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Calorimetric studies of the interaction of 4-biphenylacetic acid and its β-cyclodextrin inclusion compound with lipid model membrane

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Summary

The thermotropic properties of hydrated dispersions of dipalmitoylphosphatidylcholine (DPPC) containing 4-biphenylacetic acid (BPAA) or β-cyclodextrin–4-biphenylacetic acid (BPAA–β-Cyd) at different molar ratios, have been investigated by differential scanning calorimetry (DSC). Addition of increasing amounts of BPAA or BPAA–β-Cyd to phospholipidic dispersions leads to a main transition peak temperature (T_m) shift towards lower temperatures with a concomitant broadening of the peak. The main transition enthalpy ΔH remains almost constant increasing BPAA concentration. T_m shift in the presence of BPAA, observed for DPPC dispersions, was interpreted in terms of increasing membrane "fluidity". The interaction between BPAA–β-Cyd and DPPC liposomes depends on the fraction of BPAA exchanged from the complex with membranes and it is allowed by the amphiphatic nature of this molecule. Kinetic experiments were carried out and the diffusion rate of BPAA coming from the complex into liposomes was slower than that shown by free BPAA. The results demonstrate a possible interaction of BPAA with pospholipids in cell membranes and the potential effects of such an interaction in regulating adsorption in natural membranes modulating the fluidity.

Introduction

The recent development of new "host-guest" systems attained some remarkable issues in several industrial and application fields (Takemoto, 1969; Saenger, 1980), including pharmaceutics (Uekama, 1981; Katal and Antal, 1984; Jones et al., 1984; Fenyvest et al., 1984; Chown and Karara, 1986).

The use of agents able to form inclusion structures with drugs can optimize some biopharmaceutical parameters, such as solubility (Lach and Cohen, 1963; Ikeda et al., 1975), delivery (Habon et al., 1984), membrane permeability and bioavailability (Frömming and Weyermann, 1973; Tokomura et al., 1985) of the therapeutic component.

In such a view, in a previous paper the preparation of an inclusion complex of 4-biphenylacetic acid with β-cyclodextrin (β-Cyd), a cyclic oligosaccharide widely used in complexating pharmaceutical agents (Cramer and Hettler, 1967; Fröm-

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ming, 1973; Szejtli, 1982), has been presented (Puglisi et al., 1989).

4-Biphenyl acetic acid (BPAA) is an efficacious non-steroidal anti-inflammatory drug (NSAID) which showed to possess, also in "in vivo" studies, an activity comparable to that of the most common anti-inflammatory drugs and even 10 times greater than aspirin (Tolman et al., 1976). In vitro experiments on guinea pig lung homogenates confirmed that BPAA can inhibit prostaglandin biosynthesis (Tolman and Partridge, 1975). Like other NSAIDs, BPAA proved to be able to reduce inflammatory pain and hyperthermal substances effects, as well as to attenuate UV light-induced skin erythema in experimental animals (Sloboda and Osterberg, 1976).

Complexation of BPAA by means of β-Cyd has been primarily tested by us to enhance water solubility of the drug, in order to extend its therapeutical applicability, and to lower its weak local irritating action.

Inclusion product was analysed by several techniques (see Materials and Methods) and all results agree with assigning a structure for the system in which BPAA and β-Cyd are in 1 : 1 molar ratio. β-Cyd reveals itself by a 4,2-fold increase in BPAA solubility and a dissolution rate improvement of about 18 times in the first 12 min of the essay.

Since the interaction of BPAA with lipidic membranes has been never examined, and by considering that only few studies have been reported about the interaction of β-Cyd inclusion complexes with membranes (Stezowski et al., 1978), and nothing on the characterization by differential scanning calorimetry (DSC) of such a phenomenon, we thought to extend our investigation to the effects that BPAA complexation could have, with respect to BPAA alone, on the interaction with a lipidic model membrane, as dipalmitoylphosphatidylcholine (DPPC) liposomes.

