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CYCLODEXTRINS AND SMALL UNILAMELLAR LIPOSOMES: A COMPARATIVE THEORETICAL APPROACH

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ABSTRACT. Cyclodextrins and liposomes are generally used for protecting and controlled release of bioactive compounds. This is the first attempt to compare the cavity characteristics of cyclodextrins and small unilamellar liposomes by using molecular modeling techniques. The volume of the liposome cavity was 524 $Å^3$. In the case of β-cyclodextrin this cavity volume was only 87 $Å^3$, but with a slightly higher molecular volume (878 $Å^3$). As a conclusion, the smaller unilamellar liposome (theoretically modeled) could encapsulate bioactive compounds 5-6 fold bigger than β-cyclodextrin, while bioactive compound/cyclodextrin/liposome systems could be also obtained.

Keywords: *liposome, β-cyclodextrin, molecular modeling*

INTRODUCTION

Enhancing active compounds bioavailability is a permanent goal of the scientific community. Micro- and nanoencapsulation of biologically active compounds in various matrices is one of the most used techniques for this process [1,2]. Liposomes are widely used for micro- and nano-encapsulation of bioactive compounds, being empty micro- or nanospheres resulted by assembling of phospholipidic compounds in aqueous phases [3-6]; the liposome walls are formed by two or more double lipidic layers containing aqueous phase inside. Many liposome types are known such as multilamellar and unilamellar liposomes. Liposomes are obtained especially by inverse phase evaporation (large unilamellar liposomes) or ultrasonication (small unilamellar liposomes) [7]. The stability of liposomes can be enhanced by using various additives (polymers, cholesterol etc.) [3,8]. They are used especially in pharmaceutical, cosmetic, and food fields [3,4,9-13].

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Other matrices used for encapsulation are cyclodextrins (the most used natural ones are α-, β-, and γ-cyclodextrin which are cyclic oligosaccharides with 6-8 glucopyranose moieties, having hydrophobic inner cavity and outer hydrosolubilizing hydroxyl groups)[14-17]. The encapsulation process is determined by the geometry and hydrophobicity of the bioactive molecule. As a result, cyclodextrins can encapsulate only small or thin molecules [15], while liposomes can encapsulate also bigger molecules by various processes such as by inserting in the lipidic bilayer, adsorption on the membrane surface or physical encapsulation in the (usually) aqueous inner cavity [3,18,19]. Generally, cyclodextrins encapsulate hydrophobic molecules, while liposomes can encapsulate even hydrophobic or less hydrophobic molecules. In both cases, the advantages of new formulations are obvious: enhancing the bioactive compound transportation in biological environments (by hydrosolubilization of hydrophobic compounds with cyclodextrins, or by physical transportation by liposome micelles), protection against degradation factors (oxygen/air, light, and other chemical or biochemical reagents from the environment), and controlled release.

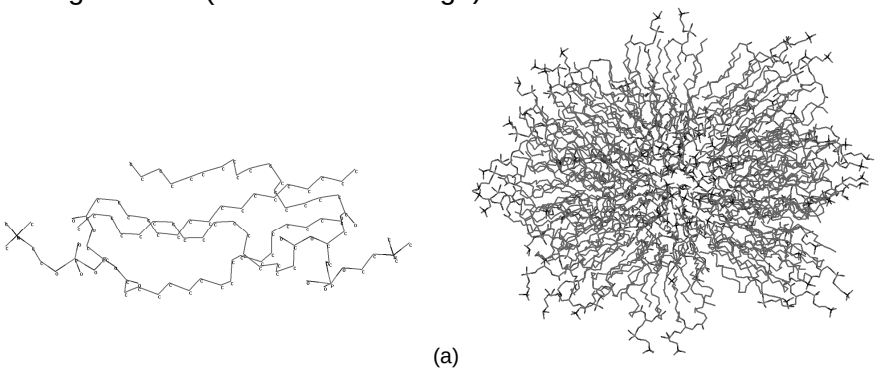
Molecular modeling of biologically active compounds or even supramolecular assemblies could be very useful for evaluation of various expected properties such as bioactivity, reactivity, hydrophobicity, solubility, docking properties [20-24]. The molecular modeling of singular compounds is easier to perform, in comparison with molecular assemblies such as liposomes. The present study is the first attempt to compare the cavity characteristics of β-cyclodextrin (bCD) and small unilamellar liposomes by using molecular modeling techniques.

