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β-Cyclodextrins influence on *E*-3,5,4′-trimethoxystilbene absorption across biological membrane model: A differential scanning calorimetry evidence

Maria Grazia Sarpietro, Sara Ottimo, Maria Chiara Giuffrida, Carmela Spatafora, Corrado Tringali, Francesco Castelli*

Dipartimento di Scienze Chimiche, Università degli Studi di Catania, Viale Andrea Doria 6, I-95125 Catania, Italy

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ABSTRACT

E-3,5,4′-trimethoxystilbene (TMS) is a naturally occurring analog of resveratrol. The anti-neoplastic, antiallergic and anti-angiogenic activities of TMS have been recently reported. From the viewpoint of metabolism, TMS may be more favourable than resveratrol because all of its hydroxyl groups, which are subjected to extensive glucuronide or sulphate conjugation in the metabolic pathways of resveratrol, are protected by methylation. Moreover, methylation increases lipophilicity and may enhance cell membrane permeability, but it decreases its solubility in aqueous media. A way to increase TMS solubility can be represented by complexation with β-cyclodextrins. In the present paper, the differential scanning calorimetry technique has been used to study the interaction of TMS with a biomembrane model constituted by dimyristoylphosphatidylcholine multilamellar vesicles. Furthermore, kinetic experiments have been carried out to follow the uptake of TMS by biomembranes in the presence of β-cyclodextrins to gain information on the effect of β-cyclodextrins on the uptake process. Our results indicate that opportune concentrations of β-cyclodextrins greatly improve the uptake of TMS by biomembrane models.

1. Introduction

E-3,5,4′-trimethoxystilbene (TMS) is a naturally occurring analog of resveratrol (Scheme 1) (Blair et al., 1969; MacRae and Towers, 1985). The anti-neoplastic, antiallergic and anti-angiogenic activities of TMS have been recently reported (Bader et al., 2008; Belleri et al., 2005; Pan et al., 2008). From the viewpoint of metabolism, TMS may be more favourable than resveratrol because all of its hydroxyl groups, which are subjected to extensive glucuronide or sulphate conjugation in the metabolic pathways of resveratrol (Lançon et al., 2007), are protected by methylation. Moreover, methylation increases lipophilicity and may enhance cell membrane permeability. Hence, improved pharmacokinetic profile, could be postulated. On the other hand, methylation decreases aqueous solubility and could hinder the oral bioavailability of TMS. In a previous paper (Sarpietro et al., 2007) we compared the interaction and the absorption of resveratrol and two analogs (TMS and 3,5,4′-tri-*O*-triacetylresveratrol) with biomembrane model. From this study it emerged a strong interaction of TMS with the biomembrane model but a very poor absorption. Therefore, it should be of interest to use cyclodextrins to increase the aqueous solubility of TMS and hence its absorption by biomembrane model.

Cyclodextrins (CD) are a family of cyclic oligosaccharides, obtained from starch by enzymatic degradation, composed of α-1,4-linked glucopyranose subunits (Uekama, 2004). These macrocyclic carbohydrates possess apolar internal cavities which can form complexes with various guest molecules via non-covalent interactions; the binding of guest molecules with the CD host is not permanent but rather it is a dynamic process whereby the guest molecule continuously associates and dissociates from the CD host (Lesieur et al., 2000; Shimpi et al., 2005). Because of their properties, CD are widely used in several fields. In particular, hydrophilic CD can lead to enhanced solubility, dissolution rate, membrane permeability and bioavailability of poorly water-soluble drugs (Másson et al., 1998; Redenti et al., 2001; Karathanos et al., 2007). Furthermore, CD possess most of the characteristics (quality, cost performance, bioadaptability, and negligible toxic effects) required for drug carriers from the safety viewpoint (Szejtli, 1998).

β-CD, which are composed of seven α-1,4-linked glycosyl units, are the ones most commonly used for pharmaceutical applications since their central cavity has good affinity for many hydrophobic drug compounds (Loftsson et al., 2005; Singh et al., 2002). In this research, we have performed different series of experiments to detect the interaction between TMS and dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV) used as biomembrane models and to follow the uptake of TMS by the biomembrane models with and without β-CD, with the aim to demonstrate that the presence of β-CD improves the uptake process.

* Corresponding author. Tel.: +39 095 221796; fax: +39 095 580138.
E-mail address: fcastelli@dipchi.unict.it (F. Castelli).

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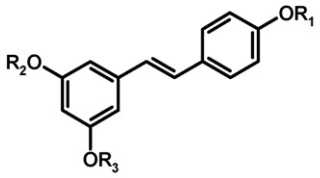

	R_1	R_2	R_3
Resveratrol	H	H	H
***E*-3,5,4'-trimethoxystilbene**	CH_3	CH_3	CH_3

Scheme 1. Resveratrol and *E*-3,5,4′-trimethoxystilbene structure.

DMPC MLV are characterized by a sharp phase transition from an ordered gel-like structure to a disordered fluid-like structure upon heating. This phase change happens at a characteristic transition temperature (T_m), revealed by the differential scanning calorimetry (DSC) technique as an endothermic peak associated to the enthalpy change (ΔH). The presence of foreign substances dissolved in the phospholipid bilayers modifies the MLV thermotropic parameters (T_m, ΔH) the effects being related to the amount of compound dissolved in the phospholipid matrix (Mabrey-Gaud, 1981; Silvius, 1991; Bach, 1984; Marsh, 1996; Huang and Li, 1999). The interaction and the absorption of TMS by the MLV have been monitored via the modification of the T_m and ΔH due to the molecule insertion in the MLV phospholipid bilayers.

