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Reduction of Phytotoxicity of Nonionic Tensides by Cyclodextrins

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Abstract. The effect of nonionic tenside nonylphenylnonylglycolate and its α-, β-, γ-cyclodextrin, 2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) and 2,3,6-tri-*O*-methyl-β-cyclodextrin (TRIMEB) complexes was tested on the potassium influx of wheat seedling roots. Tenside alone inhibited strongly the potassium influx. This noxious effect was alleviated by cyclodextrins. The alleviating effect increased with increasing cyclodextrin: tenside molar ratio, in the order: DIMEB > βCD > γCD > αCD $\approx$ TRIMEB.

Key words: Tenside, phytotoxicity reduction, cyclodextrin.

1. Introduction

Nonionic tensides are widely used for many purposes, as e.g. in pesticide formulations. The concomitant toxic phenomena are also varied. Although they are easily biodegradable they nevertheless decrease the performance of activated sludge systems [1, 2], damage pine trees by increasing the uptake of sodium ions [3, 4, 5], and by dissolving the surface wax layer of pine needles [6]. Tensides exert an inhibitory effect on the growth of *Spirodela polyrrhiza* (L.) Schleiden [7] and they are lethal to *Medaka Oryzias latipes* [8].

Their biological activity is based on their interaction with membrane phospholipids [8, 9] resulting in increased excretion of glutamate and in enhanced uptake of various substances through stomata. Besides the membrane damaging effect tensides influence markedly the activity of enzymes [10] and enhance the biological activity of insecticides [11] by improving their solubility.

Because cyclodextrins form inclusion complexes with tensides [12, 13, 14, 15] it was expected that they could modify their biological activity. The prognosticated use of cyclodextrins in pesticides formulations [16, 17] eventually together with nonionic tensides, rendered necessary the study of the damaging effect of some nonionic

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tensides on the membrane function (K^+ influx) in wheat seedling roots and to assess the preventative effect of cyclodextrins.

2. Material and Methods

Nonylphenylethyleneoxide polymers containing on average 4, 9 and 30 ethyleneoxide groups per molecule (further T_4, T_9 and T_{30}) were purchased from Hoechst (FRG) and were used without further purification. The cyclodextrins: alpha-cyclodextrin (αCD), beta-cyclodextrin (βCD), gamma-cyclodextrin (γCD), 2,6-di-*O*-methyl-beta-cyclodextrin (DIMEB) and 2,3,6-tri-*O*-methyl-beta-cyclodextrin (TRIMEB) were produced by Chinoin Pharmaceutical Works (Hungary) and were chromatographically pure.

Excised roots of 4-day old seedlings of winter wheat (*Triticum aestivum* L.cv. GK Szeged), grown in darkness at ambient temperature, under low salt concentration (0.5 mM $CaSO_4$) were used for the potassium influx experiments. Fragments of roots (0.8 g) were allowed to absorb potassium from 100 cm^3 of 0.1 mM KCl (^{86}Rb) + 0.5 mM $CaSO_4$ + 50 mg/dm^3 tenside for 2 hours then the non-absorbed ions were removed by rinsing the roots with 100 cm^3 of non-labelled 0.1 mM KCl + 0.5 mM $CaSO_4$ solution. The radioactivity of roots was measured directly in a scintillation counter and influx values (μM potassium per g fresh weight/2 hours) were calculated. Cyclodextrins were added to the most active T_9 tenside in the molar ratio 5:1, 1:1, 0.5:1, 0.2:1 and 0.1:1 cyclodextrin:tenside. Due to its limited solubility in water the effect of βCD was not tested at the highest molar ratio. The activity of T_9 was checked also

