Modulation of the Activities of Membrane Enzymes by Cereal Grain Resorcinolic Lipids

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Cereal Grains, Resorcinolic Lipids, Alk(en)ylresorcinols Membrane Enzymes, Acetylcholinesterase, Ca-ATPase

Resorcinolic lipids, amphiphilic compounds from cereal grains show strong effects upon the activity of membrane enzymes. The concentrations for 50% inhibition of erythrocyte membrane acetylcholinesterase were in the range of 18–90 μM and were dependent on the length of the aliphatic side chain of the homologue and on the modification of hydroxyl groups in the benzene ring. Sulfonation of OH groups resulted in a drastic decrease of the inhibitory potency. The effect of resorcinolic lipids on the activity of Ca^{2+}(calmodulin)-ATPase was the opposite. Up to concentrations of 50 μM alk(en)ylresorcinols stimulated the activity of this enzyme and only slight inhibition (approx. 30%) was observed above 100 μM. The results suggest that the effect of resorcinolic lipids might depend on their ability to alter the bilayer properties. Most probably these compounds decrease the mobility of membrane phospholipid molecules.

Introduction

Long chain derivatives of resorcinol (**I**) as well as phenol and catechol are naturally occurring amphiphilic compounds isolated from many plant materials [1]. Members of the *Anacardiaceae* are the main sources of these compounds. Since 1964 the occurrence of 1,3-dihydroxy-5-*n*-alk(en)ylbenzenes in cereal grains has been known [2]. Phenolic lipids were supposed to be responsible for deleterious nutritional effects of rye to animals. Alkyl and alkenyl catechols present in plants such as poison ivy, poison oak and poison sumach are well known for their allergenic and inflammatory properties (see *e.g.* ref. [3]). However, the role of resorcinolic lipids in cereal grain biology is not known, yet they might participate in the nutritional properties of cereals and cereal products (*e.g.* high fiber products based on bran milling fraction). Due to the amphiphilic character of phenolic lipid molecules they very likely interact with biological membranes [4] resulting in a strong disturbance of membrane barrier properties. Hitherto performed studies concerned mainly one aspect of phenolic lipids – membrane interaction, namely the lipid–lipid interaction. The results showed that these compounds upon incorporation into membranes induced a strong increase of their permeability for water, ions and small non-electrolyte molecules as well as the formation of intramembraneous non-lamellar structures [5–8]. It was an interesting question if and how the disturbance of the membrane environment caused by resorcinolic lipids will affect the properties of membrane proteins, especially membrane enzymes. In this paper the effects of resorcinolic lipids upon the activity of two integral erythrocyte membrane enzymes (acetylcholinesterase and Ca^{2+}(calmodulin)-dependent ATPase) are described. The effects of chain length, unsaturation of side chains and modification of the hydroxyl groups of resorcinolic lipids in relation to its activity upon the membrane enzyme are also demonstrated.

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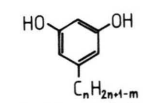
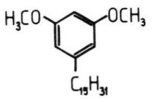
Materials and Methods

Individual homologues of 5-*n*-alk(en)ylresorcinol were isolated from rye grains as described earlier [9], 5-*n*-pentadecylresorcinol (Aldrich, Milwaukee, Wis., U.S.A.) was purified by column chromatography [10]. Methylation of hydroxyl groups of 5-*n*-alkylresorcinol was done as described by Wenkert *et al.* [2] (**II**) and sulfonation with SO_3-pyridine as described by Kumagai *et al.* [11] (**III**). Modified alkylresorcinols were purified



I



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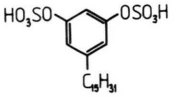


III

chromatographically on silica gel columns. The preparations were dissolved in ethanol as 5 mM stock solutions.