It is commonly assumed that lipids in biological membranes play structural roles, providing a matrix for functional membrane proteins and maintaining a permeability barrier between external and internal environments. However, a single common phospholipidic species such as DPPC could maintain the required liquid-crystalline bilayer envelope, for this reason synthetic DPPC liposomes usually represent a good membrane model.

DSC is a standard method for the study of membranes endothermic gel–liquid crystal phase transition, observed for lipid bilayers. Drugs presence in ordered bilayer structure results in melting of lipid chains at lower temperature in respect to the pure lipid with changes in enthalpy of melting (Bach, 1984).

To gain an idea on the role of BPAA in modifying the physical properties of natural membranes, we studied the thermotropic behaviour of binary mixtures of BPAA/DPPC and BPAA–β-Cyd/DPPC, carrying out kinetic experiments on the adsorption of BPAA in a model membrane.

Materials and Methods

Chemicals

Synthetic L-α-dipalmitoylphosphatidylcholine, was obtained from Fluka Chemical Co. (Buchs, Switzerland). Solutions of lipids were chromatographically pure as assessed by bidimensional thin layer chromatography (TLC) on silica gel plates (E. Merck, Darmstadt, F.R.G.) using a solvent system consisting of chloroform/methanol/water/acetic acid (60 : 35 : 4 : 1, v/v/v/v). Phospholipids phosphorous content was essayed as inorganic phosphate as described by Bartlett (1959).

4-Biphenylacetic acid was obtained from Janssen (Belgium), analytical grade and was recrystallized from ethanol.

β-Cyd was purchased from Fluka Chemical Co. (Buchs, Switzerland) and was used after recrystallization from water and drying with P_2O_5 in vacuo.

Preparation of BPAA–β-Cyd complex

A solid inclusion complex of BPAA with β-Cyd was obtained by an homogeneous coprecipitation method. Molar ratio, which was found 1 : 1, formation constant and the possible structure of the inclusion compound were studied using both in solid state characterization methods (IR, DSC, X-ray diffraction) and in aqueous solution analytical procedures (^{1}H-NMR, UV). Solubility studies showed a significant enhancement of the dissolution rate and of the solubility of BPAA-β-

Cyd complex in respect to BPAA alone (Puglisi et al., 1989).

Preparation of liposomes

Dispersions of DPPC were prepared in the presence and absence of BPAA, BPAA-β-Cyd-and β-Cyd, as it is common practice at a temperature above that of the gel-liquid crystalline phase transition that the samples are fully hydrated.

Lipidic and BPAA solutions in CH_3OH–$CHCl_3$ 1 : 1, v : v, were mixed in order to obtain different molar ratios of solutes. The solvent was removed at 30 °C on a rotary evaporator by a nitrogen stream following by overnight high vacuum storage.

BPAA–β-Cyd and β-Cyd dispersions in DPPC liposomes were prepared by adding the compounds to the lyophilized DPPC film in a sufficient amount to obtain the same relative mole fraction of compounds in respect to DPPC.

Liposomes were prepared by adding to the film 50 mM Tris buffer at pH 7.4. The samples were heated at 60 °C, vortexed twice for 1 min and shaken for 1 h at 55 °C in a water bath in order to homogenize the liposomes. Of each sample, 120 μl containing 5 mg of DPPC, were sealed in an aluminium pan and submitted to DSC analysis.

The kinetic experiments were carried out putting on the bottom of the DSC pan the exact amount of BPAA or BPAA–β-Cyd in order to obtain a 0.48 mol fraction in BPAA, and afterwards adding 120 μl of DPPC aqueous dispersion prepared as above.

Differential scanning calorimetry

Calorimetric data were obtained using a Mettler TA 3000 differential scanning calorimeter, equipped with a DSC 30 cell and a TC 10 processor. The plotting range, as full scale deflection, was set to 1.71 mW. Palmitic acid was employed to calibrate the temperature scale and the ΔH. A pan containing 120 μl of pH 7.4 Tris buffer was used as reference. Samples were subjected to several heating and cooling cycles in the temperature range 10–60 °C at a scanning rate of 2 °C/min. Enthalpy changes were calculated from the peak areas. After the calorimetric runs the pan content was extracted and the inorganic phosphate analyzed.

