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The effect of ionic and non-ionic surfactants on the growth, nitrate reductase and nitrite reductase activities of *Spirodela polyrrhiza* (L.) Schleiden

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Abstract

Inclusion into the medium of 5 $mg \cdot dm^{-3}$ of non-ionic (ENF) or ionic (DBST) surfactant caused 50-60% inhibition of nitrite reductase (NiR) activity in *S. polyrrhiza*. At the same time, increased accumulation of NO_2^- in the plant tissues and lowering of the total and soluble protein contents were found. DBST also lowered the nitrate reductase (NR) activity and the dry mass of the plants.

Key words: Spirodela polyrrhiza, surfactants, nitrate reductase, nitrite reductase

INTRODUCTION

The effect of surfactants on plants is not well understood, although it is known that many of these substances modify the growth of aquatic (Haapala 1970, Tatkowska and Toporowska 1978, Gumiński et al. 1978) and terrestrial plants (Paar and Norman 1964, Gumiński et al. 1972). Subcellular structures and membrane permeability, which is concerned with ion transport, are particularly sensitive to surfactants (Paar and Norman 1964, Cantliffe and Wilcox 1969, Gumiński et al. 1972). According to Ruesink (1971), ionic surfactants caused lysis of plasma membranes in oat coleoptiles. Haapala (1970) found changes in membrane permeability both in algae cells and in higher plants in the presence of non-ionic surfactants. It would seem then, that pollution of the aquatic environment by different

Abbreviations: NR — nitrate reductase, NiR — nitrite reductase, ENF — alkylphenoxypolyethoxy ethanol, DBST — trimethylamine alkylbenzenesulfonate.

types of detergents creates a hazzard for the development of aquatic plants.

The aim of this study was to examine the effect of a non-ionic surfactant (ENF) and an ionic one (DBST) introduced into the medium, on the growth and protein content of *S. polyrrhiza* and the activity of its nitrate and nitrite reductases.

MATERIAL AND METHODES

Sterile cultures of *S. polyrrhiza* (L.) Schleiden were cultivated in a photothermostat room under conditions of 16 h light (10.3 $W \cdot m^{-2}$ obtained from white fluorescent tubes) at 25° C and 8 h darkness at 21° C. The plants grew in 300 cm^3 Erlenmayer flasks containing 100 cm^3 of medium of the following composition (in mM): KNO_3 — 3; $Ca(NO_3)_2 \cdot 4H_2O$ — 2; $MgSO_4 \cdot 7H_2O$ — 1; NaH_2PO_4 — 1; $NaNO_3$ — 1; Fe-citrate — 0.0045. The medium contained microelements in amounts given in a previous paper (Tatkowska and Buczek 1983) and 0.1% sucrose and $CaCO_3$ (50 mg per flask). After sterilization of the mediums, surfactants were added in the amount of 5 mg active substance per dm^3 of medium and 3 plants (2-leaf stage) from the basic culture (Tatkowska and Buczek 1983) were innoculated into the medium. The non-ionic surfactant — ENF (alkylphenoxypolyethoxy ethanol with an alkyl radical R = C_9 and a polar group with C = 8) and ionic — DBST (triethylamine alkylbenzenesulfonate, R = C_{11}) were used. The ENF preparation contained 100% active substance, DBST however, 50% dissolved in deionized water. Both substances were obtained from the Pollena Household Chemical Works in Wrocław.

Enzyme extracts were made from 14 day-old cultures of *S. polyrrhiza* (exponential phase) according to the method described in a previous paper (Buczek 1984), using the same solution to extract NR and NiR. The extraction solution contained 50 mM K-phosphate buffer (pH 7.5), 5.0 mM cysteine, 3 mM EDTA and 3% casein. Enzyme activity was determined in supernatants obtained after centrifugation of the homogenate at 20,000 × g for 15 min. Extraction and centrifugation were carried out at 0-4° C.

The activity of nitrate reductase (EC 1.6.6.1) was assayed according to the method of Hageman and Flesher (1960) in 3 replicates, incubating the samples at 27° C for 30 min in an incubation solution containing: 0.1 M K-phosphate buffer (pH 7.5), 0.1 M KNO_3, 2 mM NADH, 0.2-0.4 cm^3 (appox. 0.8 mg protein) enzyme extract and distilled water to a final volume of 2.0 cm^3. The reaction was initiated by adding NADH and stopped by adding 1 cm^3 0.1 M zinc acetate. After 15 min the solutions were centrifuged (10,000 × g, 10 min) and nitrite was de-

termined in the supernatant. The assay was done by adding to the sample 1 cm^3 of 1% (w/v) sulfanilamide in 1 N HCl and 1 cm^3 of a 0.01% (w/v) of N-(1-naphtyl)-ethylenediamine dihydrochloride and diluted to 5 cm^3 with water. After 30 min the absorption was measured at 540 nm.

