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Interactions between selected photosensitizers and model membranes: an NMR classification

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ABSTRACT

Membrane interactions of porphyrinic photosensitizers (PSs) are known to play a crucial role for PS efficiency in photodynamic therapy (PDT). In the current paper, the interactions between 15 different porphyrinic PSs with various hydrophilic/lipophilic properties and phospholipid bilayers were probed by NMR spectroscopy. Unilamellar vesicles consisting of dioleoyl-phosphatidyl-choline (DOPC) were used as membrane models. PS-membrane interactions were deduced from analysis of the main DOPC ^{1}H-NMR resonances (choline and lipid chain signals). Initial membrane adsorption of the PSs was indicated by induced changes to the DOPC choline signal, i.e. a split into inner and outer choline peaks. Based on this parameter, the PSs could be classified into two groups, Type-A PSs causing a split and the Type-B PSs causing no split. A further classification into two subgroups each, A1, A2 and B1, B2 was based on the observed time-dependent changes of the main DOPC NMR signals following initial PS adsorption. Four different time-correlated patterns were found indicating different levels and rates of PS penetration into the hydrophobic membrane interior. The type of interaction was mainly affected by the amphiphilicity and the overall lipophilicity of the applied PS structures. In conclusion, the NMR data provided valuable structural and dynamic insights into the PS-membrane interactions which allow deriving the structural constraints for high membrane affinity and high membrane penetration of a given PS.

1. Introduction

Photosensitizers (PSs) are a class of compounds which performs an important role in photodynamic therapy (PDT). PDT is a widely accepted method for treatment of several diseases (mainly different types of cancer), and is used in medical fields like oncology and dermatology [1,2]. The mechanism behind this method is the photochemical reaction of the PS with oxygen which leads to the formation of highly oxidative species (mainly singlet oxygen, $^1O_2$) which trigger a sequence of oxidation reactions finally reaching cell death. This treatment is highly selective, because the tissue damage is achieved only if three components (PS, oxygen and light) are combined [3,4].

More recently, PSs are also used in photochemical internalization (PCI), in combination with "normal" drugs. PCI is a new approach based on the release of active molecules from endocytosed vesicles after photodynamic break-down of the irradiated vesicle [5]. Lately it has been reported that PCI improves the biological activity of several active macromolecules [6].

An ideal PS should have several features [7]: minimal dark toxicity, preferential uptake and/or retention by tissues of interest, high quantum yield for the generation of singlet oxygen ($^1O_2$), strong absorbance with a high extinction coefficient in the 600–900 nm range where penetration of light into tissue is optimal, rapid excretion leading to low systemic toxicity, low aggregation tendency and chemical properties conducive to efficient drug administration.

Chemical research continues to search novel PSs with improved combinations of chemical, photophysical and biological properties. Up to now, hundreds of different PSs are known [8]. Except for the common porphyrin or chlorin skeleton-based core, PSs can have different chemical structures. Important differences are the presence (or absence) of a metal ion [9], the presence of polar or unpolar lateral substituents [10] and the presence of anionic or cationic lateral side chains [11]. PS properties like solubility, aggregation tendencies and singlet oxygen yield are strictly related to the chemical structure. Therefore, there is currently great ongoing interest in understanding which molecular design of a PS is favorable for PCI and PDT [12]. In particular, it has been demonstrated that PSs with amphiphilic

Abbreviations: PSs, photosensitizers; PDT, photodynamic therapy; PCI, photochemical internalization; DOPC, dioleoyl-phosphatidyl-choline; CE, chlorin e6; RG7, rhodin G7; CEMED, chlorin e6 monoethylene diamine monoamide; m-CEMED, mesochlorin e6 monoethylene diamine amide; MACE, mono-L-aspartyl-chlorin e6; Arg-CE, arginine amide of chlorin e6; Tyr-CE, monotyrosine amide of chlorin e6; HPIX, hematoporphyrin IX; DPIX-DSME, deuteroporphyrin IX 2,4-disulfonic acid dimethyl ester; CPIII, coproporphyrin III; DPIX-DS, deuteroporphyrin IX 2,4-disulfonic acid; TCPhP, 5,10,15,20-Tetrakis-(4-carboxyphenyl)-21,23H-porphyrin; TSPhP, 5,10,15,20-Tetrakis (4-sulfonatophenyl)-21,23H-porphyrin; TMPyP, 5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)-21,23H-porphyrin; THPhP, 5,10,15,20-Tetrakis-(3-hydroxyphenyl)-21,23H-porphyrin; PL, phospholipid; PBS, phosphate buffered saline

