIBILITY

Preliminary biocompatibility data show that the replacement of the hydrocarbon fatty chains by perfluoroalkylated ones, in spite of increasing the surface activity of the phospholipid, does not result in any increase in hemolitic activity.[3] This is in contrast with the observation that short chain saturated hydrocarbon analogs are highly hemolytic. Neither the growth nor the viability of lymphoblastoïd cells of the Namalva strain is affected by the *F*-alkyl-PCs. Furthermore, some of the compounds (e.g. **4**) demonstrate i.v.LD_{50} in mice as high as 8 g/Kg body weight, which falls in the 7.5 to 10 g/Kg range usually reported for EYP.

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