The activity of nitrite reductase (EC 1.6.6.4) was determined in 3 replicates according to the method described by Hucklesby et al. (1972) by incubating the samples at 27° C for 30 min. The incubation medium contained 0.1 M K-phosphate buffer (pH 6.8), 1 mM $NaNO_2$, 0.2 cm^3 enzyme extract (aprox. 0.8 mg protein), 0.5 cm^3 metyl viologen solution (6 mg in 10 cm^3 K-phosphate buffer, pH 6.8) and 0.5 cm^3 dithionite (72 mg $N_2S_2O_3$ and 10 cm^3 0.1 M $NaHCO_3$). The reaction was started by adding the dithionite solution and stopped by shaking the tube with the reaction mixture until the blue color disappeared.

Further procedure was the same as in the case of NR assay. The activity of NR and NiR was expressed in nmoles NO_2^- formed (NR) or utilized (NiR) per g fresh weight per hour.

The amount of soluble protein and NO_2^- accumulation were determined in the supernatant used to measure enzyme activity. The extract sample was precipitated at 4° C for 24 h with an equal volume of 10% trichloroacetic acid. After centrifugation, the precipitate was dissolved in 1 N NaOH and the protein content determined by a modified method of Lowry et al. (Markwell et al. 1978), using BSA as the standard. Nitrite was determined after precipitating protein in the supernatant with 0.1 M zinc acetate and centrifugation as described above. The total protein content in plants was calculated from the amount of protein nitrogen using the multiplier 6.26. Protein nitrogen was calculated from the difference between total N and soluble N. Total N was determined by the Kjeldahl micromethod, after digestion of a fresh weight sample in concentrated H_2SO_4 with an addition of hydrogen peroxide. Soluble N was determined by digesting the filtrate obtained after boiling a fresh weight sample with water (10 min) and precipitating protein with 10% trichloroacetic acid in the cold overnight.

Nitrate uptake was measured from the decrease in the amount of NO_3^- in the medium in accordance with the method described by Cataldo et al. (1975). The sample (0.2 cm^3) was mixed very exactly with 0.8 cm^3 5% (w/v) salicylic acid in undituled H_2SO_4. After 20 min, 19 cm^3 2 N NaOH were added and, after chilling, the absorption was read at 410 nm. The amount of NO_3^- was calculated from a standard curve. The credibility of the results was calculated from the standard deviation of averages (S_1 and S_2) using the formula for SE difference = $\sqrt{(S_1)^2+(S_2)^2}$. Differences which exceeded twice the standard deviation of the difference (SE) were considered significant at the 5% level.

Reagent sources: NADH (Reanal), metyl viologen (Koch-Light), bovine serum albumin (Biomed), vitamin-free casein (Fluka AG), dithionite (UCE, Bruxelles) the remaining chemicals, chemically pure grade, were from POCh Gliwice.

RESULTS

Figure 1 presents the kinetics of the changes in the growth of *S. polyrrhiza* measured by the increase in fresh and dry weight in the presence of 5 mg·dm^{-3} of the studied compounds introduced into the medium. It was found that only DBST decreased by 30 and 40% the increase in fresh and dry weight between the 14th and 21st days of culture, respectively. A small but significant decreasing of dry weight in the presence of DBST was found after 14 days, which was not found in respect to fresh weight. The studies showed that a concentration of DBST greater than 5 mg·dm^{-3} was lethal for the plants, however, they were more resistant to ENF. Introducing into the medium 25 and 50 mg·dm^{-3} ENF reduced the increase in mass of the plants only by 20 and 30%.