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properties are the most efficient in photochemical internalization of functional genes complexed to polylysine [13]. Amphiphilic PSs were localized mainly in the membrane of the endocytic vesicles, while ionic PSs remained mainly in the matrix and therefore were found to be less active toward membrane damage. Another study [14] has revealed that when incorporated in liposomes, apolar PSs showed better efficacy in terms of lipid peroxidation than the amphiphilic ones. On the other hand, the photoinduced permeation (process that takes place in PCI) of the same liposomes was higher when amphiphilic PSs were involved. These results demonstrate that amphiphilicity seems to be an important characteristic that a PS suitable for PCI should have. In a different work the in vitro effects of a series of dihydroxychlorins with different degree of amphiphilicity have been studied [15]. The aim of this study was to better understand the influence of amphiphilicity on intracellular uptake, subcellular localization and photosensitizing activity of some PSs. The results have shown that an increased amphiphilicity of the sensitizer molecules is correlated with an increased sensitizer uptake and an increased PDT efficiency.

Besides the chemical structure of the PSs, there is currently also great interest in the development of efficient and specific carrier delivery platforms for PDT [16] as, for example, PS-polymer conjugates [17] and PS-fullerene adducts [18].

Processes involved in PDT comprise several steps: first, the PS is injected into the blood stream, second, the PS binds to the blood vessel wall, and third, the PS penetrates the wall and diffuses into the extracellular medium of the tissue and finally penetrates into the tumor cells and locates in organelles. Owing to these processes, conditions like pH and potential protein-binding can vary a lot [19]. Considering also that different PSs have different pharmaco-kinetic and distribution properties, it is easy to understand that there are so many variables involved in the complete process that finding a perfect PS is quite a complex aim [20]. Because of that, effective photosensitizers are often discovered by "trial and error" procedures. As the cellular response to PDT is strictly related to the subcellular localization of the PS, and as the vesicle membrane distribution of PSs is a key step in PCI, the behavior of PSs towards the membrane is currently a very attractive research topic [21]. Despite numerous publications around this topic, there is still no clear knowledge of all the mechanisms involved. Therefore, there is great ongoing interest in understanding the factors modulating the interactions between photosensitizers and membranes, and several studies in this field have been carried out, mainly involving fluorescence spectroscopy [22].

We previously demonstrated that NMR can be an efficient method to understand certain processes involved in the interactions between PSs and model membranes [23,24]. Up to now several NMR studies by other groups have been applied to study the transport and the dynamics of various non-porphyrinic compounds across lipid bilayer membranes [25–27].

In this work we studied the interaction between a series of commercially available PSs and model membranes probed by NMR spectroscopy. The main aim was to find correlations between molecular structure of the PS and its interactions with membranes.

As membrane models, unilamellar vesicles consisting of dioleoyl-phosphatidyl-choline (DOPC) were used. Several chlorin and porphyrin skeleton-based PSs having different chemical properties were employed (Fig. 1):

Chlorin e6 (CE (1)), Rhodin G7 (RG7 (2)), Chlorin e6 monoethylene diamine monoamide (CEMED (3)), Mesochlorin e6 monoethylene diamine amide (m-CEMED (4)), Mono-L-Aspartyl-Chlorin e6 (MACE (5)), Arginine amide of chlorin e6 (Arg-CE (6)), Monotyrosine amide of chlorin e6 (Tyr-CE (7)), Hematoporphyrin IX (HPIX (8)), Deuteroporphyrin IX 2,4-disulfonic acid dimethyl ester (DPIX-DSME (9)), Coproporphyrin III (CPIII (10)), Deuteroporphyrin IX 2,4-disulfonic acid (DPIX-DS (11)), 5,10,15,20-Tetrakis-(4-carboxyphenyl)-21,23H-porphyrin (TCPhP (12)), 5,10,15,20-Tetrakis(4-sulfonatophenyl)-21,23H-porphyrin (TSPhP (13)), 5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)-21,23H-porphyrin (TMPyP (14)) and 5,10,15,20-Tetrakis-(3-hydroxyphenyl)-21,23H-porphyrin (THPhP (15)).

