Chloroacetamide Mode of Action, I: Inhibition of Very Long Chain Fatty Acid Synthesis in *Scenedesmus acutus*

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Herbicidal chloroacetamides cause a very sensitive inhibition of fatty acid incorporation into an insoluble cell wall fraction of *Scenedesmus acutus*. The molecular basis was investigated in more detail.

After incubation of the algae with [^{14}C]oleic acid and saponification, the remaining pellet was solubilized and fractionated consecutively with chloroform/methanol, phosphate buffer, amylase, pronase, and finally with dioxane/HCl. By acid hydrolysis in dioxane a part of the cell wall residue was solubilized showing inhibition of exogenously applied oleic acid and other labelled precursors such as stearic acid, palmitic acid, and acetate. After extraction of this dioxane-soluble subfraction with hexane, HPLC could separate labelled metabolites less polar than oleic acid. Their formation was completely inhibited by chloroacetamides, e.g. 1 μM metazachlor. This effect was also observed with the herbicidally active *S*-enantiomer of metolachlor while the inactive *R*-enantiomer had no influence. These strongly inhibited metabolites could be characterized by radio-HPLC/MS as *very long chain fatty acids* (VLCFAs) with a carbon chain between 20 and 26. Incubating a metazachlor-resistant cell line of *S. acutus* (Mz-1) with [^{14}C]oleic acid, VLCFAs could not be detected in the dioxane/HCl-subfraction. Furthermore, comparing the presence of endogenous fatty acids in wild-type and mutant Mz-1 the VLCFA content of the mutant is very low, while the content of long chain fatty acids (C16–18) is increased, particularly oleic acid.

Obviously, the phytotoxicity of chloroacetamides in *S. acutus* is due to inhibition of VLCFA synthesis. The resistance of the mutant to metazachlor has a bearing on the higher amount of long chain fatty acids replacing the missing VLCFAs in essential membranes or cell wall components.

Introduction

Chloroacetamides are biologically active substances which inhibit the germination of grass and some dicotyledonous weeds in a number of crops. This feature has led to commercialization of several compounds as successful preemergence herbicides. In the last few years, the introduction of new chloroacetamide and oxyacetamide herbicides, such as dimethenamid, mefenacet and flufenacet, has shown that this herbicide class is still going strong in agricultural applications, although allidochlor, the first chloroacetamide herbicide, was commercialized 40 years ago (Hamm, 1974). In part, the success of these compounds is due to the absence of resistance problems and to their broad application through the use of safeners.

Much work has been done to localize the primary target. Many physiological effects in various crops and weeds have been reported, as well as many biochemical modifications in diverse biosynthesis pathways (for a review see Fuerst, 1987; LeBaron *et al.*, 1988; Böger, 1997). The primary target site, however, has not been resolved as yet. Effects obtained with rather high concentrations

Abbreviations: alachlor, 2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide; cafenstrole, 1-(diethylcarbamoyl)-3-(2,4,6-trimethylphenylsulfonyl)-1,2,4-triazole; Chl, chlorophyll; dimethenamid, 2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)-acetamide; EPTC, *S*-ethyldipropylcarbamothioate; flufenacet (formerly fluthiamid, FOE-5043), *N*-(4-fluorophenyl)-*N*-(1-methylethyl)-2-[(5-trifluoromethyl-1,3,4-thiadiazol-2-yl)oxy]acetamide; mefenacet, 2-(2-benzothiazolyloxy)-*N*-methyl-*N*-phenylacetamide; metazachlor, 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(1*H*-pyrazol-1-ylmethyl)acetamide; metolachlor; 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide; NLF, non-lipid fraction; VLCFA, very long chain fatty acid.

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up to mM have been reported like (*i*) in *de novo* fatty acid synthesis, such as the inhibition of malonic acid incorporation into hypocotyl lipids (Mann and Pu, 1968), (*ii*) in fatty acid metabolism, such as the influence on desaturation and elongation of seed storage lipids in microspore-derived embryoids of *Brassica napus* (Möllers and Albrecht, 1994), and (*iii*) in secondary lipid biosynthesis pathways, shown by the reduction of epicuticular waxes (Ebert and Ramsteiner, 1984; Tevini and Steinmüller, 1987).

