Acta
Biochimica
Polonica

Vol. 42 No. 2/1995

241–246

QUARTERLY

# Interaction of alkylresorcinols with proteins*

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Received: 21 December, 1994

Key words: phenolic lipids, 5-alkylresorcinols, monolayer, lipid-protein interaction

**Alkylresorcinol homologs form stable monomolecular layers at air-water interface. Their interaction with proteins present in the subphase results in an increase of alkylresorcinol molecular packing in the extent dependent upon the protein studied. Strongest effects were observed for proteins with large hydrophobic regions, e.g. glycophorin or serum albumin. Interaction of proteins with alkylresorcinol monolayers is stronger than with phospholipids. A decrease and a shift of intrinsic protein fluorescence upon interaction with the compounds studied support their involvement in alteration of hydrophobic regions. For trypsin, 50% quenching was observed at the alkylresorcinol/trypsin ratio of 0.75. Concomitantly, an apparent inhibition of the enzymatic activity was noted. These results indicate that direct interaction of alkylresorcinols and modulation of enzymatic activities should be recognised as a significant part of the biological effect of these cereal bran components.**

Alkylresorcinols (AR)[1] belong to the class of secondary metabolites named non-isoprenoic phenolic lipids. The occurrence of these compounds has been demonstrated in a large number of species in the plant and bacterial kingdoms [1]. It is interesting that a broad spectrum of alkylresorcinol homologs was also found in many graminaceous plants, including cereals [2–5]. The biological role of these compounds, that are consumed in human and animal diets in average amounts of 40 mg/cereal serving, is not completely understood. The results obtained hitherto indicate that alkylresorcinols, due to their amphiphilic properties, interact with biological membranes inducing significant alterations of their structure and function (e.g. [6–14]). There are sets of data suggesting that direct interaction of alkylresorcinols with proteins could be also responsible for alteration of enzymatic activities and cellular physiological functions (e.g. [15–17]). The data presented in this work indicate that direct interaction of alkylresorcinols with proteins seems to play also an important role in the biological effect of these compounds.

## MATERIALS AND METHODS

*Alkylresorcinol homologs* were isolated chromatographically from rye grain acetone extracts according to the procedure published elsewhere [18]. Bovine serum albumin was from POCh (Gliwice, Poland) trypsin, chymotrypsin A and cytochrome *c* from Sigma (U.S.A.) and glycophorin was a kind gift from Dr. R.A. Demel (CBLE, The Netherlands).

*Monolayer experiments.* For the measurements of changes of monolayer surface tension NIMA TECHNOLOGY (U.K.) tensiometer employing Wilhelmy plate method, connected to personal computer and chart recorder was used. Monolayers were formed by spreading known microliter amounts of alkylresorcinol

*This work was supported by the State Committee for Scientific Research grant No. 4 1294 91 01
[1]Abbreviations: AR, alkylresorcinol(s)

homologs stock solutions in chloroform (1 mg/ml) onto the subphase (10 mM Tris/acetic acid, pH 7.0). The measurements were performed at 25°C, basically in the same way as described before [6]. For determination of the force-area curves a teflon trough (32 cm × 17 cm) with motor-driven movable bar was used as described earlier [6]. For alkylresorcinol-protein interaction studies the trough of 5 cm × 5 cm with a total subphase volume of 25 ml was used. The subphase was gently stirred during the experiments with a magnetic stirrer. Monolayers of individual alkylresorcinol homologs were formed by stepwise additions of chloroform solution until the desired initial surface pressure was reached. After 5 min of equilibration an aliquot of protein solution was injected into the subphase (final protein concentration of 0.5 µg/ml) and changes of surface pressure were recorded for 45 min. Each experiment was repeated 3–5 times and the curves presented in Figures represent the average values.

***Spectrofluorimetric method.*** Changes of intrinsic fluorescence spectra of proteins were followed in SFM-25 (KONTRON, Italy) spectrofluorimeter. The excitation was set at 280 nm and the spectra were taken in the range 300–370 nm. The measurements were made at 25°C. Briefly, 3 ml of buffered protein solution was put into cuvette and left for 5 min under constant stirring until temperature was equilibrated, then an aliquot of alkylresorcinol ethanolic solution was added and thoroughly mixed. The spectrum was recorded after 5 min incubation. For corrections of the trypsin-alkylresorcinol spectra for sample turbidity and alkylresorcinol fluorescence the spectra of the control samples containing identical concentrations of alkylresorcinol in the buffer were recorded and then subtracted (hardware baseline correction procedure) from the spectra of the samples.

