Calorimetric Study on the Interactions of 5-*n*-Heptadec(en)ylresolcinols from Cereal Grains with Zwitterionic Phospholipid (DPPC)

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Z. Naturforsch. **46c,** 423–427 (1991); received July 18, 1990/March 11, 1991

Alk(en)ylresorcinols, Lipid Phase Transitions, Microcalorimetry

The effect of two 5-*n*-alk(en)ylresorcinol* (17:1 and 17:0) homologs at the concentrations of 5–20% on the thermotropic properties of dipalmitoylphosphatidylcholine in hydrated bilayers has been studied. The effect is different, depending on unsaturation of the aliphatic chain of resorcinol derivative. Saturated homologue (5–20 mol%) induces disappearance of the pretransition, increase of the main transition temperature and a half-width of the transition peak. Unsaturated homologue shifts pretransition towards higher temperatures, and similarly to the saturated one increases transition half-width but decreases the transition temperature, decreases the transition heat content and induces some phase separation.

Introduction

Resorcinolic lipids (5-*n*-alk(en)ylresorcinols) are long chain derivatives of 1,3-dihydroxybenzene, mainly of the plant origin. The homologs with the chain of 13 to 29 carbon atoms in the length [1, 2] are unique components of cereal grains [3–5]. Their localization in the hull of the grain [6] makes them almost present exclusively in the bran milling fraction. Since human population today is concerned about having an adequate amount of fiber in the diet and therefore consumes various high fiber products prepared with cereal brans, the explanation of the possible effects of all constituents present in the bran should be given. It should be noted that these high fiber products contain up to three-fold higher level of alk(en)ylresorcinols than the rye grains.

Hitherto obtained results show that due to their amphiphilic character resorcinolic lipids interact with biological membranes [7–11], affecting their properties. Although experiments indicated that resorcinolic lipids (particularly their enoic homologs) at higher concentrations were hemolytic and membrane disturbing [7, 12], the low concentration effects of these compounds, which might be of the biological relevance, remain to be elucidated.The effects of resorcinols at the levels below 20 μM or several percent in the membrane (which are well below their hemolytic activity) are to be considered.

Our recent studies indicate that low concentrations of resorcinolic lipids significantly modulate activity of membrane-bound enzymes [13], receptors [14] and the fluidity of the membrane lipids [15].

In this paper the effects of low concentrations (5–20 mol%) of a given alk(en)ylresorcinol homologs on thermotropic properties of phospholipid are presented.

Experimental

Materials

17:0 AR and 17:1 AR were extracted from rye grains according to the procedure described previously [16]. Their ethanolic solutions of 5 mM concentration were used for experiments. DPPC (Calbiochem) was used as delivered, without further purification.

Abbreviations: 17:0 AR, 5-(heptadecyl)resorcinol; 17:1 AR, 5-(heptadecenyl)resorcinol; DPPC, dipalmitoylphosphatidylcholine; T_p, T_m, pre- and main transition temperatures, respectively; ΔH, transition enthalpy; $\Delta T_{1/2}$, transition half-width.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/0500–0423 $ 01.30/0

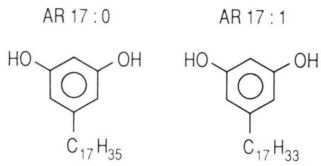
Methods

Samples were prepared as follows. To 6 mg of lipid appropriate, to obtain required AR:DPPC molar ratio, amount of resorcinol solution was added and solution was filled up to 0.5 ml with ethanol. After short mixing, solution was dried under stream of nitrogen and then evaporated under vacuum for at least 3 h. To dry AR:DPPC mixture 60 µl of bidistilled water was added. For proper hydration samples were heated to about 50 °C and shaken vigorously until homogenous suspension was obtained. Then 20 µl of AR:DPPC dispersion was placed in the aluminum sample pan and scanned at least three times. For each AR:DPPC molar ratio studied three separate samples were examined. Samples of pure DPPC were prepared and scanned as a control.

Microcalorimetric measurements were performed using Unipan type 600 microcalorimeter equipped with modified measuring thermostat. Scanning rate was 1 °C/min, temperatures were determined with 0.1 °C accuracy. Experimental error of phase transition enthalpy estimation was less then 10%. Phospholipid content of the samples was determined after experiment by phosphorus determination, using the method described by Bartlett [17].

