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ARKADIUSZ KOZUBEK

THE EFFECT OF 5-(n-ALK(EN)YL)RESORCINOLS ON MEMBRANES. II. DEPENDENCE ON THE ALIPHATIC CHAIN LENGTH AND UNSATURATION*

Institute of Biochemistry, University of Wroclaw, Tamka 2; 50-137 Wroclaw, Poland

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The effect of chain length and unsaturation on the haemolytic properties of cereal resorcinolic lipids, (5-n-alk(en)ylresorcinols), was studied using isolated saturated, monoenoic and dienoic homologues. The haemolytic activities of the homologues studied were proportional to the degree of the side chain unsaturation and inversely proportional to the chain length. At temperatures close to physiological of animal organisms the most active were mono- and di-enoic homologues of 5-n-heptadecyl and 5-n-nonadecyl resorcinols. The results might point to the importance of short-chain cereal resorcinolic lipids in animal and human nutrition.

Many plants contain amphiphilic 5-(n-alk(en)yl)resorcinols (cardols, bilobols) [1 - 9], as well as other phenolic lipids: alkylphenols (cardanols) and alkylcatechols (urushiols, laccols, thitsiols) [1]. *Gramineae*, in contrast to other plant families, are rich in alkylresorcinol analogues with aliphatic chains of more than 15 carbon atoms. In cereal and grass grain substantial amounts of C17 - C25 homologues were found [2 - 5], the homologues with saturated aliphatic chains being predominant in the former. However, rye grain contains in addition significant amounts of mono- and diolefinic homologues [2, 3, 5 - 7].

Among cereal alk(en)ylresorcinols, the effect of olefinic homologues on the properties and structure of biological membranes was shown to be stronger than that of saturated homologues [8 - 11]. Many functional and

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structural defects of membranes and cells incubated in the presence of micromolar concentration of these homologues were earlier demonstrated [9, 11].

The present paper describes a comparative study on the effect of isolated homologues of alk(en)ylresorcinol of various chain length and unsaturation on erythrocyte membranes.

MATERIAL AND METHODS

Various saturated, monoenoic and dienoic homologues of alkylresorcinol were isolated from total rye grain alk(en)ylresorcinols by the chromatographic procedure described elsewhere [5]. The homologues were found to be homogeneous and of purity exceeding 96% on two-dimensional thin-layer chromatography [7]. For experiments 5 mM stock ethanolic solutions of the homologues were used. The concentration of the compounds in solution was determined colorimetrically with the use of Fast Blue B [12]. The membrane-disturbing activity of the homologues was determined turbidimetrically by following the rate of human erythrocyte haemolysis [11]. Briefly, to 5 ml of 0.14 M NaCl, 10 mM Tris/HCl (pH 7.3) solution, microlitre amounts of ethanolic solution of the homologues were added. After 60 s 25 μl of fresh washed erythrocyte suspension was injected and changes in transmittance at 600 nm were recorded for several minutes.

Fluorescence titration of Rhodamine 6G was performed by several additions of small aliquots of the homologues to 4 ml of 7.3 μM Rhodamine 6G solution in buffered 0.14 M NaCl (pH 7.3) equilibrated at desired temperature. The emission intensity was recorded continuously for several minutes after addition of the homologue solution to observe any time-dependence of fluorescence quenching. Fluorescence measurements were performed at excitation wavelength of 480 nm and emission wavelength of 550 nm.

RESULTS

Recorder tracings exemplifying absorbance of erythrocyte suspension in the presence of 10 μM 5-n-heptadec(en)ylresorcinol homologues (saturated, monoenoic and dienoic derivatives) are shown in Fig. 1. The homologue with saturated side chain did not cause any evident haemolysis within at least 2 min, which is in agreement with the previous observations on total alkylresorcinols [10, 11]. However, in the presence of this homologue permeability of cell membrane increased, as manifested by a progressive decrease in absorbance of erythrocyte suspension. Unsaturation of the aliphatic chain dramatically enhanced the membrane-disturbing properties of the homologue. Heptadecenyl-resorcinol at 10 μM concentration lysed erythrocytes within 2 min. The presence of two double bonds in the side