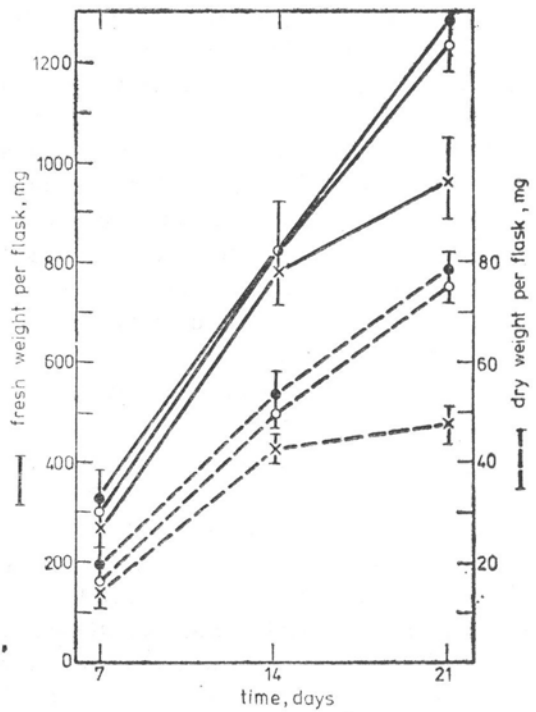

Fig. 1. The kinetics of the changes in fresh (solid line) and dry mass (broken line) of *S. polyrrhiza* as a function of the presence of 5 mg·dm^{-3} ENF and DBST in the medium, ● — control, O — ENF, × — DBST

The effect of ENF and DBST on the level and kinetics of changes in the activity of nitrate and nitrite reductases is shown on Figs. 2A and B. The results show that between the 14th and 21st days of culture, ENF stimulated the activity of NR by about 30%, whereas DBST permanently lowered the activity of nitrate reductase (Fig. 2A). Both of these surfactants significantly affected the changes in the activity of NiR. The activity of this enzyme in plants cultured in the presence of ENF and DBST was about 50-60% lower compared with NiR activity in control plants. Measurements of NO_3^- uptake by *S. polyrrhiza* in the presence of ENF did not show significant differences compared with the control, whereas DBST slightly lowered the absorption of nitrate. Plants which grew in the presence of DBST for 21 days, taken up of 395 μmoles NO_3^- per g fresh weight, whereas control plants, 479 μmoles NO_3^- per g fresh weight. Therefore, the reduction in nitrate uptake was low and did not exceed 20% of the control. Both ENF and DBST present in the

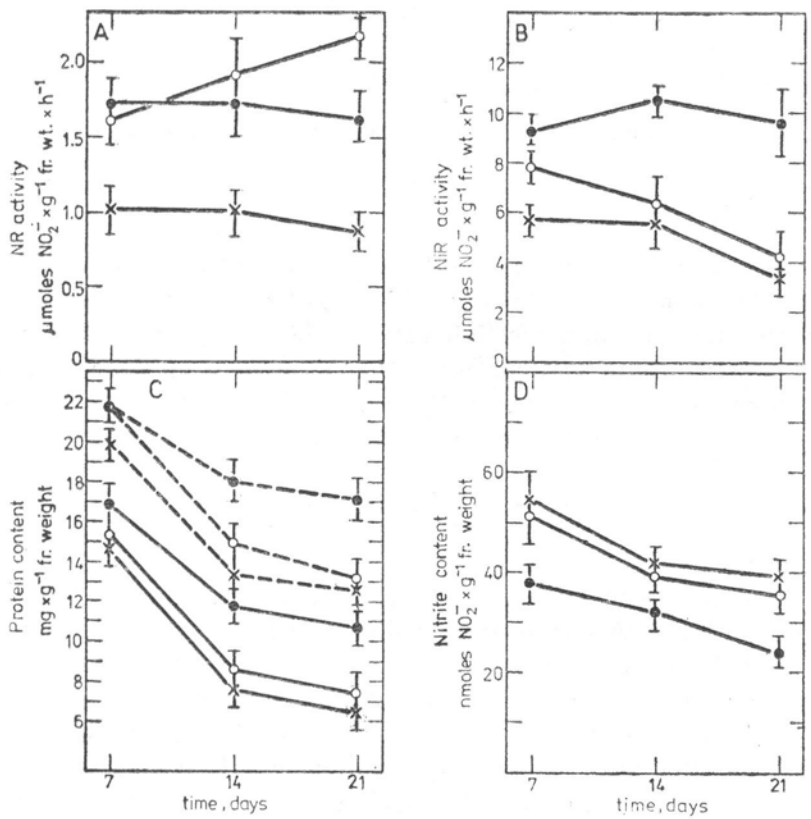

Fig. 2. The *in vivo* effect of 5 mg·dm^{-3} of ENF and DBST on the activity of nitrate reductase (A), nitrite reductase (B), soluble protein content (solid line) and total protein (broken line) (C), and the accumulation of nitrate (D) in *S. polyrrhiza*. ● — control, O — ENF, × — DBST

medium increased the concentration of NO_2^- in plant tissues (Fig. 2B). A lowering of total protein contents in the fresh weight and soluble protein tissue extracts from *S. polyrrhiza* cultivated in the presence of ENF and DBST (Fig. 2C) were also found.

