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Synthesis and Liposome Insertion of a New Poly(carboranylalkylthio)porphyrazine to Improve Potentiality in Multiple-Approach Cancer Therapy

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Porphyrins and related macrocycles are efficient sensitizers in anticancer treatments such as photodynamic therapy (PDT) and photothermal therapy (PTT).[1−6] Recently, these molecules have been derivatized with boron containing functions for possible use in boron neutron capture therapy (BNCT).[3−5] All of these therapies require a "bullet", that is, light or neutrons, and an active compound, such as a strong neutron or light absorber, preferentially located inside malignant cells.[6] Here we describe a new poly(carboranylalkylthio)porphyrazine, (H_2HECASPz, Figure 1), synthesized with the purpose to contribute to this issue. The choice of H_2HECASPz was dictated by several factors. First, the notion that in the context of carboranyl-tetrapyrroles, polycarboranyl-alkylthio-porphyrazines represent an interesting variant, in that they conjugate high peripheral carboranyl substitution (80 boron atoms per macrocycle) with considerable flexibility of the azaporphyrin core and with the presence of eight electron rich sulfur atoms.[7] Second, the experimental evidence that this class of tetrapyrroles, depending on the peripheral substituents and the coordinated metal, may show intense absorbance in the "therapeutic window", near-IR luminescence or rapid radiationless decay of the photogenerated S_1 (Q) state.[1,2,6,8] These properties make them well suited for anticancer multiple approach.

H_2HECASPz was obtained by template cyclotetramerization of the 2,3 *bis*[6-(2-allyl-1,2-*closo*-dodecarboran-1-yl) hexylthio] maleonitrile over $Mg(OPr)_2$, followed by mild demetallation with trifluoroacetic acid (see Supporting Information). H_2HECASPz exhibits a strong Q-band at 712 nm ($\epsilon = 2.8 \times 10^4$ M^{-1} cm^{-1}) and is not luminescent. So, we have a case of tetrapyrrole for which the S_1 (Q) state decays nonradiatively, even in the absence of a quenching coordinated metal.[6,8]

Vectors to deliver and concentrate the active molecules at target sites are extensively looked for and many delivery agents have been proposed, including surfactant-based structures,[9] polymers,[10] and, more recently, nanospheres[11] and nanotubes.[12] However, only liposomes are currently accepted for clinical tests on humans.[13] Here we loaded liposomes with H_2HECASPz. The loaded vectors were characterized by dynamic light scattering (DLS), zeta potential (ζ), and synchrotron small-angle X-ray scattering (SAXS). Valuable information on the host−guest interactions at the molecular level were obtained by density functional theory (DFT) calculations.

As liposome constituents, we used 1,2-dioleoyl-*sn*-glycero-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-phosphoethanolamine (DOPE). PC and PE polar heads are the most abundant in plasma membranes and are known to be compatible with cells. Long and flexible dioleoyl chains were required to accommodate the bulky H_2HECASPz molecule (~40 Å in its extended form). Liposomes were prepared by two different methods, that is, detergent depletion with sodium cholate, and extrusion. Typical SAXS curves at the absolute scale are reported in Figure 2. Plain and porphyrazine loaded liposomes prepared by detergent depletion (Figure 2A) showed $I(q)$ versus q diagram characteristic of monolamellar liposomes, which could be fitted by using the form factor of a bilayer:

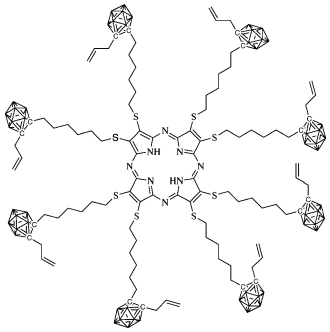

Figure 1. Molecular structure of H_2HECASPz.

$$I(q) = \frac{4\pi}{q^4}\Sigma\left[(\rho_c - \rho_h)\sin\left(q\frac{t_c}{2}\right) + (\rho_h - \rho_s)\sin\left(q\frac{2t_h + t_c}{2}\right)\right]^2 \quad (1)$$

where ρ_c, ρ_h, t_c, and t_h are the electronic density and the thickness of the hydrophobic and hydrophilic layer, respectively, ρ_s is the electronic density of the solvent, and Σ is the interface extension per unit volume. A Gaussian distribution of t_c (with half-height width δt_c) was allowed to blur out the sharp minima in eq. 1 and to take into account a small degree of polydispersity. The relevant parameters extracted from the fitting are reported in Table 1 together with the aggregate mean diameter (D), size polydispersity, and ζ-potential, which is directly related to the total surface charge. All these physical properties showed that progressive insertion of H_2HECASPz slightly affected the host liposomes, but did not disrupt their basic structure nor modified their monolamellar nature. In particular, the electronic density decreased in the hydrophobic core and increased in the hydrophilic layer. This meant that less polar and/or smaller units were intercalated among the lipid chains, whereas association occurred between the polar heads and electron rich units.

The SAXS diagrams of liposomes prepared by extrusion (Figure 2B) showed correlation peaks due to interactions among lipid bilayers. Therefore, oligolamellar aggregates were formed, and Bragg peaks at q spacing 1:2:3 became more evident with increasing H_2HECASPz content. This was probably favored by the well-known tendency of porphyrin-like rings to interact with each other and build stacking structures.[2] DLS measurements did not show any definite trend in this case (see Supporting Information).