Erythrocyte membranes were isolated from fresh pig blood according to Dodge *et al.* [12]. Acetylcholinesterase activity was analyzed by the method of Ellman *et al.* [13] and Ca^{2+}(calmodulin)-ATPase activity according to Szyja *et al.* [14]. The effect of resorcinolic lipids was studied after injecting small amounts (up to 10 µl) of ethanolic solutions of the compounds before initiation of the reaction with the substrate. The concentration of alcohol in the samples was always kept below 0.5%. Protein was determined according to Lowry *et al.* [15].

Results and Discussion

The hydrolysis of acetylthiocholine iodide by erythrocyte membrane acetylcholinesterase is decreased in the presence of resorcinolic lipid in the incubation mixture which indicates the inhibitory action of these compounds. As shown in Fig. 1 the effectiveness of this inhibition is dependent on the preincubation time of the membrane with alk(en)-ylresorcinol. The time necessary to reach the half-maximal effect is short (about 2 min) indicating relatively fast incorporation of resorcinolic lipid into the membrane and interaction with the enzyme in good agreement with the high value of membrane-buffer partition coefficient (about 100,000 [4]). Further experiments were performed using 5 min equilibration of added compounds with the membranes. This time was sufficient for incorporation of most of the added resorcinolic lipids as their membrane-buffer partition coefficient is high (about 100,000 [4]). Dependence of the apparent inhibition of erythrocyte membrane acetylcholinesterase on the concentration of individual homologues and some of their derivatives show (Fig. 2) their different abilities to inhibit the activity of the studied enzyme. In the concentration range of $1 \times 10^{-5} - 10 \times 10^{-5}$ M the strongest effect was observed for homologues having the longer aliphatic chains (above C17). The activities of shorter-chain homologues (C15 and C17) as well as of homologues in which hydroxyl groups were methylated were significantly lower. Contrary to the previous results, presented *e.g.* in ref. [16] the ability of resorcinolic lipids to decrease the acetyl-

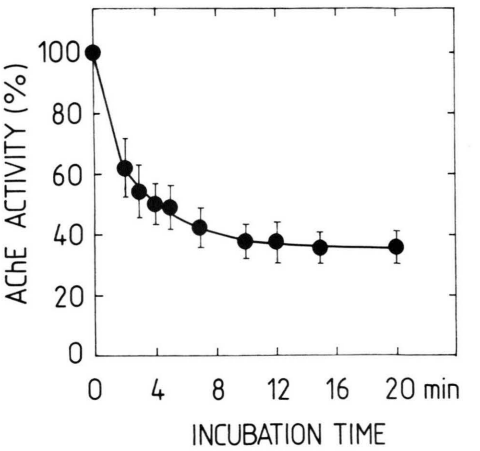


Fig. 1. Effect of the incubation time of erythrocyte membranes with heptadecenylresorcinol on membrane acetylcholinesterase activity. Concentration of membrane in the sample: 100 µg of protein. Concentration of resorcinolic lipid 28 µM. Temperature of the reaction 37 °C.

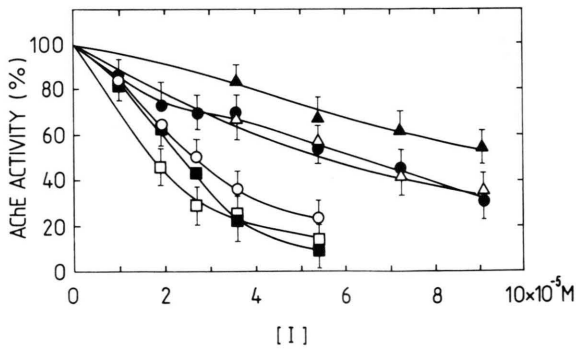
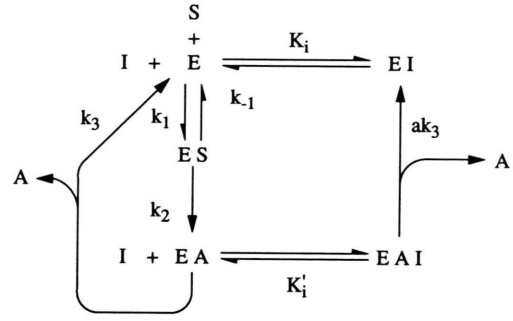