Table 1

Effect of surfactants ENF and DBST (5 mg $\cdot dm^{-3}$) added either to the extraction or incubation medium on the *in vitro* activities of NR and NiR (μmoles $NO_2 \cdot g^{-1}$ fresh weight $\cdot h^{-1}$) in extracts from 14 day-old (exponential phase) *S. polyrrhiza* cultures

Treatment	Activity	
	NR	NiR
Extraction medium	1.55 (100)	11.08 (100)
—"— +ENF	1.65 (106)	8.05 (73)
—"— +DBST	0.75 (47)	6.04 (54)
Incubation medium	1.34 (100)	10.43 (100)
—"— +ENF	1.56 (116)	6.35 (61)
—"— +DBST	0.58 (43)	4.10 (30)

The results are averages from 3 replicates. The numbers in parentheses indicate the percentage of the control activity.

The effect of ENF and DBST *in vitro* on NR and NiR activity is presented in Table 1. ENF (5 mg $\cdot dm^{-1}$) added to the extraction or incubation mediums did not affect NR activity, however, DBST at the same concentration, significantly lowered the activity of nitrate reductase. Both surfactants clearly lowered the NiR activity, however, the inhibitory effect of DBST did not exceed that of ENF.

DISCUSSION

The results presented above indicate that the inclusion of surfactants used in industry into media, causes disturbances in the assimilation of nitrates in cultures of *S. polyrrhiza*. Nitrite reductase which, as is known, catalyses the reduction of nitrite to ammonia, showed itself to be exceptionally sensitive to the presence of ENF and DBST in the medium. The plants reacted with a two-fold reduction in NiR activity. The reason for increased accumulation of NO_2^- and lowering of the protein content in *S. polyrrhiza* therefore seems to be evident.

The possible mechanism of ENF and DBST action on NiR activity is unknown. Taking into account, however, the fact that detergents act on subcellular structures, it can be supposed that DBST and ENF can

modify a number of metabolic processes. Mac Dowall (1963) observed the effect of non-ionic surfactants on the respiration of *Nicotiana tabacum* roots. Neumann and Jagendorf (1965) found uncoupling of photophosphorylation processes and changes in the structures of chloroplasts in *Spinacia oleracea* leaves. Tatkowska and Toporowska (1978), studying the effect of ENF and DBSS (sodium salt of dodecylbenzenesulfonate) on *S. polyrrhiza,* found the biosynthesis of chlorophyll to be inhibited and the accumulation of iron to be reduced. Because nitrite reductase in the green parts of plants is localized in chloroplasts (Ritenour et al. 1967, Swader and Stocking 1971), and because it uses reducing equivalents (NADPH, reduced ferredoxin) formed during photosynthesis to reduce nitrite (Beevers and Hageman 1969, Hewitt 1975), it seems probable that ENF and DBST act on the structure and function of chloroplasts, and indirectly, on the activity of NiR. However, the *in vitro* inhibition of this enzyme by ENF and DBST favor a more complicated effect of these substances on NiR.

DBST was shown to be especially harmful to *S. polyrrhiza*. This surfactant also inhibited the activity of NR both *in vivo* and *in vitro*. The slight lowering of NO_3^- uptake as an effect of DBST rather excludes the effect of this compound on the activity of NR by reducing the flow of its substrate (NO_3^-).

There is therefore no doubt that the presence of surfactants in water reservior may affect the development of plants. DBST at a concentration of 0.005% lowered the increase in dry mass of *S. polyrrhiza* and clearly lowered the activity of NR and NiR, the basic enzymes in the reduction of NO_3^- to ammonia. The inhibition of the activity of both enzymes resulted in the decreasing of the total and soluble protein content. ENF, a non-ionic surfactant introduced into the medium at the same concentration as DBST, was less harmful. However, the evident inhibition of NiR activity, which was probably connected with the increased accumulation of NO_2^- an the decreased protein content, indicates the harmful effect of this non-ionic surfactant on the development of plants.

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Wpływ niejonowej i jonowej substancji powierzchniowo czynnej na wzrost oraz aktywność reduktazy azotanowej i redukatazy azotynowej Spirodela polyrrhiza (L.) Schleiden

Streszczenie

Wprowadzenie do pożywki 5 $mg \cdot dm^{-3}$ niejonowej (ENF) i jonowej (DBST) substancji powierzchniowo czynnej, wpłynęło na zahamowanie o 50-60 procent aktywności reduktazy azotynowej (NiR) w *S. polyrrhiza*. Stwierdzono równocześnie zwiększoną akumulację NO_2^- w tkankach roślin oraz zmniejszenie ilości białka całkowitego i rozpuszczalnego. DBST zmniejszył również aktywność reduktazy azotanowej (NR) oraz suchą masę roślin.