RESULTS AND DISCUSSION

Molecular modeling and conformational analysis of distearoyl-phosphatidylcholine (PC) structure revealed that the most stable conformation in vacuum has a helicoidal conformation for both hydrophobic moieties, but the best interaction between two opposite PCs appear in the case of pseudolinear conformations of these moieties (Figure 1a). In the last case, choline moieties are disposed outside and will forms the inner and outer hydrophilic micelle sides, while the fatty acid moieties will forms the hydrophobic micelle wall. The interaction energy for this unit is 27 kcal/mole (computed as difference between energies of unitary PCs and 2×PC unit).

Small energetically stable unilamellar liposome can be built and optimized by using these PC units. Micelle moieties consisting of 4, 2×2, 4×2, 4×4, 16×4, and 24×4 PCs were built in order to obtain an energetically stable unilamellar micelle (maximum of 96 PCs) (Figure 1b). The interaction energy increased with the number of PCs, the dependence being linear

(Figure 2). This demonstrates that the liposome stability is enhanced by increasing the PCs (in the studied range).



(a) (b)

Figure 1. The most stable assemblies of the liposome PC unit, 2×PC (a) and the optimized smaller unilamellar liposome consists of 96 PC units (b)

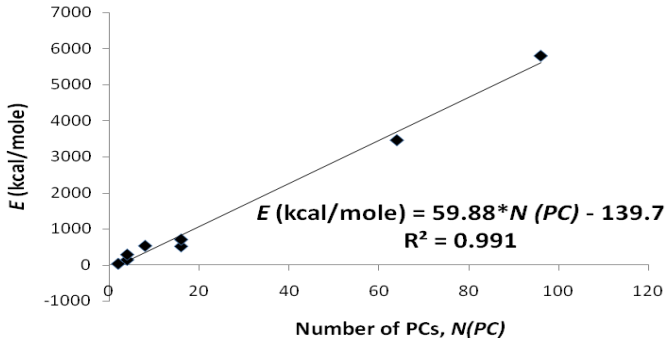


Figure 2. Variation of the interaction energy, *E (kcal/mole)*, in the micellar moieties with the number of PCs, *N (PC)*

The smaller unilamellar liposome which could be built has a pseudospherical cavity with an approximate volume of 524 $Å^3$; this volume was calculated considering the mean diameter of the closer opposite hydrogen envelopes of the choline moieties (see Experimental section).

In the case of β-cyclodextrin, the minimum energy conformation looks like a “pseudotruncated cone”, with the primary (from C6 positions) and secondary (from C2 and C3 positions) hydroxyl groups oriented to the outside of the molecule, while the tetrahydropyrane moieties corresponding to the glucopyranose units were oriented to the interior. The external hydroxyl groups confer water solubility, while the cavity has hydrophobic properties. The geometric characteristics of the most stable conformation of bCD could be evaluated by knowing the Cartesian coordinates of all atoms (including hydrogen atoms). Thus, the mean external diameters of the primary

and secondary faces have approximate values of 12.9 Å and 17.5-18.3 Å, respectively. The mean interior diameters are relatively close for both faces (5.5 Å). The bCD stable conformation was evaluated in vacuum as well as in water periodic box and the main bCD characteristics could be determined (*i.e.* the torsion angles between the C_2-C_1-O_e-C_4' of alternative glucoside moieties of -140º and -122º in vacuum). The interior volume of bCD can be calculated by using the Cartesian coordinates of atoms for the most stable conformation (see Experimental section). The approximate volume of bCD cavity is only 87 $Å^3$, six times lower than the unilamellar liposome.