Analysis of the ^{1}H-NMR phospholipid (PL)-vesicle resonances permits to understand the membrane affinity and localization of the PS, and is a useful tool to obtain an approximate model of the diffusion of PSs within the bilayer.

The method takes advantage of the PS ring current effect inducing major shift changes to the ^{1}H-NMR signals of PL-molecules in spatial proximity. This shifting effect is – within certain limits – proportional to the amount of PS close to the PL molecules. Analyzing these induced chemical shift changes enables to obtain approximate information on the adsorption, the time-dependent movement and on the penetration of PSs into the lipid bilayer.

In this paper, we propose a classification of the investigated PSs with respect to their interactions with model membranes. Two main groups (called Model-A and Model-B) can be defined based on the initial and fast adsorption of the PS to the outer membrane layer. Each group can be subsequently divided into two further sub-groups (called Model-A1, Model-A2, Model-B1, Model-B2) based on the slower diffusion of the PS into and within the two membrane layers. A correlation between PS structure and type of membrane interaction is suggested.

2. Materials and methods

2.1. Materials

18:1 PC (cis) 1, 2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids Inc. Deuteroporphyrin IX 2,4-disulfonic acid dihydrochloride (DPIX-DS), Coproporphyrin III dihydrochloride (CPIII), Chlorin e6 (CE), Mono-L-Aspartyl-Chlorin e6 tetrasodium salt (MACE, Npe6), Rhodin G7 sodium salt (RG7), Hematoporphyrin IX dihydrochloride (HPIX), Deuteroporphyrin IX 2,4-disulfonic acid dimethyl ester disodium salt (DPIX-DSME), Monotyrosine amide of chlorin e6 trisodium salt (Tyr-CE), Arginine amide of chlorin e6 trisodium salt (Arg-CE), Chlorin e6 monoethylene diamine monoamide disodium salt (CEMED) and Mesochlorin e6 monoethylene diamine amide disodium salt (m-CEMED) were purchased from Frontier Scientific. 5,10,15,20-Tetrakis-(3-hydroxyphenyl)-21,23H-porphyrin (THPhP), 5,10,15,20-Tetrakis-(4-carboxyphenyl)-21,23H-porphyrin (TCPhP), 5,10,15,20-Tetrakis(4-sulfonatophenyl)-21,23H-porphyrin (TSPhP) and 5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)-21,23H-porphyrin tetratosylate were purchased from Porphyrin Systems GbR. The 4 tosylate counterions in 5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)-21,23H-porphyrin tetratosylate were replaced by chloride ions using an ionic exchange resin in order to obtain 5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)-21,23H-porphyrin tetrachloride (TMPyP). MeOH, CH_2Cl_2 and $CHCl_3$ were purchased from Sigma-Aldrich. Deuterated water (D_2O, D 99.9%) and DMSO-d6 were obtained from Cambridge Isotopes Laboratories, Inc. Trimethyl-silyl-3-propionic acid-d4 sodium salt (TMSP-d4, D 98%), obtained from Euriso-Top, was used as internal ^{1}H-NMR reference. All chemicals and solvents were used without further purification. PS stock solutions were freshly prepared in DMSO-d6 at a concentration of 15 mM. Phosphate buffered saline (PBS) solution of pH-values of 6.9 was prepared by mixing different aliquots of 50 mM solutions of KH_2PO_4 and Na_2HPO_4 (both Sigma-Aldrich) in D_2O containing 0.9% NaCl.

2.2. Solubility of selected PS compounds

The water solubility of the applied PS for our study was quite heterogeneous: some were water soluble, others were water soluble after the formation of the salt and some were water insoluble. Therefore, we decided to prepare all PS stock solutions in DMSO (good solvent for all PSs) to keep the experimental conditions constant. A