The effect of alkylresorcinols upon proteolytic activity of trypsin was determined at pH 7.6 using casein as a substrate by the method of Kunitz [19]. Controls did not contain alkylresorcinols but identical amounts of ethanol.

## RESULTS AND DISCUSSION

Figure 1 shows the force-area curves of alkylresorcinol homologs. The area occupied by the homolog studied depends on the length of its aliphatic chain. At surface pressure of 15 mN/m a short chain homolog (C15) occupies the area of 0.38 $nm^2$/molecule whereas C19 and C23 homologs of 0.32 and 0.35 $nm^2$/molecule, respectively. At maximal condensation of alkylresorcinol molecules (at 40 mN/m) limiting areas between 0.22 $nm^2$ for the C25 homolog and 0.27 $nm^2$ for C15 homolog were observed. These results are in good agreement with the data obtained for 4-n-hexadecylresorcinol by Adam [20] and 5-n-heptadecenylresorcinol (irisresorcinol) by Kato *et al.* [21]. These authors also showed the temperature dependence of the limiting area occupied by alkylresorcinol molecules. At 5°C, saturated-chain homologs showed condensation to solid films in which flat dihydroxybenzene rings were oriented vertically to the aqueous surface. Raising of temperature or the presence of *cis*-unsaturation in the middle of the side chain prevented the monolayer from solidifying at low temperatures and resulted in a significant increase of the area occupied by unsaturated homologs [6, 21].

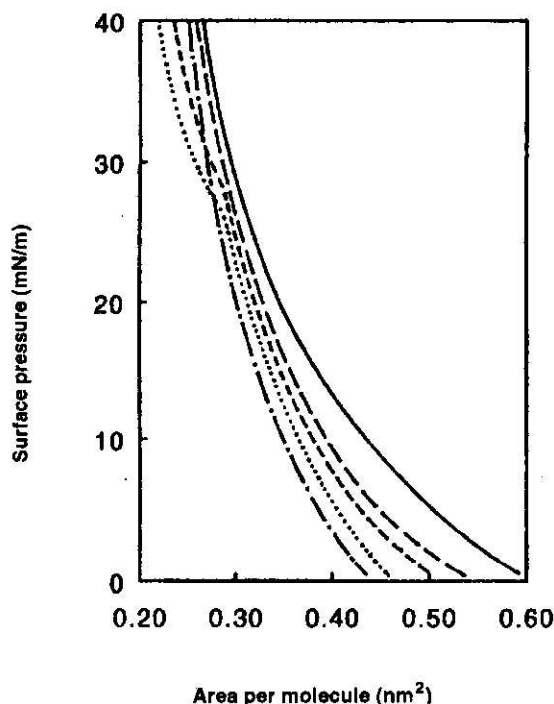


*Fig. 1. Force-area curves for resorcinolic lipid homologs.*
———, 5-n-Pentadecylresorcinol; – – –, 5-n-heptadecylresorcinol; –•–•–, 5-n-nonadecylresorcinol; - - -, 5-n-tricosylresorcinol; • • •, 5-n-pentacosylresorcinol. Measurements were done at 25°C, subphase was of pH 7.0 buffered with Tris/acetic acid.