Results

Because resorcinolic lipids are known to affect the membrane properties and structure at micromolar concentrations [7, 12–15], in the present



study we also used AR:DPPC low molar ratios, in the range 5–20 mol%. In Fig. 1, enthalpies of the DPPC main phase transition are plotted as a function of the AR molar fractions in the mixtures. As it can be seen in this figure for both resorcinol lipids studied we observed almost linear dependence of ΔH on AR molar ratio to DPPC, but courses of obtained plots are opposite. Namely, saturated compound (AR 17:0) caused a progressive increase of the transition enthalpy, while unsaturated one (AR 17:1) its progressive decrease. It is also

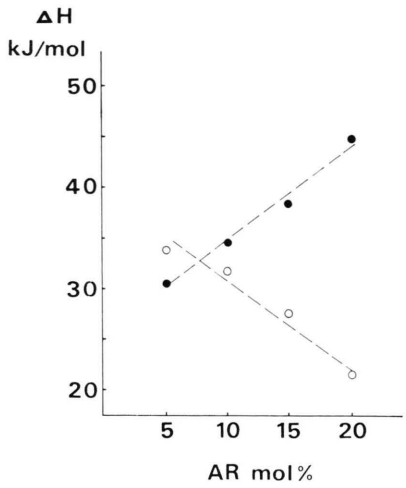


Fig. 1. Dependence of the enthalpy of main phase transition (ΔH) of DPPC/AR on the molar fraction of alk(en)ylresorcinols in mixtures. (●) AR 17:0 and (○) AR 17:1 added.

worth to note, that in the case of the lowest of the studied concentrations both of these compounds caused slight but definite decrease of ΔH, when compared with transition enthalpy for pure lipid. This finding seems to be particularly interesting in the case of AR 17:0, which at higher concentrations (15 and 20 mol%) induced an increase of ΔH above the value obtained for pure DPPC.

The opposite direction of the effects induced by the presence of resorcinol lipids in mixtures with DPPC was not observed in the case of other phase transition parameters. Both compounds induced an increase of the transition half-width (see Fig. 2),

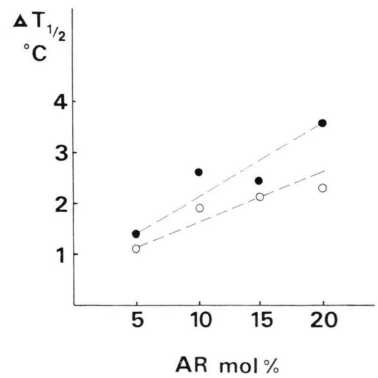


Fig. 2. Plot of DPPC/AR mixtures main transition half-width ($\Delta T_{1/2}$) as a function of molar fraction of alk(en)ylresorcinols. (●) AR 17:0 and (○) AR 17:1 added.

which was almost linearly dependent on molar fractions of ARs in mixtures. However, the influence of saturated AR 17:0 was more pronounced, thermograms obtained for mixtures of this compound with DPPC were broader then those obtained for AR 17:1/DPPC systems (compare also thermograms in Fig. 4a and 4b, respectively).

Temperatures of the pre- and main phase transitions were also affected by the presence of the resorcinol lipids in the studied systems, but the effects of saturated and unsaturated one on these parameters were different. As it can be seen in Fig. 3 (and also in Fig. 4), pretransition of phospholipid studied was abolished for AR 17:0 content higher then 5 mol%, whereas increasing concentration of AR 17:1 caused a progressive increase of T_p. However, together with mentioned increase of T_p, simultaneous decrease and broadening of pretransition peaks were observed (see Fig. 4b). In contrast to the described above effects of resorcinol lipids on DPPC pre-transition, which show linear dependence on AR molar fraction, the T_m changes induced by AR were more complicated (see upper part of Fig. 3). For both compounds at lower concentrations (5 and 10 mol% in the case of AR 17:0; 5–15 mol% in the case of AR 17:1) a progressive decrease of T_m, which was more pronounced in the case of AR 17:1, was observed. This effect was reverse for higher concentrations (15–20 mol% for AR 17:0; and 20 mol% for AR 17:1), when an increase of T_m was found. Similarly as it was in the case of ΔH, the T_m values obtained for saturated AR/DPPC mixtures were higher, while for unsaturated AR/DPPC mixtures lower than the value of main phase transition temperature obtained for pure DPPC.

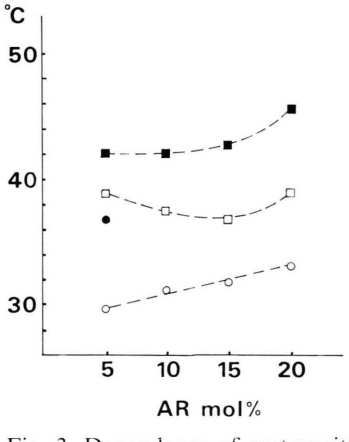


Fig. 3. Dependence of pretransition (circles) and main transition (squares) temperatures of DPPC/AR mixtures on molar fraction of alk(en)ylresorcinols. Open symbols represent data for DPPC/AR 17:1, filled symbols data for DPPC/AR 17:0 mixtures.