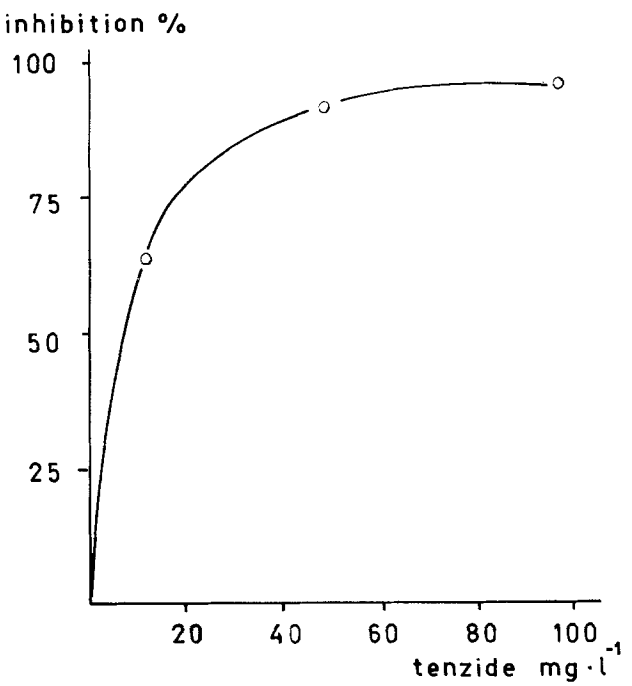

Fig. 1. Effect of nonylphenyl-nonylglycolate on the potassium influx of wheat seedling roots.

at 10 and 100 mg/dm^3 concentrations. The % decrease of potassium influx was considered as the measure of biological activity.

Each experiment was made in triplicate, corresponding to three separate experiments.

3. Results and Discussion

The dependence of the inhibition of K^+ influx on the concentration of T_9 is shown in Figure 1. The tenside inhibits the K^+ influx very strongly even at 10 mg/dm^3 concentration, the inhibition is nearly complete at 50 ppm. The probable explanation is that the tenside damages the membrane structure around the ion channels disrupting them partially or completely. The fact that this effect was the highest for T_9 (94,4% inhibition) and lower for T_4 (70,7%) and T_{30} (25,0%) indicates that the noxious effect of tensides depends considerably on the length of the hydrophilic ethyleneoxide chain of the tensides. These observations are in good agreement with other results [18, 19, 20].

The alleviating effect of cyclodextrins is summarized in Figures 2 and 3. Each

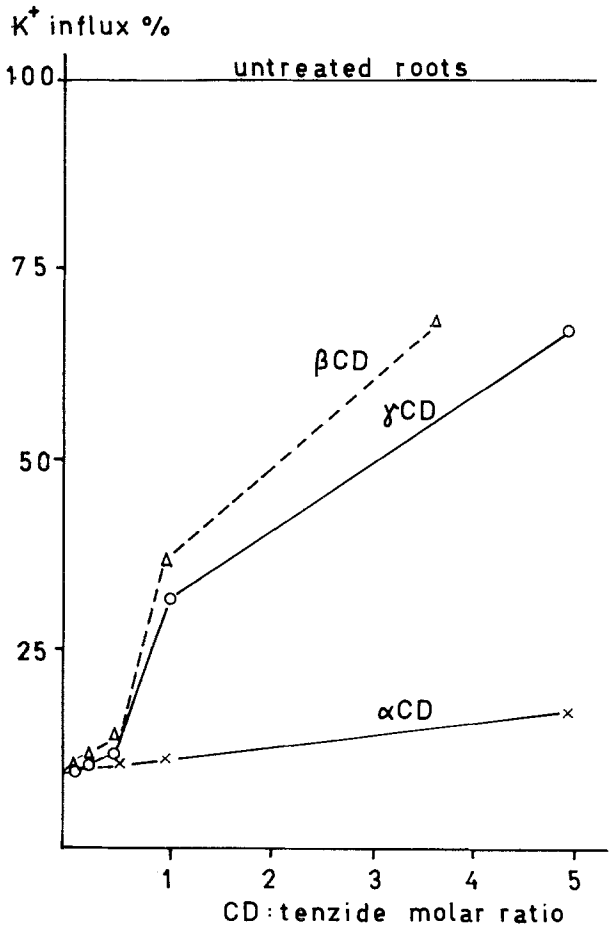

Fig. 2. Effect of nonylphenyl-nonylglycolate and its α-, β-, and γ- cyclodextrin complexes on the inhibition of K^+ influx of wheat seedling roots.