Fig. 2. Concentration dependence of the effect of various resorcinolic lipid homologues on activity of erythrocyte membrane acetylcholinesterase. The reactions were initiated with the substrate (final concentration 0.5 mM) after 5 min incubation of membrane suspensions (100 µg of protein) with the homologue studied at 37 °C. ▲ – pentadecylresorcinol, △ – dimethylpentadecylresorcinol, ● – heptadecylresorcinol, ○ – heptadecenylresorcinol, □ – tricosylresorcinol, ■ – tricosenylresorcinol.

cholinesterase activity was not as strongly dependent upon the number of double bonds in the chain as it was observed for their hemolytic effect. This is especially well seen for long chain homologues – both inhibitory activity of C23:0 and C23:1 homologues and the concentration *vs.* inhibition relationships are very similar (Fig. 2). The comparison of the effects of the various homologues and derivatives studied to the effect of sodium dodecyl sulfate as an example of a detergent upon the activity of acetylcholinesterase is shown in Fig. 3. The apparent inhibition of the enzyme by sodium dodecyl sulfate is very low. The homologue, with OH groups sulfonated, exhibits also very weak effects upon the acetylcholinesterase activity. Even at the concentration above 200 μM 15:0 disulfopentadecylresorcinol induced a decrease of the enzyme activity only by approx. 20%. On the other hand this compound due to the higher polarity of its polar head shows strong detergent-like properties when the effect on membrane permeability was studied [4]. The decrease of the headgroup polarity by methylation of the hydroxyl groups of pentadecylresorcinol molecule resulted in an opposite effect: slight enhancement of their inhibitory activity (Fig. 3). Our previous findings showed that alk(en)ylresorcinols exhibit also the capability for direct interaction with proteins [4, 17, 18]. Therefore, a kinetic analysis of the inhibition was made based on the assumption that the compounds studied would act as small, simple enzyme inhibitors. For kinetic considerations the scheme of inhibition proposed by Krupka *et al.* [19] for long chain ammonium salts was taken:



where: E, enzyme; ES, enzyme-substrate complex; EA, acetylated enzyme; EI, enzyme-hinhibitor complex; EAI, acetylated enzyme-inhibitor complex; A, acetic acid; S, substrate.

According to this scheme Miyagawa and Fujita [20] showed that the slope of the Lineweaver-Burk plot at a given inhibitor concentration will be equal to K_m/V_{max} $(1 + [\mathbf{I}]/K_i)$ and proportional to the inhibitor concentration. Therefore, the abscissa intercept of the plot of the values of slopes of plots $1/V = f(1/S)$ determined for various inhibitor concentrations *versus* inhibitor concentration will give the value of $-K_i$. This value could be considered as a measure of affinity of inhibitor to the enzyme. Several parameters characterizing the inhibitory effect of some of the resorcinolic lipids studied on acetylcholinesterase activity are presented in Table I. An increase of the apparent Michaelis constant in the presence of resorcinolic lipids is very characteristic and is higher for unsaturated homologues. Resorcinolic lipids despite the unsaturation of the side chain or modification of hydroxyl groups in the ring show high affinity for the enzyme which is depicted by K_i values. This affinity is similar to that obtained for other compounds known to be acetylcholinesterase inhibitors [20]. It might be supposed that resorcinolic lipids bind to a hydrophobic "pocket" close to the ester binding site in a similar way as *m*-substituted benzyltriammonium salts [20].