Introduction of proteins into the subphase underneath the alkylresorcinol monolayer the initial surface pressure of which was set at 15 mN/m, resulted in a gradual increase of the monolayer molecular packing (Fig. 2). The extent of this increase was dependent upon the protein tested. The strongest effect was observed for glycophorin, and somewhat lower for serum albumin and cytochrome *c*, proteins that are known to posses large hydrophobic regions interacting with lipids or other amphiphilic molecules. The intensity of the interaction of alkylresorcinol homologs with protein was higher than that shown by such phospholipids as phosphatidylglycerol and phosphatidylcholine (Fig. 3). The increase of the monolayer surface pressure during the interaction with proteins was dependent on initial packing of the monolayer (Table 1). The highest penetration (expressed as the increase of the surface pressure, $\Delta\pi$) was observed for low initial surface pressures of the monolayer (over 170% increase after 50 min incubation). When the initial surface pressure was set at 30 mN/m an only 3% increase of the surface pressure resulting from interaction with protein was observed. These observations indicate that protein molecules during their interaction penetrate the alkylresorcinol monolayer resulting in its condensation even at an already high level of molecular packing. When the surface pressure of the monolayer interacting with proteins was kept on a constant level by a gradual

Table 1

*Increase of pentadecylresorcinol monolayer surface pressure after 50 min interaction with trypsin for various initial monolayer packing.*

The data are means of 3–5 experiments ± S.E. Experimental details as in Fig. 2.

| Initial surface pressure $\pi$ (mN/m) | Increase of surface pressure $\Delta\pi$ (mN/m) |
|---|---|
| 10 | 17 ± 1.9 |
| 15 | 14 ± 2.1 |
| 20 | 10 ± 1.3 |
| 25 | 6 ± 0.8 |
| 30 | 3 ± 0.6 |

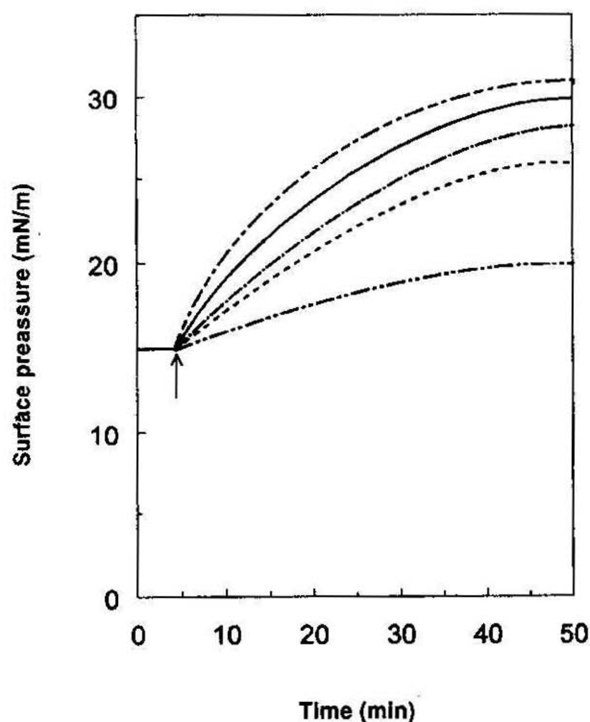


*Fig. 2. Increase of pentadecylresorcinol monolayer surface pressure in the presence of 0.5 μg protein in the subphase, pH 7.0, 25°C.*

Initial surface pressure was adjusted to 15 mN/m. — - -, Glycophorin; ———, bovine serum albumin; – • – • –, cytochrome *c*; - - -, trypsin; — - - —, chymotrypsin A.

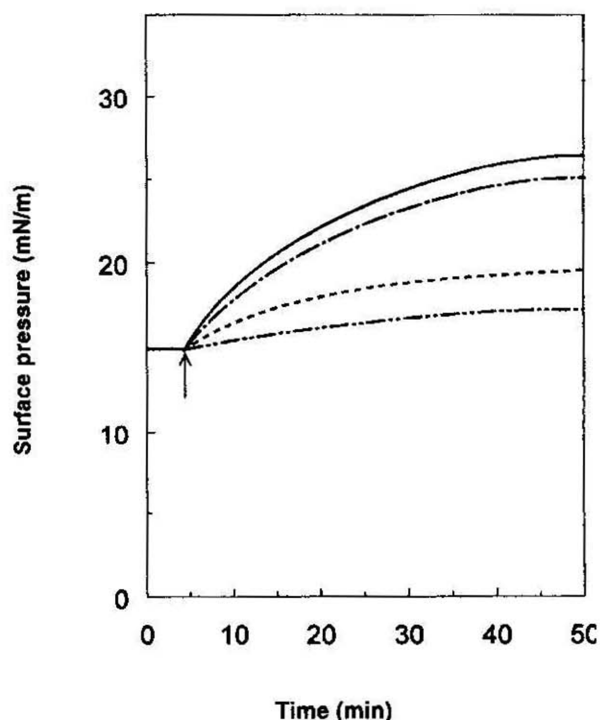


*Fig. 3. Increase of surface pressure during interaction of alkylresorcinol and phospholipid monolayer with trypsin (0.5 μg in the subphase), pH 7.0, 25°C.*