Results and Discussion

Some typical DSC runs, in heating mode, of pure DPPC dispersions in Tris buffer (pH = 7.4) containing different molar ratios of BPAA are reported in Fig. 1. The pretransition and the main transition peaks for pure DPPC were observed at 36.9 and at 42.2 °C, respectively. Addition of BPAA to DPPC bilayers produced evident changes in their thermotropic behaviour. By increasing the BPAA mole fraction in the aqueous dispersion, the main transition peak for the pure lipids shifts towards lower temperatures with a concomitant peak broadening, while the related ΔH remains constant. At a 0.24 BPAA mole fraction the T_m value of the main endothermal peak in the heating scan goes 5–6 °C below that observed for the pure DPPC and the enthalpy changes were negligible. By increasing the mole fraction to upper values no further variation was observed both in T_m and ΔH (see Tables 1 and 2).

These results clearly demonstrate that the BPAA molecule is able, during the liposome preparation, to fit into the DPPC bilayer causing a "fluidify-

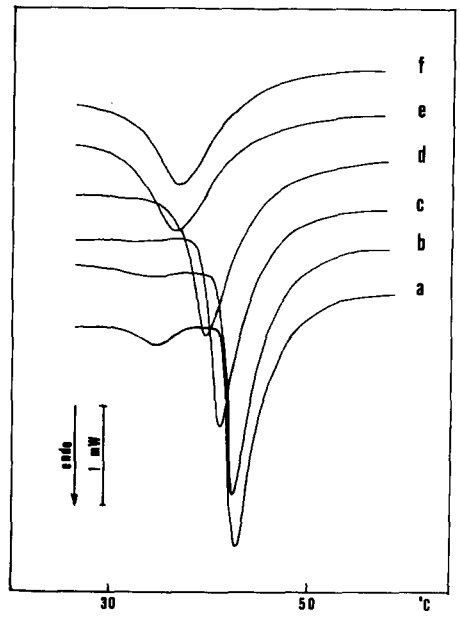

Fig. 1. Typical DSC heating curves of hydrated DPPC containing BPAA at mole fraction: a = 0; b = 0.06; c = 0.12; d = 0.18; e = 0.24; f = 0.48.

TABLE 1

Main transition peak temperature (T_m expressed in °C) of DPPC dispersions for different molar fractions of BPAA and BPAA-β-Cyd

Mol. fraction	BPAA	BPAA–β-Cyd
0.00	42.2	42.2
0.06	41.7	42.2
0.12	40.6	41.4
0.18	38.8	40.6
0.24	36.0	40.2
0.48	36.0	39.2

The figures are the mean values obtained from DSC heating curves. The percentual standard deviation was better than 0.5.

ing" effect on this model membrane by engaging hydrogen bonds with the polar head group of PC, whereas the apolar biphenyl rings could be introduced in the apolar tails of DPPC, causing a destabilization of the lipidic aggregate as well as a loss in cooperativity leading to a broadening of the curves without variation in the ΔH.

The influence of BPAA on the vesicles constituted by synthetic DPPC is then exerted as a spacer of the lipidic structure as already demonstrated for other molecules (Cater et al., 1974; Estep et al., 1978; Castelli et al., 1984) because of the presence of an amphiphatic structure.

The thermotropic behaviour of BPAA–β-Cyd complex dispersed in DPPC appears slightly different with respect to that previously shown by BPAA. Also in this case it seems evident that as the BPAA–β-Cyd mole fraction increases, the

TABLE 2

Main transition enthalpy changes (ΔH expressed in kcal/mol) of DPPC aqueous dispersions for different molar fractions of BPAA and BPAA–β-Cyd

Mol. fraction	BPAA	BPAA–β-Cyd
0.00	8.4	8.4
0.06	8.2	8.7
0.12	7.2	7.8
0.18	7.8	7.9
0.24	6.8	7.4
0.49	6.9	8.0

The figures are the mean values calculated from DSC heating curves. The percentual standard deviation was better than 5.