CONCLUSIONS

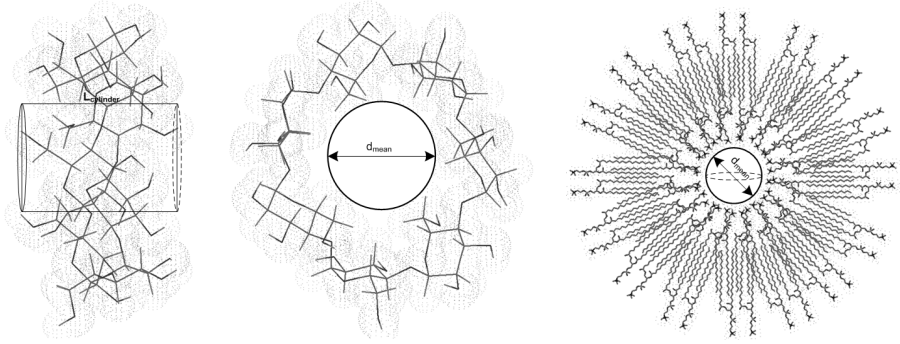
The following conclusion can be drawn from studies among molecular modeling of β-cyclodextrin structure and the smaller unilamellar liposome which can be builded by using distearoyl-phosphatidylcholine as unit: (1) the smaller unilamellar liposome has a volume of the cavity of 524 $Å^3$, little bit lower than the β-cyclodextrin molecular volume (878 $Å^3$); the interior volume of β-cyclodextrin is 5-6 fold lower than the liposome cavity (87 $Å^3$). As a result, a small unilamellar liposome can encapsulate bigger molecules than β-cyclodextrin; moreover, the flexibility of liposome is higher than in the case of β-cyclodextrin and facilitates the encapsulation process; (2) the liposome micelle has a hydrophilic cavity and can better interact with the β-cyclodextrin exterior; further, β-cyclodextrin can encapsulate hydrophobic small molecules and it is possible to obtain hydrophobic bioactive compounds / β-cyclodextrin / liposome systems with enhanced bioavailability.

EXPERIMENTAL SECTION

Molecular modeling and conformational analysis. Molecular modeling of biocompatible matrices (liposomes – phosphatidylcholine, PC, and β-cyclodextrin, bCD) was performed by using HyperChem 5.1 package (MM+ molecular mechanics program), with a RMS gradient of 0.005 kcal/mole and a Polak-Ribiere conjugate gradient algorithm. Phosphatidylcholine structure has a great number of flexible bonds which must be considered for identifying the most stable conformations, while bCD have only seven flexible bonds, corresponding to hydroxymethyl moieties. The following aspects must be considered in order to obtain the most stable conformations by using Conformational Search program from the HyperChem package: variation of the flexible torsion angles of $\pm 60º \div \pm 180º$, criterion of energy acceptance of 4 kcal/mole above best, all conformations which have distances between equivalent atoms lower than 0.5 Å and differences between torsion angles lower than 15º were neglected.

Structural descriptors. The capacity of encapsulation of the most stable small unilamellar liposome and bCD was evaluated by means of

cavity volume. This volume was calculated by knowing the Cartesian coordinates of all atoms from the most stable conformations; thus, the interior cavity of bCD was approximated with a cylinder having the diameter (d_{mean}) evaluated as the mean of interior distances between opposite hydrogen atoms, considering also the radius of these atoms (1.20 Å, according to Bondi [25]), and the cylinder length ($L_{cylinder}$) was that corresponding to the cyclodextrin height, including atoms radius (Figure 3a). The same algorithm was used in the case of small unilamellar liposome, but the cavity was approximated with a sphere (considering the mean diameter as the distance between the opposite hydrogen atoms envelope of the choline moieties, d_{mean}, Figure 3b).



(a) (b)

Figure 3. Calculation of the cavity volume for β-cyclodextrin (a) and smaller unilamellar liposome (b)

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