Initial surface pressure was adjusted to 15 mN/m. ———, 5-n-Pentadecylresorcinol; — - —, 5-n-pentacosylresorcinol; - - -, phosphatidylglycerol; — - - —, phosphatidylcholine.

increase of monolayer surface, it was possible to determine the maximal increase of the area occupied by an alkylresorcinol molecule after its interaction with protein. After 30 min incubation with protein the area per molecule of the homologs studied was increased by 0.096–0.063 $nm^2$ (Table 2). This increase was higher for short-chain homologs than for homologs with longer chains. In mixed phosphatidylcholine-alkylresorcinol monolayers the interaction with protein was dependent on the amount of alkylresorcinol present in the mixtures (Fig. 4). This suggests that alkylresorcinols incorporated into a biological membrane might interact specifically with some membranous proteins.

In opposition to the phospholipids studied, binding of proteins to alkylresorcinols was slightly pH-dependent and was more accentuated at neutral pH (Fig. 5). Similarly a weak effect of the ionic strength of the subphase upon interaction (Fig. 6) together with stronger binding of such proteins as glycophorin or serum albumin suggest that alkylresorcinols interact preferentially with hydrophobic domains present in protein molecules. A decrease and shift of trypsin intrinsic fluorescence maximum observed after incubation of this protein with alkylresorcinol (Figs. 7 and 8) support this supposition. It is worth mentioning that the interaction was strong as 50% quenching was

Table 2

*Increase of molecular area occupied by a single alkylresorcinol molecule at the air-water interface after interaction with trypsin.*

The data are means of 3–5 experiments ± S.E.

| Resorcinolic lipid | Increase of molecular area $\Delta$ $nm^2$ ($\times 10^{-2}$) |
|---|---|
| 5-n-Pentadecylresorcinol | 9.6 ± 1.1 |
| 5-n-Heptadecylresorcinol | 9.2 ± 0.8 |
| 5-n-Nonadecylresorcinol | 9.0 ± 0.5 |
| 5-n-Heneicosylresorcinol | 7.2 ± 0.4 |
| 5-n-Pentacosylresorcinol | 6.3 ± 0.5 |

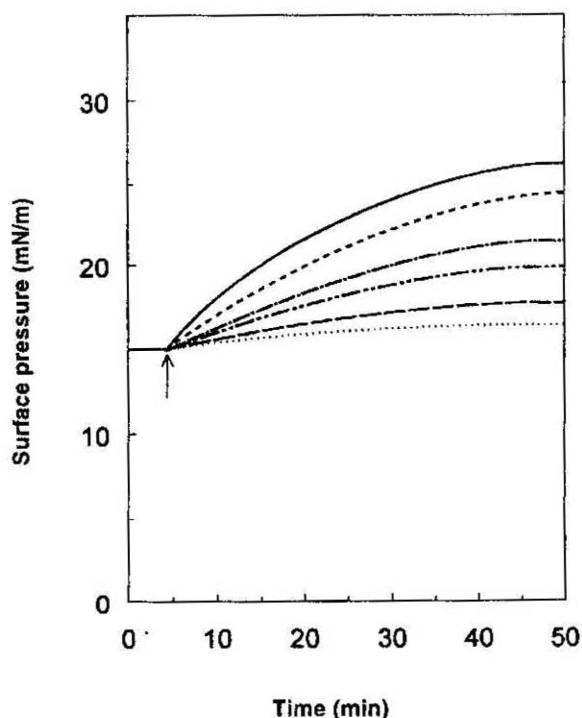


*Fig. 4. Increase of surface pressure during interaction of mixed pentadecylresorcinol-phosphatidylcholine monolayer with trypsin (0.5 µg in the subphase), pH 7.0, 25°C.*

Initial surface pressure was adjusted to 15 mN/m. Alkylresorcinol-phospholipid: ———, 1:0; - - - , 2:1; – • –, 1:1; — - - —, 1:5; — — —, 1:10; • • •, 0:1.