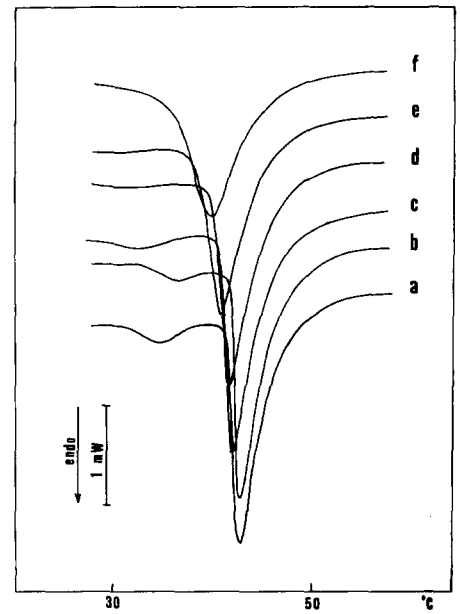

Fig. 2. Typical DSC heating curves of hydrated DPPC containing BPAA–β-Cyd at mole fraction: a = 0; b = 0.06; c = 0.12; d = 0.18; e = 0.24; f = 0.48.

main peak of DPPC gel-liquid crystal phase transition shifts towards lower temperatures and the curves become broader (Fig. 2 and Tables 1 and 2).

These effects are due to BPAA that leaves the "host" β-Cyd molecule when the solubilized complex BPAA–β-Cyd comes near to the liposomes surface. The effect exerted by this kind of free BPAA in the Tris-buffered solution is less powerful than that observed when an exactly equal amount of BPAA was dispersed in liposomes (Figs. 1, 2 and 3) because a fraction of BPAA remains entrapped in the β-Cyd and is not available to interact with the liposome surface. The assertion that these effects are due only to BPAA coming from BPAA–β-Cyd complex is also supported by experiments using DPPC liposomes in the presence of β-Cyd and submitting them to calorimetry. It was evident that β-Cyd did not interact with the bilayer. In fact no variation in the shape of the pretransition and transition peaks was observed, and consequently the T_m and ΔH did not change (see Fig. 3), according to the literature (Szejtli et al., 1986).

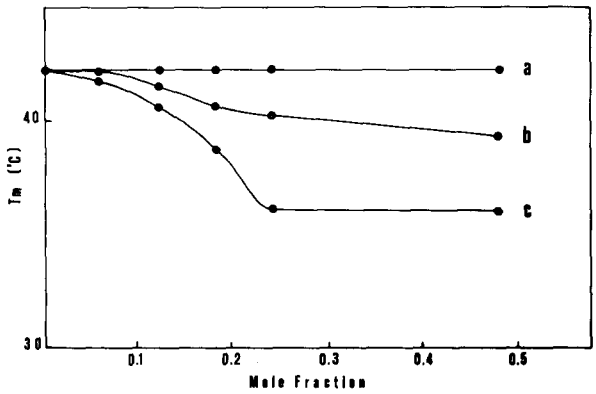

Fig. 3. Transition temperature (T_m, °C) values (average of at least 5 runs), in heating mode, as a function of mole fraction of a, β-Cyd; b, BPAA–β-Cyd; c, BPAA–β-Cyd.

To verify if the difference in the mutual interaction between BPAA-DPPC and BPAA coming from the inclusion complex and DPPC could be caused by the different preparation procedures for the two dispersions, we carried out kinetic experiments on DPPC liposomes in the presence of BPAA or BPAA–β-Cyd without mixing the aqueous suspensions.