2. Materials and methods

2.1. Materials

Reagents were of commercial quality and were used as received (Merck and Sigma–Aldrich); only solvents were distilled using standard techniques. The ^{1}H NMR spectra were recorded on a Varian Unity Inova spectrometer at 500 MHz and performed at constant temperature (27 °C). Analytical thin-layer chromatography was performed on silica gel (Merck 60 F254) plates using cerium sulphate as developing reagents.

β-CD (purity ≥99%) were purchased from Fluka (Germany). 1,2-Dimyristoyl-sn-glycero-3-phosphatidylcholine (purity = 99%) was supplied by Genzyme Pharmaceuticals (Liestal, Switzerland). Lipids were chromatographically pure as assessed by two-dimensional thin-layer chromatography. 50 mM tris(hydroxymethyl)aminomethane (TRIS) solution, adjusted to pH 7.4, was employed.

TMS was synthesized, as previously reported, through on a Arbuzov rearrangement followed by Horner–Emmons–Wadsworth reaction; spectral data are in perfect agreement with those obtained previously (Spatafora et al., 2009).

2.2. DSC analysis

A Mettler Toledo STAR^e system equipped with a DSC-822^e calorimetric cell and a Mettler TA-STAR^e software was used. The sensitivity was automatically chosen as the maximum possible by the calorimetric system, and the reference pan was filled with TRIS solution. The calorimetric system was calibrated, in transition temperature and enthalpy changes, by using indium, stearic acid, and cyclohexane by following the procedure of the DSC 822 Mettler TA STAR^e instrument.

2.3. Liposome preparation

Stock solutions of DMPC and *E*-3,5,4′-trimethoxystilbene in chloroform/methanol (1:1, v/v) were prepared. Aliquots of DMPC solution were distributed in glass tubes to have the same amount of DMPC in all of the tubes; then aliquots of TMS solution were added to have a defined molar fraction (X) (0.00, 0.015, 0.03, 0.045, 0.06, 0.09, 0.12 and 0.15) of the examined compound with respect to the phospholipid. The solvents were removed under nitrogen flow, and the resulting films were dried under vacuum to eliminate eventual solvents residues. TRIS solution was added to the films (to have 0.06125 mmoles/ml of phospholipid), and the samples were heated at 37 °C for 1 min and successively shaken for 1 min, for three times, and kept at 37 °C for 1 h to permit the MLV to homogenize and allow the compounds to partition between phospholipid and aqueous phases.

2.4. TMS/MLV interaction

120 μl of MLV with or without TMS was transferred into a 160 μl aluminum DSC pan, which was sealed, and submitted to calorimetric analysis as follows: (i) a heating scan between 5 and 37 °C at 2 °C/min; (ii) a cooling scan between 37 and 5 °C at 4 °C/min; for at least four times to check the results reproducibility. After the DSC analysis, aliquots of all samples were extracted from the calorimetric aluminum pans and used to determine, by the phosphorus assay (Rouser et al., 1970), the exact amount of phospholipids present in each sample.

2.5. Kinetic experiments

These experiments were carried out according to four experimental patterns: (i) in order to evaluate the TMS ability to diffuse through the aqueous medium, reach the biomembrane model and cross it, an exact amount of powdered TMS (to have a 0.12 molar fraction of compound with respect to the phospholipid) was weighed in the bottom of the DSC pan where 120 μl of DMPC MLV aqueous dispersion was added; (ii) in order to evaluate the possibility to increase the rate and extent of these processes, the previously described kinetic measurements were also done in the presence of β-CD solutions, keeping unchanged the TMS molar fraction (0.12) and varying the amount of β-CD in order to get 1:0.5, 1:1, 1:2, TMS/β-CD molar ratios; (iii) to monitor eventual interactions between MLV and β-CD, powdered β-CD (to have the same amount used in the above experiments) was weighted in the bottom of the DSC pan and added with 120 μl of DMPC MLV aqueous suspension; (iv) to detect the stability of the TMS dispersion in the lipid matrix and the β-CD capability to extract the compound from DMPC MLV, kinetic experiments were also carried out on DMPC MLV loaded with 0.12 molar fraction of TMS left in contact with solid β-CD to have 1:0.5, 1:1, 1:2 TMS/β-CD molar ratios. The samples were hermetically sealed and gently vortexed for a couple of seconds, and the interaction between TMS and DMPC (with or without β-CD) and between β-CD and DMPC was detected submitting the samples to the following calorimetric analysis: (a) a heating scan between 5 and 37 °C at the rate of 2 °C/min; (b) an isothermal period (1 h) at 37 °C; and (c) a cooling scan between 37 and 5 °C at the rate of 2 °C/min.

The procedure was repeated eight times to follow the variations in the calorimetric curves, which indicate an interaction between the tested compounds and the DMPC bilayers occurs.

3. Results and discussion

It has been reported that TMS, due to its lipophilicity, is unable to migrate towards the aqueous medium and interact with biomem-