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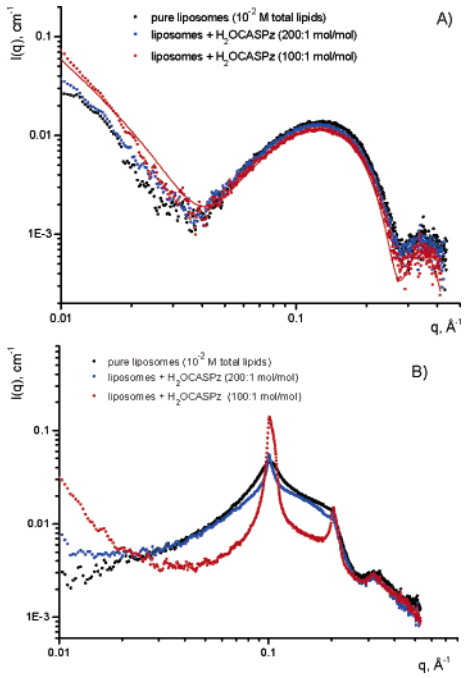

Figure 2. SAXS diagrams of plain and porphyrazine loaded DOPC/DOPE 1/1 liposomes prepared by detergent depletion (2A) and by extrusion (2B). The same H_2HECASPz/lipid starting ratios were used for the two preparation methods, as indicated in the legend. Spectrophotometric quantitative analysis showed that 6−10% loading was obtained by extrusion and 38−55% by detergent depletion (see Supporting Information). The curves in panel A were fitted with eq 1, as stated in the text. For clarity, only the simulated curve of the liposomes + H_2HECASPz 200:1 system has been reported (continuous red line).

Table 1. Best Fit Parameters for SAXS Curves of Liposomes Prepared by Detergent Depletion. The Initial Lipid/H_2HECASPz Molar Ratios Are Indicated for Loaded Liposomes

system	liposomes	liposomes + H_2HECASPz (200:1)	liposomes + H_2HECASPz (100:1)
ρ_c (cm^{-2})	$(7.8 \pm 0.1) \times 10^{10}$	$(6.6 \pm 0.1) \times 10^{10}$	$(6.0 \pm 0.1) \times 10^{10}$
ρ_h (cm^{-2})	$(1.32 \pm 0.01) \times 10^{11}$	$(1.41 \pm 0.01) \times 10^{11}$	$(1.46 \pm 0.01) \times 10^{11}$
t_c (Å)	26.4	25.3	24.8
δt_c	2.3	2.8	3
t_h(Å)	5.7 ± 0.1	8.2 ± 0.1	9.1 ± 0.1
D (nm)	134 ± 5	200 ± 10	190 ± 15
polyd	0.26	0.31	0.38
ζ (mV)	-11 ± 3	-8 ± 2	-6 ± 2

Interestingly, insertion of H_2HECASPz into liposomes was accompanied by marked hypochromism of the porphyrazine Q-band and by the appearance of an "extra" band at 585 nm (see Supporting Information). The above-mentioned structural and spectroscopic variations suggested that, besides substantially aspecific intermolecular forces, localized host−guest interactions could be crucial in driving the supramolecular architecture. To confirm this hypothesis we turned to density functional theory (DFT) calculations. Unconstrained geometry optimization at the BP86/TZ2P level of the {H_2OMSPz[$NH_3(CH_2)_2HPO_4$]} model complex obtained by replacing the peripheral tails with methyl groups, gave the stable C_1 symmetry structure displayed in Figure 3. In this complex, nearly linear hydrogen bonds bridge a pyrrole N_pH proton to *one* oxygen atom of the phosphate group and a NH proton of the PE head to a porphyrazine aza-methyne, N_b. The $N_p\cdots O$ and $N_b\cdots N_{PE}$ distances (2.674 Å and 2.739 Å, respectively) fit in with experimental and theoretical data reported for similar structures.[14] Hydrogen-bond formation enhances the distortion of the porphyrazine core, and the involved pyrrole ring is tilted 23.5° and 17.8° from the adjacent pyrrole planes. The energy required for this distortion amounts to

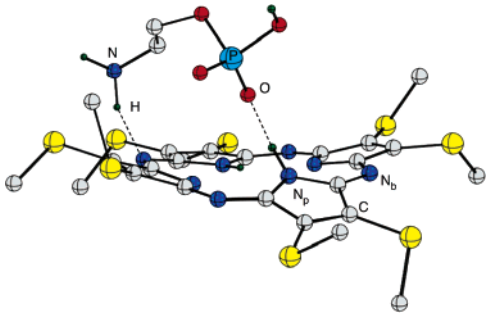

Figure 3. DFT optimized structure of the model complex {H_2OMSPz-[$NH_3(CH_2)_2HPO_4$]}. For clarity, the hydrogen atoms of the porphyrazine sulfanylmethyl groups are omitted.

$\sim$13 kcal mol^{-1} and is paid back by the hydrogen-bond interactions, which are exothermic by 40.6 kcal mol^{-1}. The changes in the porphyrazine optical spectrum induced by supramolecular interactions were well reproduced by time dependent-DFT (TDDFT) calculations. Indeed, a 70% reduction of the oscillator strength is predicted for the Q-band, and a new absorption with oscillator strength of 0.107 is computed at 593 nm.

In summary, experimental and theoretical data showed that effective interactions set up between the newly synthesized polycarboranyl porphyrazine and the polar heads of liposome constituents, indicating a useful strategy for the design of new tetrapyrrolic systems to be loaded in liposomes and delivered through the membrane of cancerous tissues. This is a favorable starting point for anticancer multiple-approach therapy.

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Supporting Information Available: Synthetic and computational details, liposome preparation and characterization, electronic spectra of H_2HECASPz loaded in liposomes. This material is available free of charge via the Internet at http://pubs.acs.org.

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