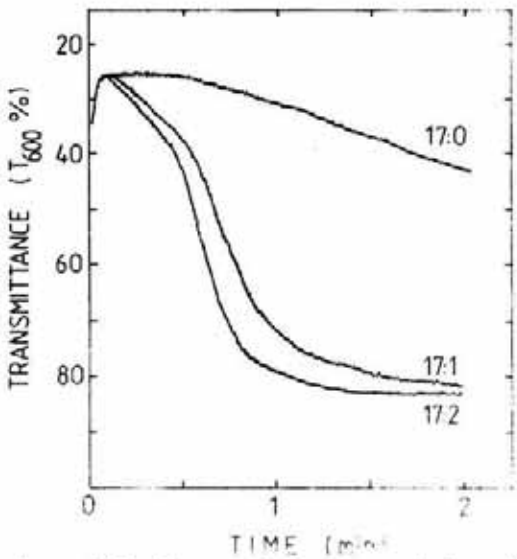

Fig. 1. Recorder tracings of the changes of absorbance of the red blood cell suspension in the presence of 10 μM 5-n-heptadec(en)ylresorcinols. The process was followed at 37°C in isotonic NaCl buffered with 10 mM Tris/HCl, pH 7.3. 17:0, 5-n-heptadecylresorcinol, 17:1, heptadecenylresorcinol; 17:2, heptadecdienylresorcinol

chain of this homologue resulted in a further increase of the rate of lysis.

The maximum rates of haemolysis by various saturated and enoic homologues isolated from rye grain are given in Fig. 2. For all homologues studied the lytic activity decreased with increasing number of carbon atoms. Irrespective of the number of double bonds in the chain, the shorter the aliphatic chain (C17) the more active was the homologue. These results indicate high effectiveness of the short-chain and enoic homologues in the mixture of native resorcinolic lipids in cereal grain, and are in good agreement with the findings of other authors on biological activities of urushiols [13].

Previous results indicating higher haemolytic activity of total saturated alkylresorcinol suspensions prepared at elevated temperature suggested the effect of temperature on the activity of resorcinolic lipids [10]. Therefore this effect on haemolytic activity of resorcinolic lipids on Rhodamine 6G fluorescence was examined. It is well known that incorporation of Rhodamine 6G into amphiphilic micelles results in quenching of its fluorescence. Thus, quenching reflects to some extent the number of micelles formed and, indirectly, informs on the temperature-dependent changes in concentration of monomers available for micelle formation. As shown in Fig. 3, the haemolytic activities at the same concentration of saturated and unsaturated homo-

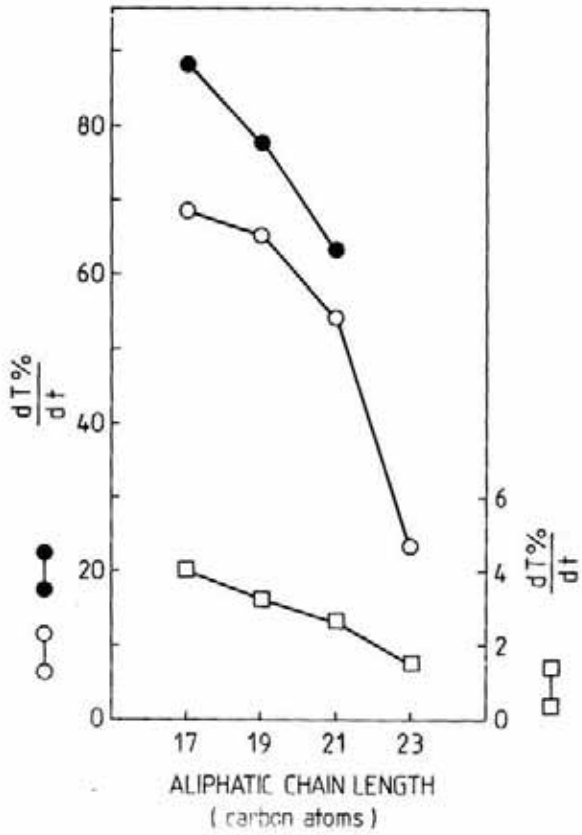

Fig. 2. Relationship between the rate of red blood cell haemolysis and the length and unsaturation of the aliphatic chain of homologue; Experimental conditions as in Fig. 1. □, saturated homologue; ○, monoenoic homologue; ●, dienoic homologue

logues were different at varying temperature. Monoenoic homologues were very active over a wide range of temperature; however, the temperature of the maximal activity was characteristic for each homologue. These temperatures were higher for the homologues with longer side chains. The short-chain homologues exhibited their maximal activities at temperatures below 35°C. Similarly, the short-chain homologues displayed their maximal effect on Rhodamine 6G fluorescence at the same temperatures (not shown). The haemolytic activities of saturated homologues were almost unchanged at temperatures between 20 and 35°C (Fig. 3). In order to assess the effect of elevated temperatures (40 - 70°C) on the activities of these homologues, the resorcinolic lipid-induced quenching of Rhodamine 6G fluorescence was examined. The results (Fig. 4) show that the ability of saturated homologues to quench fluorescence was also both temperature and chain length dependent. Raising of the temperature enhanced quenching of Rhodamine

