Relationship Between Hydrophobicity Parameters and the Strength and Selectivity of Phytotoxicity of Sulfosuccinic Acid Esters

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Abstract. The strength and selectivity of the phytotoxicity of 11 sulfosuccinic acid ester surfactants were determined on the leaves of *Tradescantia bicolor*, and the data were evaluated by multivariate mathematical-statistical methods. Spectral mapping technique combined with stepwise regression analysis indicated that both the strength and the selectivity of the effect depend significantly on the specific hydrophobic surface area of the anionic surfactants determined in the presence of ions. The significant relationship between this hydrophobicity parameter and phytotoxic activity suggests the involvement of apolar (hydrophobic) forces in the plant – surfactant interaction. It was assumed that the apolar alkyl chains of the surfactants may insert in the hydrophobic part of the phospholipid bilayers causing membrane disorder and malfunction.

Key words: Phytotoxicity — Anionic surfactants — *Tradescantia bicolor*

Introduction

Due to their favorable physicochemical characteristics anionic surfactants are frequently used in pharmaceutical preparations (Ashton et al. 1992; Wells and Parrott 1992), pesticide formulations (Seaman 1990; Sanchez-Ferrer et al. 1993) and cosmetics. However, anionic surfactants also show significant human and environmental toxic effects. The eye irritation potential of anionic surfactants has been reported many times (Grant et al. 1992; Roguet et al. 1992). Bioaccumulated alkylbenzenesulfonate inhibits alkaline and acid phosphatase in liposomes (Bragadin et al. 1996) and increases the mortality of juvenile *Folsomia fimetaria* in soil (Holmstrup and

Krogh 1996). Sodium dodecyl sulfate damaged estuarine crustaceans (Whiting et al. 1996) and inhibited the growth of *Lemna minor* L (Dirilgen and Ince 1995). The toxicity of anionic surfactants has been shown to depend both on the length of the apolar alkyl chain and the character of the hydrophobic head group (Versteeg et al. 1997).

The impact on anionic surfactants on the environment is ambivalent. They have beneficial effects including facilitation of the removal of pesticides (Iglesias-Jimenez et al. 1997), lead (Huang et al. 1997) and trinitrotoluol from soil (Taha et al. 1997). They enhance biodegradation of hydrocarbons in soil (Walter et al. 1997) and promote adsorption of phenanthrene on alumina (Sun and Jaffe 1996).

As anionic surfactants are released into the environment in high quantities they are potential environmental pollutants (Shimp and Larson 1996) accumulating in rivers (Souza and Wasserman 1996) and lakes (Muramoto et al. 1996) and increasing the solubility of hydrophobic organic contaminants in ground water (Di Vicenzo and Dentel 1996).

The objectives of the present work were to determine the phytotoxicity of some sulfosuccinic acid ester anionic surfactants using the leaves of *Tradescantia bicolor* as model materials, and the elucidation of the relationships between phytotoxicity and the physicochemical parameters of sulfosuccinic acid esters.

Materials and Methods

The anionic surfactants tested included di-*n*-butyl- (compound I), di-*iso*-butyl- (compound II), di-*n*-pentyl- (compound III), di-*n*-hexyl- (compound IV), di-cyclo-hexyl- (compound V), di-(2-ethyl- hexyl)- (compound VI), di-*n*-octyl- (compound VII), di-*iso*-octyl- (compound VIII), *iso*-decyl- (compound IX), *n*-dodecyl- (compound X), and di-*n*-tridecyl- (compound XI) esters of sulfosuccinic acid. Stock solutions (0.2 mol/l) of surfactants were prepared in methanol-water mixture (1:1 v/v) and stored at below −25 °C.

Tradescantia bicolor plants were grown in soil:perlite (2:1 w/w, one plant per 0.75 kg pot) and cultivated in a greenhouse. Solutions of surfactants ranging from 40 mmol/l to 0.16 mmol/l concentrations were infiltrated into leaf sheets of plants (about 10 × 10 mm areas). Infiltration with distilled water was used as control. Each experiment was run in triplicate. The phytotoxic effect was checked after 24 and 48 hours and evaluated by 3 point (0 = no effect, the infiltrated area is similar to that infiltrated with distilled water; 1 = no necrosis induced, the infiltrated area became yellow; 2 = necrosis induced in the infiltrated area). *T. bicolor* was selected as the model plant because variations in the responses within individual plants is negligible. Furthermore, the injection of leaves is easy to carry out due to their structure.