Fig. 4 and Tables 3 and 4 show that BPAA and BPAA–β-Cyd, present in the pan, are able to diffuse in the aqueous medium reaching the liposomes surface and interacting with them, but even when the diffusion of the complex should be faster than BPAA alone for its higher solubility, the

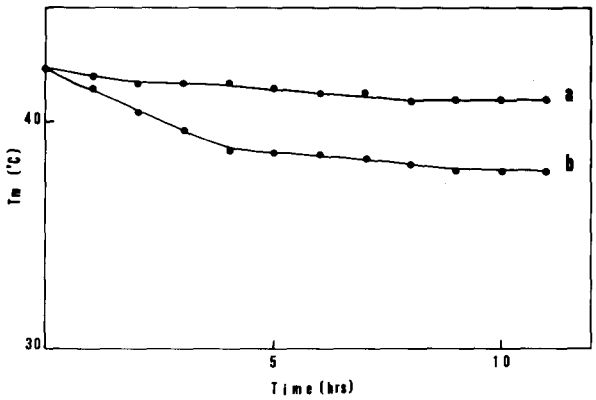

Fig. 4. Transition temperature (T_m, °C) values (average of at least 5 runs), in heating mode, as a function of time for 0.48 mol fraction of a, BPAA–β-Cyd; b, BPAA.

TABLE 3

Main transition peak temperature (T_m expressed in °C) of DPPC dispersions for 0.48 mole fraction of BPAA and BPAA–β-Cyd as a function of time

Time (h)	BPAA	BPAA–β-Cyd
0	42.2	42.2
1	41.5	42.0
2	40.4	41.7
3	39.6	41.7
4	38.8	41.6
5	38.6	41.4
6	38.6	41.2
7	38.3	41.1
8	38.1	40.9
9	37.8	40.9
10	37.8	40.9
11	37.8	40.9

interaction with membranes is slowed by the stability of the complex that seems to avoid the fast exchange of BPAA with DPPC bilayer. In both cases we observed that, after a first period of 5–6 hours where the interaction is relatively fast, a near-constant T_m and ΔH were reached meaning that BPAA could be incorporated in the model membrane following a very slow process, that could permit the T_m to reach the lower values reported in Fig. 3 only after a very long time. The slow decrease of T_m for a longer time may be due, besides to the slow diffusion of BPAA or its

TABLE 4

Main transition enthalpy changes (ΔH expressed in kcal/mol) of DPPC aqueous dispersions for 0.48 mol fractions of BPAA and BPAA–β-Cyd as a function of time

Time (h)	BPAA	BPAA–β-Cyd
0	8.5	8.3
1	8.4	8.6
2	8.8	8.1
3	8.1	8.6
4	8.5	8.3
5	8.0	8.6
6	7.6	8.4
7	7.3	8.3
8	7.1	8.1
9	7.6	8.0
10	8.0	7.9
11	7.8	8.1

inclusive complex through the water, also to diffusive processes between the interior bilayers of liposomes and a flip–flop exchange of BPAA inside them. We can have an idea of the time required by this process looking at other molecules: i.e. cholesterol needs several hours to pass through lipidic bilayer and days to get through the inner bilayers (Houslay and Stanley, 1982).

Conclusions

It is indisputable that cyclodextrins are raising in the last years a more and more qualified role as prolonged-release carrier for drugs (Hirayama et al., 1988), and the results presented here show that BPAA-β-Cyd complex is able to interact with the model membrane constituted by DPPC liposomes and even if its interaction is not so strong as when BPAA alone was employed, it is important to underline that BPAA-β-Cyd represents a way to bring in solution the usual insoluble BPAA molecule and that this complex could exchange with the membrane surface the "guest" BPAA molecule, thus representing a system to regulate the adsorption rate of hydrophobic molecules in cells.

It should also be interesting to compare these results with the response of "in vivo" pharmacological tests that we are carrying out at the moment and which may clarify the significance of complexation on the tissue interactions of this drug.

It should be pointed out that the calorimetric procedure used here can represent a good approach to the experimental study of the physical relations between a pharmaceutical compound and an artificial lipidic membrane.

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