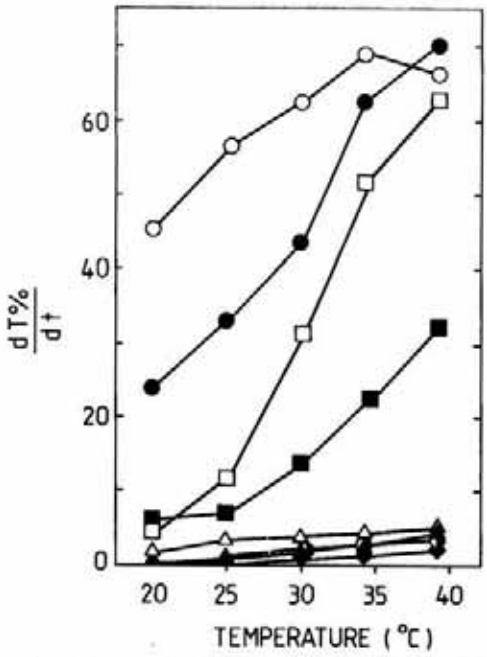

Fig. 3. Temperature dependence of the lytic activity of 10 μM 5-n-alk(en)-ylresorcinols expressed as the rates of the decrease of absorbance. △, C17:0, ○, C17:1; ▲, C19:0; ●, C19:1; ◑, C21:0; □, C21:1; ◆, C23:0; ■, C23:1

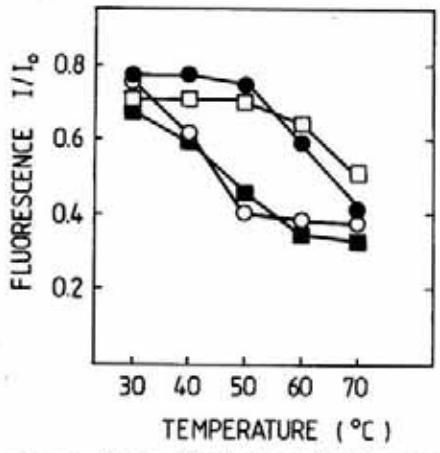

Fig. 4. Temperature dependence of the Rhodamine 6G fluorescence in the presence of 5-n-alkylresorcinols at the concentration of 50 μM. ○, C17:0;, ■, C19:0; ●, C21:0; □, C23:0. I_o, Fluorescence of Rhodamine 6G in absence of homologue; I, fluorescence of Rhodamine 6G in presence of homologue

6G fluorescence by short-chain homologues more than quenching by long-chain ones. 5-n-Tricosylresorcinol, the homologue with the longest side chain studied, did not exhibit its highest activity even at 70°C. On the other hand, all saturated homologues showed a time-dependent decrease of quenching, which indicated instability and secondary aggregation of resorcinolic micelles during incubation. Unsaturated homologues did not display such properties.

DISCUSSION

Since the work of Wieringa [2] resorcinolic lipids of rye grain were believed to be responsible for the antinutritive properties of rye when fed to farm animals: poultry and swine. Recently, Sedlet *et al.* [14] have demonstrated an evident growth-depressing and toxic effect of 5-n-pentadecylresorcinol on rats at concentration of 0.13% in fodder, i.e. equivalent to the content of resorcinolic lipids in rye grain. Moreover, the results of recent studies showed that alkenylresorcinols present in mango and philodendron induced allergies [15, 16].

The resorcinolic lipids of rye differ from those of wheat by average chain length and degree of unsaturation [17]. The amount of short-chain homologues (C13 - C17) is significantly higher in rye than in wheat. The present study indicates that at temperatures close to physiological ones for mammalian organisms, the short-chain unsaturated homologues exhibit high haemolytic activity at very low concentration, whereas the saturated homologues are significantly less active. The enhancement of the membrane-perturbing effect at higher temperature implies the importance of solubility of these amphiphiles and the role of monomer concentration in their interaction with biomembrane [17]. Usually, for active amphiphilic compounds the plots illustrating the relation between their structure and activity are biphasic with maximum which shows the homologue of the highest activity. The lack of the biphasic shape of the plot (Fig. 2) indicates low activity and suggests that active homologues should contain less than 17 carbon atoms in their chains. The presence of double bonds of phenolic lipids seems to be of key importance for their biomembrane-perturbing properties. Basing on the antimicrobial activities of 4-n-alkylresorcinols [18, 19] one can conclude that the most most active homologues may be those having 11 to 15 carbon atoms in their side chains. This can also explain the very high biological effect observed with other phenolic lipids, e.g. urushiols, highly unsaturated derivatives of C15 carbon atom homologues. The low activity of saturated homologues observed by us despite their hydrophilicity and affinity for biological membranes, [17, 20] cannot exclude the possibility that their effect might be stronger, e.g. when dissolved in fat in animal fodder.

In conclusion, the results obtained hitherto point to the importance of short-chain unsaturated homologues of 5-n-alk(en)ylresorcinols in the biological effect of cereal resorcinolic lipids on animal organism. Also this effect might be important in the human high-fiber diet as it is based on bran rich in resorcinolic lipids [21, 22].

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