The strength and selectivity of the phytotoxic effect of anionic surfactants were separated by the spectral mapping technique (SMP) (Lewi 1989). SMP was developed for quantitative structure-activity relationship calculations and was successfully applied for the elucidation of the relationship between chromatographic

retention data and biological activity of nonionic surfactants (Oros et al. 1997), and for the characterization of chromatographic stationary phases (Hamoir et al. 1994). The spectral mapping technique divides the information into two matrices using the logarithm of the concentration values. The first one is a vector containing so-called potency values proportional to the overall effect, i.e. it is a quantitative measure of the effect. The second matrix (selectivity map) contains the information related to the spectrum of activity, i.e., the qualitative characteristics of the effect. SMP was applied separately to the phytotoxicity data obtained after 24 and 48 hours. As the evaluation of the multidimensional selectivity map is difficult the dimensionality of the spectral maps was reduced to two by the nonlinear mapping technique (Sammon 1969). The iteration of the two-dimensional nonlinear maps was carried out to the point when the difference between the last two iterations was smaller than 10^{-8}.

In order to compare the phytotoxic effects after 24 and 48 hours, linear correlations were calculated between the corresponding potency values, and the first and the second coordinates of the two-dimensional nonlinear selectivity map. The relationship between the strength and selectivity of the phytotoxic effect, lipophilicity, and specific hydrophobic surface area of surfactants was analyzed by stepwise regression analysis (Mager 1982). In the traditional multilinear regression analysis, the presence of independent variables that have no significant influence on the dependent variable (strength and selectivity of the phytotoxic effect of surfactants) lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, stepwise regression analysis automatically eliminates the insignificant independent variables from the selected equation, increasing in this manner the information power of the calculation. The dependent variables included the potency values, the first and second coordinates of the two-dimensional nonlinear selectivity map. The independent variables were respectively, the lipophilicity (R_M values) and specific hydrophobic surface area (b values) of sulfosuccinic acid esters determined in ion-free environment and in the presence of 0.1 mol/l NaCl, KCl, MgCl$_2$ and CaCl$_2$. The hydrophobicity parameters listed above were taken from Cserháti et al. (1997). The number of accepted independent variables was not limited and the acceptance limit was set to the 95% significance level.

In order to compare the results obtained by using the spectral mapping technique with those obtained by traditional quantitative structure-activity relationship (QSAR) methods linear correlations were calculated between the means of the inhibitory effects of the individual surfactants measured after 24 and 48 h and the hydrophobicity parameters listed above.

The calculations were carried out on an IBM AT computer, spectral mapping and nonlinear mapping software were developed by Dr. Barna Bordás, Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary. Software for stepwise regression analysis was developed by CompuDrug Ltd, Budapest, Hungary.

Results and Discussion

The phytotoxicity data obtained after 24 and 48 h exposure are compiled in Tables 1 and 2, respectively. As 40 mmol/l concentration of sulfosuccinic acid ester surfactants always caused necrosis (degree of damage = 2) and 0.16 mmol/l was always ineffective (degree of damage = 0) these data were omitted from the Tables.

Table 1. Phytoxic effects of sulfocuccinic acid esters on the leaves of *Tradescantia bicolor*. Exposure time 24 h. For the designation of surfactants, see Materials and Methods.

Surfactant	Tenside concentration (mmol/l)						
	20	10	5	2.5	1.25	0.625	0.3125
I	0	0	0	0	0	0	0
II	0	0	0	0	0	0	0
III	2	1	1	0	0	0	0
IV	2	2	1	1	1	0	0
V	1	0	0	0	0	0	0
VI	2	2	2	2	1	0	0
VII	2	2	2	1	1	0	0
VIII	2	1	1	1	0	0	0
IX	2	2	1	0	0	0	0
X	2	2	1	0	0	0	0
XI	1	0	0	0	0	0	0

0 = no effect, the infiltrated area is similar to that infiltrated with distilled water; 1 = no necrosis induced, the infiltrated area became yellow; 2 = necrosis in the infiltrated area.

Table 2. Phytoxic effects of sulfocuccinic acid esters on the leaves of *Tradescantia bicolor*. Exposure time 48 h. For the designation of surfactants, see Materials and Methods.

Surfactant	Tenside concentration (mmol/l)						
	20	10	5	2.5	1.25	0.625	0.3125
I	1	0	0	0	0	0	0
II	1	0	0	0	0	0	0
III	2	2	2	2	0	0	0
IV	2	2	2	2	1	1	1
V	2	2	2	1	0	0	0
VI	2	2	2	2	2	1	1
VII	2	2	2	2	2	1	1
VIII	2	2	2	2	1	1	0
IX	2	2	1	1	0	0	0
X	2	2	1	1	0	0	0
XI	2	2	1	0	0	0	0

0 = no effect, the infiltrated area is similar to that infiltrated with distilled water; 1 = no necrosis induced, the infiltrated area became yellow; 2 = necrosis in the infiltrated area.