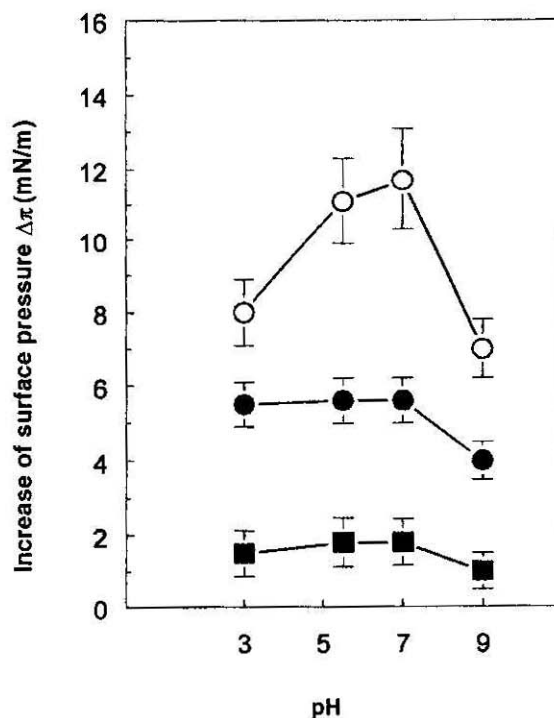


*Fig. 5. pH-Dependence of the surface pressure increase of alkylresorcinol and phospholipid monolayers upon interaction with trypsin.*

Protein amount in subphase — 0.5 µg. Initial surface pressure — 15 mN/m. Experiments were done at 25°C. ○, 5-n-Pentadecylresorcinol; ●, phosphatidylglycerol; ■, phosphatidylcholine.

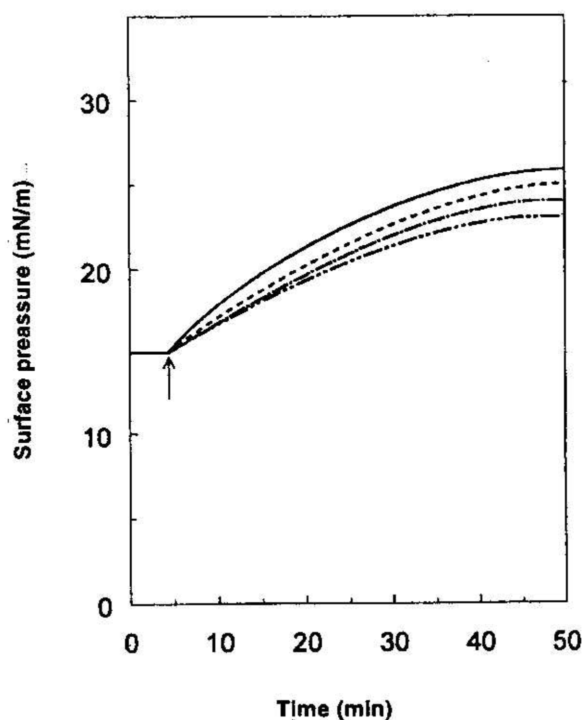


*Fig. 6. Effect of ionic strength of the subphase on the surface pressure increase during incubation of pentadecylresorcinol monolayer with the subphase containing 0.5 µg of trypsin.*
Initial surface pressure — 15 mN/m. Measurements were done at 25°C. Ionic strength of the subphase was adjusted with NaCl: ———, 10 and 50 mM; - - - , 100 mM; — - —, 200 mM; — - - —, 300 mM.