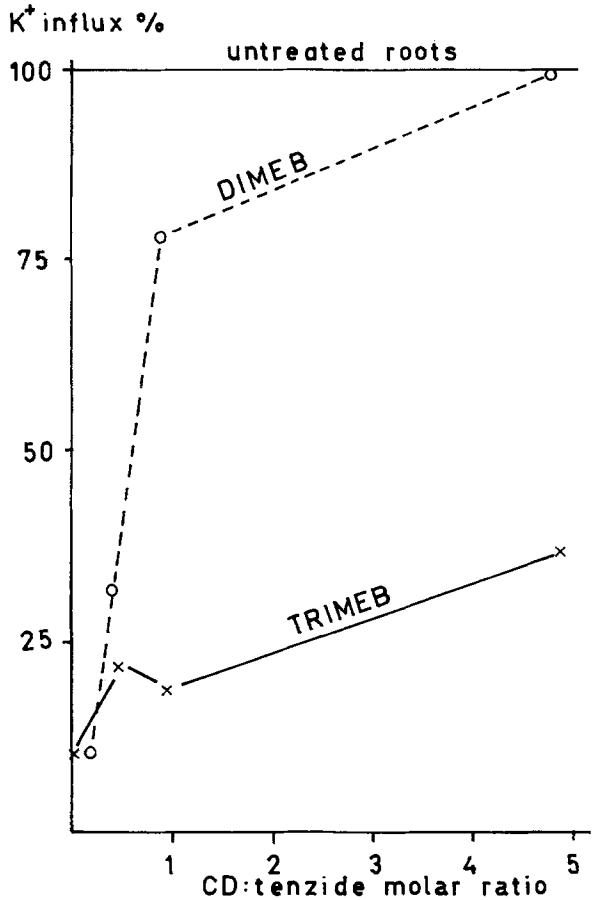

Fig. 3. Effect of nonylphenyl-nonylglycolate and its DIMEB and TRIMEB complexes on the inhibition of K^+ influx of wheat seedling roots.

tenside reduces the inhibitory effect of T_9. This result is probably due to the fact that the cyclodextrins form inclusion complexes with the tensides, lowering the concentration of free tenside molecules responsible for the biological effect. This observation also indicates that the tenside-cyclodextrin complexes exert a negligible effect on the potassium influx of wheat seedling roots. Nonionic tensides also form complexes with membrane phospholipids [21], however our data suggest that the interactive forces between cyclodextrins and T_9 are higher than those between membrane phospholipids and T_9.

The alleviating effect of cyclodextrins differs considerably from each other. Among the nonmethylated derivatives βCD showed the highest, αCD the lowest preventive effect, the difference between the activity of βCD and γCD was lower than that between γCD and αCD (Figure 1). It means that T_9 forms inclusion complexes of commensurable stability with βCD and γCD but only a less stable one with the αCD. The cavity of αCD is probably not large enough to accommodate adequately the nonylphenyl hydrophobic moiety of T_9.

As the interaction between the nonmethylated cyclodextrins and phospholipids is

rather weak and does not influence significantly the membrane permeability [22], the above data reflect entirely the effect of inclusion complex formation on the biological activity of T_9.

The alleviating effects of methylated beta-cyclodextrins deviate markedly from that of unmethylated βCD (Figure 3), the effect of DIMEB is higher, that of TRIMEB is lower than the effect of βCD. It suggests that their complex forming capacities are different. Methylation decreases the accessibility of the cyclodextrin cavity for T_9, moreover the increased lipophilicity of methylated cyclodextrins [23] favours the micelle formation like hydrophobic-hydrophobic interactions with T_9. In the case of DIMEB the favourable effect of hydrophobic-hydrophobic interactions predominates, the association with T_9 is stronger than with βCD. In the case of TRIMEB the complex formation is weaker than with βCD probably the steric hindrance caused by the numerous methyl groups overshadows the favourable effect of the outer sphere hydrophobic interactions.

The overal effectivity order of cyclodextrin derivatives was DIMEB$>\beta$CD$>\gamma$CD$>\alpha$CD$\approx$TRIMEB.

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