The data in Tables 1 and 2 indicate that the phytotoxic effect intensifies with the increasing exposure time. It can further be established that the activity of sulfosuccinic acid esters shows wide variations: derivatives with shorter hydrophobic alkyl chains are less effective; surfactants containing cycloalkyl substituents are less active than derivatives with a linear alkyl chain containing the same number of carbon atoms. Also, the branching of the alkyl chain influences the activity. Both cyclisation and branching decrease the lipophilicity of the molecules, and lipophilicity can be assumed to have considerable effect on the phytotoxicity of sulfosuccinic acid esters.

The strengths of the phytotoxic effect (potency values) of anionic surfactants after 24 and 48 h exposure times are shown in Table 3. The results entirely support the previous qualitative conclusions drawn from from the data in Tables 1 and 2. The length, branching and cyclisation of the apolar alkyl substituents equally have a significant effect on the phytotoxicity proving again the considerable role of hydrophobic interactions in the phytotoxic activity of anionic surfactants. The two-dimensional selectivity maps of surfactants after 24 and 48 h exposure are shown in Figs. 1 and 2, respectively. The distribution of the surfactants on the maps differs indicating that the exposure time not only influences the strength of the phytotoxic effect but also its selectivity. Surfactants do not form well defined clusters on the map according to the character of the alkyl substituents. This finding indicates that the length, branching and cyclisation of the alkyl substituents have a similar effect on selectivity, and the effects observed are a result of the overlapping or complementarity of the various structural characteristics.

Significant linear correlation was found between the strengths of the phytotoxic effect after 24 and 48 h exposure, the coefficient of regression being 0.8896

Table 3. Strength (potency) of the phytotoxic effects of sulfosuccinic acid esters towards the leaves of *Tradescantia bicolor*. Exposure times 24 and 48 h. Results of spectral mapping. For the designation of surfactants, see Materials and Methods.

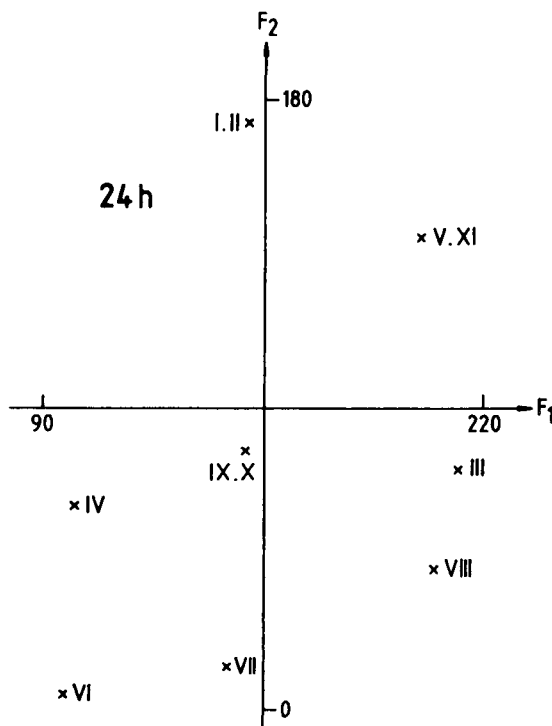| Surfactants	Potency values (arbitrary units)	
	24 h	48 h
I	0.00	0.38
II	0.00	0.38
III	1.51	3.02
IV	2.65	4.16
V	0.38	2.65
VI	3.40	4.54
VII	3.02	4.54
VIII	1.89	3.78
IX	1.89	2.27
X	1.89	2.27
XI	0.38	1.89

Figure 1. Similarities and dissimilarities between the selectivity of the phytotoxic effects of sulfosuccinic acid esters. Exposure time 24 h. Two-dimensional nonlinear selectivity map. Iterations: 225; maximal error: 5.60×10^{-2}. For the designation of surfactants, see Materials and Methods.

(significance level higher than 95%). This suggests that the strength of the phytotoxic effect is similar but not identical after 24 and 48 h exposure. No significant relationship was found between the first and the second coordinate of the two-dimensional nonlinear selectivity maps after 24 and 48 h exposure, proving again the marked role of exposure time in the selectivity of the phytotoxic effect (the individual anionic surfactants probably need different times to damage the leaves of *Tradescantia bicolor*).