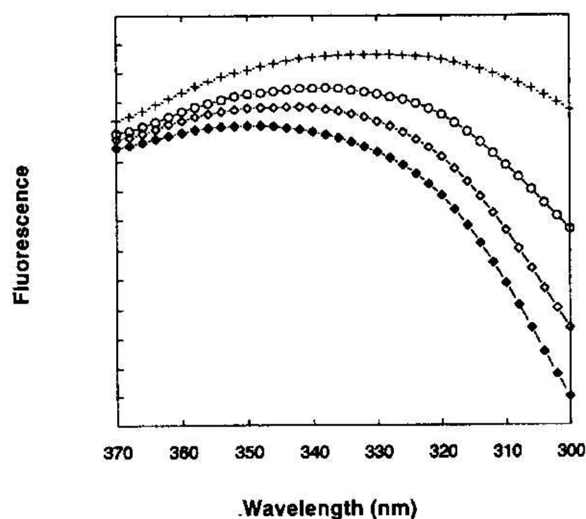


*Fig. 7. Alteration of trypsin intrinsic fluorescence spectrum in the range 300–370 nm during incubation with pentadecylresorcinol (C15AR).*
Trypsin 40 µg/ml, 25°C, pH 7.6. +, Trypsin alone; O, 25 µM C15AR; ◊, 50 µM C15AR; ■, 100 µM C15AR.

observed at alkylresorcinol/trypsin ratio of about 0.75. A similar strong effect of alkylresorcinol upon intrinsic protein fluorescence has been also observed for erythrocyte spectrin [22] and photosystems [23].

Direct interaction of proteins with alkylresorcinol affects not only its structure but also its biological activity. Almost complete inhibition of apparent trypsin activity was observed when this enzyme was incubated with alkylresorcinol (0.25 mM, Fig. 9) with the $IC_{50}$ value of 50 µM. A similar inhibition of trypsin by direct binding of amphiphilic molecules has been observed for potassium salts of long-chain fatty acids [24]. The capacity of alkylresorcinols for direct binding and alteration of biological activ-

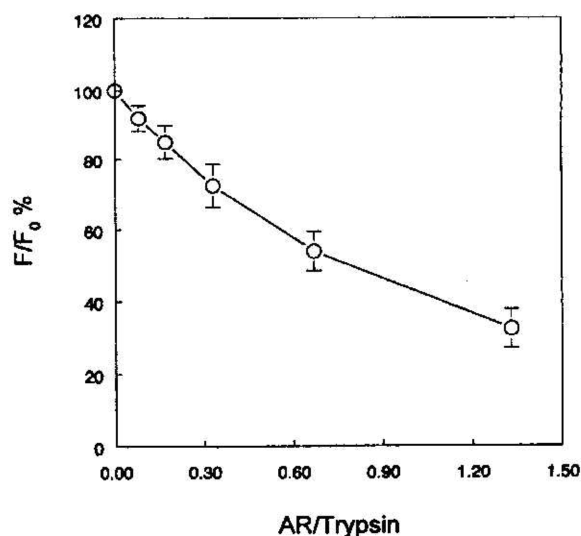


*Fig. 8. Decrease of the intrinsic trypsin fluorescence at 332 nm in its mixtures with pentadecylresorcinol.*
Conditions similar as in Fig. 7.

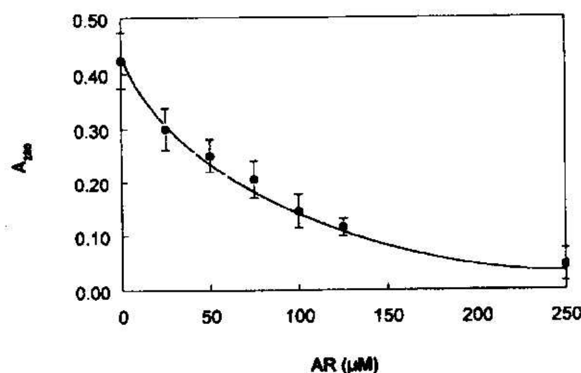


*Fig. 9. The effect of 5-n-heptadecylresorcinol on hydrolysis of casein with trypsin.*
Experimental details as in Materials and Methods.

ity of proteins, not only those membrane-bound, could explain the apparent inhibition of various enzymes [15–17] by these and related amphiphilic compounds of natural origin. Direct interaction of alkylresorcinols with enzymatic proteins and modulation of their activity should therefore be recognised as a significant part of the biological effect of these natural compounds.

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