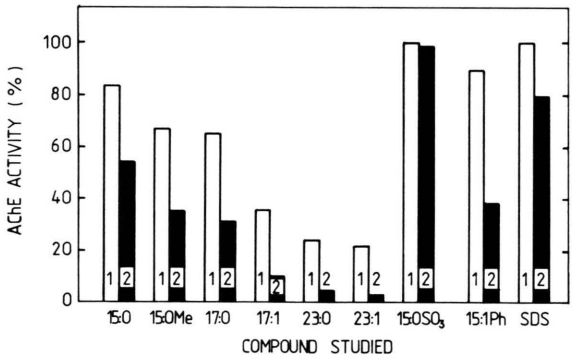


Fig. 3. Comparison of the effect of various resorcinolic lipids, sodium dodecyl sulfate and pentadecenylphenol upon acetylcholinesterase activity. Reactions were performed at 37 °C using 80 μg of membranes and 0.5 mM substrate. The concentrations of the compounds: 1–36 μM and 2–90 μM. 15:0 – pentadecylresorcinol, 15:0 Me – dimethylpentadecylresorcinol, 17:0 – heptadecylresorcinol, 17:1 – heptadecenylresorcinol, 23:0 – tricosylresorcinol, 23:1 – tricosenylresorcinol, 15:0 SO_3-disulfopentadecylresorcinol, 15:0 Ph – pentadecylphenol, SDS – sodium dodecyl sulfate.

Table I. Some parameters describing the effect of resorcinolic lipids on the activity of erythrocyte membrane acetylcholinesterase.

Resorcinolic lipid	IC_{50}* [μM]	$K_{m\,app}$** [mM]	-log K_i***
None	–	0.120	–
Pentadecylresorcinol (15:0)	90	0.200	4.45
Dimethoxypentadecylresorcinol (15:0 Me)	62	0.222	4.62
Heptadecylresorcinol (17:0)	65	0.270	4.70
Heptadecenylresorcinol (17:1)	25	0.510	5.10
Tricosylresorcinol (23:0)	18	0.357	5.35
Tricosenylresorcinol (23:1)	24	0.556	4.96

* Determined after 5 min incubation of ghosts with compound studied, substrate concentration 0.5 mM.
** In the presence of 3.6×10^{-5} M of compound studied and 0.5 mM substrate.
*** Obtained from the slope replots of Lineweaver-Burk plots at various inhibitor concentrations against |I|.

Since many enzymes are integral membrane proteins the possibility of indirect effects on enzymatic activity by alteration of the bilayer properties should also be considered. In this respect acetylcholinesterase is known to be quite sensitive to changes in fluidity of the phospholipid bilayer of the membrane. It was shown that a decrease of membrane "fluidity" or the restriction of the phospholipid acyl chains mobility causes a limitation of the enzyme conformational elasticity, a drop of its cooperativity and finally a decrease of activity [21, 22, 24, 25]. According to this resorcinolic lipids might affect also the enzyme activity *via* induction of changes of membrane fluidity. The decrease of enzyme activity is in accordance with the restriction of the motion of membrane lipids by resorcinolic lipids [23].

The inhibitory effect of resorcinolic lipids on acetylcholinesterase activity is temperature dependent which is most pronounced for saturated homologues. At 25 °C heptadecylresorcinol inhibits the enzyme by less than 20%, whereas at 42 °C by more than 50% in comparison to the control assays (Fig. 4). This temperature dependence of inhibitory action of saturated and unsaturated homologues is similar to the effect on barrier properties [26] and further supports the importance of membrane alteration in the apparent inhibition of acetylcholinesterase activity by resorcinolic lipids. Therefore resorcinolic lipid molecules will play a role in both indirect (*via* alteration of bilayer properties) and direct (*via* interaction with hydrophobic "pockets" of enzyme molecule) modulation of membrane protein activity.

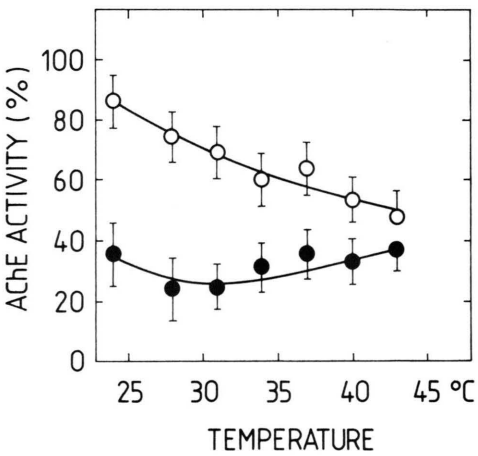


Fig. 4. The effect of temperature on apparent inhibition of acetylcholinesterase with 17:0 and 17:1 alk(en)ylresorcinols at the concentration of 36 μM. Conditions as described in Fig. 2. ○ – heptadecylresorcinol, ● – heptadecenylresorcinol.