The parameters of the multilinear relationships between the strength of the phytotoxic effect and lipophilicity and specific hydrophobic surface area of surfactants are summarized in Table 4. The hydrophobic surface area of surfactants determined in the presence of 0.1 mol/l KCl and CaCl$_2$ significantly influences the strength of the phytotoxic effect, the significance level being in both cases higher than 99% (see F values). The change of the specific hydrophobic surface area explains 75–81% of the change of the biological efficiency (see r^2 values). The path coefficients (B_i % values) indicate that the effect of both area values are com-

Table 4. Parameters of the linear relationships between the strength of the phytotoxic effects of anionic surfactants and their specific hydrophobic surface area determined in the presence of 0.1 mol/l KCl (b_{KCl}) and $CaCl_2$ (b_{CaCl_2}). ($n = 11$)
  I. Potency $= A + B_1 \cdot (b_{KCl}) + B_2 \cdot (b_{CaCl_2})$ Exposure time 24 h
 II. Potency $= A + B_1 \cdot (b_{KCl}) + B_2 \cdot (b_{CaCl_2})$ Exposure time 48 h

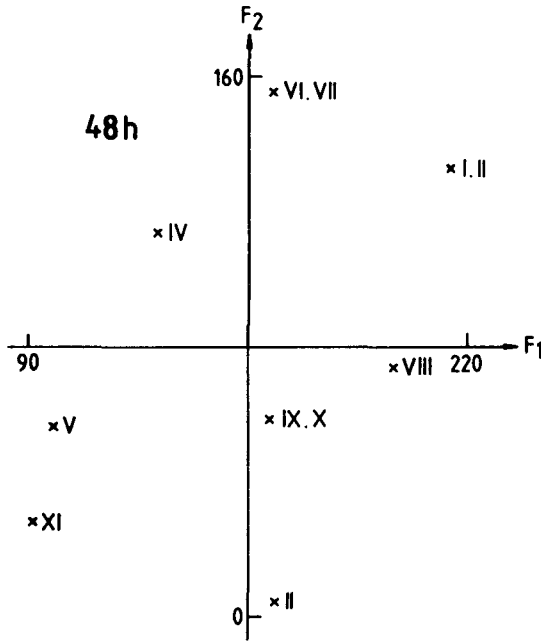| Parameter	Equation	
	I.	II.
A	−1.96	−1.83
B_1	1.13	1.38
s_{B_1}	0.27	0.29
B_2	−0.55	−0.64
s_{B_2}	0.21	0.23
B_1 %	62.07	63.29
B_2 %	37.93	36.71
r^2	0.7567	0.8106
F	12.44	17.12

Figure 2. Similarities and dissimilarities between the selectivity of the phytotoxic effects of sulfosuccinic acid esters. Exposure time 48 h. Two-dimensional nonlinear selectivity map. Iterations: 248; maximal error: 6.84×10^{-2}. For the designation of surfactants, see Materials and Methods.

mensurable. Since the hydrophobicity parameters determined in the presence of KCl and CaCl$_2$ were similar, it is probable that the interaction occurs in a similar ion-containing environment. The results also indicate that it is highly recommendable to determine hydrophobicity parameters of bioactive compounds in an ionic environment similar to that of the site of their activity.

Standard linear QSAR calculations disclosed significant linear relationships between mean inhibitory effects of the individual surfactants measured after 24 and 48 h (Mean$_{24}$ and Mean$_{48}$) and the hydrophobicity parameters:

$$\text{Mean}_{24} = -0.64 + (0.28 \pm 0.06) \cdot b_{\text{KCl}} - (0.17 \pm 0\,07) \cdot R_{\text{MCaCl}_2} \qquad (1)$$
$$F_{\text{calc}} = 11.30 \qquad r^2 = 0.7386$$
$$\text{Mean}_{48} = -0.33 + (0.20 \pm 0.05) \cdot b_{\text{KCl}} \qquad (2)$$
$$F_{\text{calc}} = 17.13 \qquad r^2 = 0.6556$$

These data are in good agreement with the results of the spectral mapping technique, the hydrophobicity parameters determined in the presence of KCl and CaCl$_2$ have a significant effect on the phytotoxicity of anionic surfactants. We assume that the application of the spectral mapping technique shows considerable advantages over the employment of common linear regression anlysis, because the former method enables separation of strength and selectivity of the phytotoxic effect which is impossible with the traditional linear QSAR model.

It can be concluded from the above data that sulfosuccinic acid esters show a marked phytotoxic effect on the leaves of *Tradescantia bicolor*. The significant relationship between hydrophobicity parameters of the anionic surfactants and their biological activity suggests the involvement of hydrophobic (apolar) forces. It can be assumed that the phytotoxicity of anionic surfactants is based on their membrane damaging effect. The apolar alkyl chain is introduced between the lipophilic bilayers of phospholipid membranes while the highly hydrophilic substructure of surfactants binds to the polar head groups of the phospholipid membrane. The interaction of anionic surfactants with mebrane phospholipids may result in the disruption of the membrane structure causing leakage and dysfunction.

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