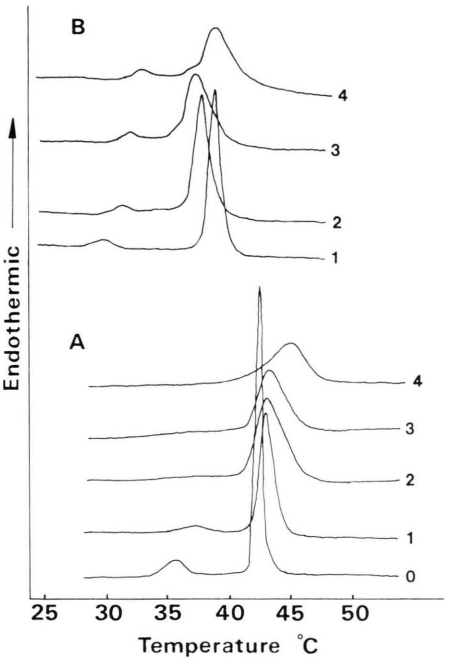


Fig. 4. Examples of thermograms obtained for A – DPPC/AR 17:0, and B – DPPC/AR 17:1 mixtures. In each part of the figure thermogram numbered 0 represent pure DPPC, traces numbered 1, 2, 3, 4 represent 5, 10, 15 and 20 mol% of the appropriate alk(en)ylresorcinol added, respectively.

Inspection of the obtained thermograms (Fig. 4b) revealed that when unsaturated resorcinol lipid was present in higher molar ratios to phospholipid (*i.e.* 15 and 20 mol%), a small hump appears on the low-temperature shoulder of the main transition peaks. This effect was not recorded for any of 17:0 AR concentrations studied.

Discussion

Presented above studies were undertaken to provide a better insight into the effect of resorcinolic lipids, the compounds interesting also from the chemical point of view (all substitutions to the ring are in the *meta*-positions), on the properties and function of the cellular membranes.

Obtained here results confirmed the different effects of saturated and unsaturated homologs observed earlier [11]. At the same time it is shown that both saturated and enoic homologs even at low membrane concentrations affect thermotropic transitions of phospholipid molecules in bilayer, although in different manner.

Biphasic effect of increasing concentrations of long-hydrocarbon chain agents on the phase transition temperature was observed also in the case of alcohols [18, 19], which chemical structures are similar to some extend to resorcinols. The authors of [19] also showed theoretically, that shape of T_m dependence on alcohol-lipid molar ratio is related to the energy of interaction between components of the mixture. Although in the case of alk(en)ylresorcinols the difference between studied compounds lies not in the length of hydrocarbon chain but in degree of unsaturation, general similarity of obtained results suggests the following: energy of interaction between AR 17:1 and DPPC is probably lower than in AR 17:0-DPPC systems due to fact that unsaturated chains of AR 17:1 do not match ordered structure of DPPC as well as saturated AR 17:0 chains do.

Humps appearing in the thermograms recorded for 15 and 20 mol% AR 17:1/DPPC mixtures should be attributed to the effect of phase separation. A calorimetric picture of this phenomenon depends on the miscibility of mixture components. When a distinct hump or splitting of the transition peak into two components is observed, presence of two separately melting phases is suggested [20–22]. It is also known [23] that unsaturated long-chain compounds reveal larger phase-separation tendency than saturated ones (in mixture with fully saturated lipids). This explain why symptoms of phase separation were not recorded for AR 17:0/DPPC mixtures. The appearance of phase separation in the case of enoic homologue might be correlated to their ability (at much higher membrane concentrations) to affect the phospholipid structural polymorphism as observed by ^{31}P NMR [8]. Saturated homologs induced formation of only highly mobile intramembraneous structures whereas enoic ones caused H_{II} phase formation. The increase of the width of the AR:DPPC transition peaks also indicate that alk(en)ylresorcinols affect the cooperativity of the transition, and the size of the phospholipid domains. This may affect the number and type of phase boundary defects responsible for proper binding and action of such enzyme as phospholipase A [24, 25].

Gradual elevation of the DPPC pretransition temperature by the presence of AR 17:1 could be explained by the influence of this compound on the structure of the gel phase ($L_{\beta'}$), as proposed by O'Leary *et al.* [26] in the case of tetradecenols. In O'Leary's model, the presence of perturbants like tetradecenols reduce the hydrocarbon chain tilt in gel phase, reducing the structural differences between gel and rippled phases, what in turn leads to elevation of pretransition temperature.

Because biological membranes are the structures in which various lipid types are involved, further studies on the role of membrane lipid polar head-group and acyl chains as well as the role of sterols in their interactions with resorcinolic lipids are to be continued.

Acknowledgements

This work was supported by grants No. RP II.11.4.4 (A.B.H.) and RP II.11.4.9 (A.K.).

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