The studies of alk(en)ylresorcinol effects on another membrane enzyme – calcium dependent ATPase support the importance of the indirect modulation of membrane properties. It was shown that at concentrations inhibiting acetylcholinesterase resorcinolic lipids activate ATPase (Fig. 5). Only above 10×10^{-5} M activities lower than controls were observed (maximal inhibition of approx. 30% at 20×10^{-5} M). The differences

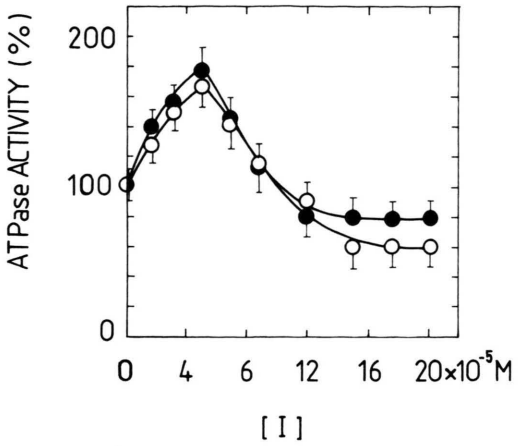


Fig. 5. The effect of heptadecyl- and heptadecenylresorcinols (17:0 and 17:1) on the activity of Ca^{+2}(calmodulin)-ATPase at 37 °C. 100 μg of membrane proteins were used and calmodulin concentration was 50 nM [14]. ● – Heptadecylresorcinol, ○ – heptadecenylresorcinol.

between saturated and unsaturated homologue activities were small and insignificant.

The results obtained in this work indicate that resorcinolic lipids inhibit acetylcholinesterase and activate (in the same concentrations) Ca^{2+}-(calmodulin) ATPase. A kinetic analysis indicated a mixed type of inhibition of acetylcholinesterase activity suggesting an important role of bilayer fluidity in the regulation of this enzyme. The data presented above are in perfect agreement with our previous findings that incorporation of alk(en)ylresorcinols into the membrane decreases its fluidity [23] and alterates phospholipid thermotropic properties [8, Hendrich and Kozubek – in preparation]. They are also capable of direct interaction with proteins *e.g.* bovine serum albumin, erythrocyte spectrin, cholera toxin [4, 17, 18] which might be responsible for the affinity changes of enzyme to substrate [20]. These two main effects would result in mixed type inhibition observed for alk(en)ylresorcinols and other amphiphiles. The observed opposite effects of resorcinolic lipids upon activity of the enzymes studied (acetylcholinesterase and Ca^{2+}-ATPase) are in good agreement with the opposite effect of membrane fluidity on acetylcholinesterase and ATPase activities described in the literature [24, 25]. Changes of the inhibitory activities caused by modification of the polar headgroup suggest the importance of proper size and/or hydrophilicity of this part of the molecule.

The effects of cereal grain resorcinolic lipids presented above are similar to those showed for a new cardiotonic compound, gingerol, the bitter constituent isolated from ginger, which is also a member of phenolic lipids [27]. Ginger is known since many years in Far East folk medicine to be cardiotonic and to be a constituent of many herbic mixtures. Gingerol was shown to activate calcium-dependent ATPases and in particular the heart-muscle Ca-ATPase [27]. A similarity of the action of resorcinolic lipids to the action of this compound opens new perspectives for further studies and suggests the necessity of solving the problem of biomedical effects of resorcinolic lipid-rich bran products in human nutrition and health.

Acknowledgements

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