We focused our efforts on the influence of chloroacetamides on lipid metabolism of the green alga *Scenedesmus acutus*. This unicellular alga is used as a model for higher plants as its lipid composition and biochemistry is very similar and, in particular, the algae is easy to grow and provides a homogeneous system for investigating molecular responses to external stimuli and stress factors. The availability of a metazachlor-resistant cell line of *S. acutus* (called Mz-1; Couderchet *et al.*, 1995) was another advantage for mode of action studies with this organism. The first striking effect by micromolar concentrations of alachlor or metazachlor was a decrease of endogenous fatty acid desaturation after a 40-hour incubation of the wild-type (Weisshaar *et al.*, 1988), while metazachlor did not cause any change in the fatty acid profile of the mutant (Couderchet *et al.*, 1995). Even stronger affected was the incorporation of exogenously applied oleic acid into a non-lipid fraction (NLF) obtained after saponification of the cells. A 50% inhibition was found between 10 and 100 nM after a very short incubation time of 3.5 hours (Couderchet and Böger, 1993a). This inhibition was not found in the mutant, relating this effect with the herbicidal activity of metazachlor. Furthermore, the inhibition of oleic acid incorporation could be positively correlated with the growth inhibition of *S. acutus* wild-type cells by various acetamide herbicides. Besides chloroacetamides such as alachlor, metazachlor and metolachlor, and oxyacetamides like mefenacet and flufenacet, the new triazole herbicide cafenstrole was found very effective. Since the influence on oleic acid metabolism is specific to herbicidal chloroacetamides and related compounds, for the first time an assay could be developed to quantify the herbicidal activity of these substances. This assay is a quick one since labelled oleic acid is incorporated into an *insoluble* residue which can be obtained by simple centrifugation and washing. No HPLC run is required (Couderchet *et al.*, 1998).

All these compounds include an *N, N*-disubstituted amide function which we postulate as the essential activity element. This moiety, however, is not responsible for herbicidal activity alone, since the *R*-isomer of metolachlor and dimethenamid is almost ineffective. Accordingly, the respective *S*-isomers are much more effective to inhibit oleic acid incorporation into the NLF, which correlates exactly with their phytotoxic activity (Couderchet *et al.*, 1997).

As shown previously the NLF could be solubilized in part (see Methods) yielding a dioxane/HCl subfraction and a residue including sporopollenin (Couderchet *et al.*, 1996). Both, residue and soluble subfraction exhibited a similar inhibition of fatty acid incorporation. Accordingly, analysis of this soluble subfraction should reveal the nature of labelled metabolites produced from applied oleic acid during its incorporation into sporopollenin, and the particular biosynthetic step which is inhibited by acetamide herbicides. Comparative investigations with the metazachlor-resistant cell line Mz-1 accompanied these studies.

Materials and Methods

Biological material

The unicellular green alga *Scenedesmus acutus* (No. 276–3a, Algae Collection, Göttingen, Germany) was grown in the light (80 μmol m^{-2} s^{-1}; 400–700 nm) in 200 ml liquid medium, constantly gassed with air containing 2% CO_2 (v/v) and subcultured every other day as described previously (Couderchet and Böger, 1993b). From this culture the metazachlor-resistant mutant Mz-1 was selected (Couderchet *et al.*, 1995) and grown under the same conditions as the wild-type in liquid medium containing 5 μM metazachlor.

One day before the experiment, the wild-type or the mutant Mz-1 was subcultured into fresh medium without herbicide. At time of harvest the culture density was approximately 15–20 μg chlorophyll (Chl) ml^{-1}.

Incubation procedure

[2-^{14}C]Acetate, [1-^{14}C]palmitic acid, [1-^{14}C]-stearic acid, or [1-^{14}C]oleic acid (16.7 kBq per sample) respectively, were used as precursors of VLCFAs (purchased from Amersham, Braunschweig, Germany with 1.85–2.29 GBq mmol^{-1}). In HPLC/MS investigations [1-^{14}C]oleic acid was diluted with unlabelled oleic acid to decrease the specific activity to 2.04 MBq mmol^{-1}. Labelled fatty acids dissolved in toluene were dried under nitrogen and then dispersed in 1 ml algae growth medium by ultrasonication for 5 min, while the aqueous [2-^{14}C]acetate solution was directly diluted into 1 ml growth medium. One day-old cultures of *S. acutus* were concentrated to 55–60 µg Chl ml^{-1}. A sample containing 9 ml of the algal suspension was preincubated for 90 min with 10 µl herbicide solution in ethanol, and with a control aliquot including 10 µl ethanol. The 1-ml growth medium containing labelled precursor was added to the 9-ml sample and incubation proceeded under growth conditions for another 16 h. The final concentration of precursors were 0.8 µM for the data of Fig. 1, 2 and 4 and 0.8 mM for the data of Fig. 3.

Solubilization

After incubation the algal suspension was centrifuged (800 × *g*, 10 min), the culture medium discarded, and the algal cells were heated at 65 °C in 5 ml methanol for 10 min. The sample was cooled and centrifuged (800 × *g*, 10 min), and the pellet washed twice with chloroform/methanol (2/1, v/v). The resulting precipitate representing the non-lipid fraction (NLF) was treated according to the four-step solubilization procedure according to Couderchet *et al.* (1996). The NLF was washed with phosphate buffer, and the pellet incubated successively with α-amylase and pronase (both from Sigma, Deisenhofen, Germany). Eventually, the remaining residue could be hydrolyzed in part with hot dioxane/HCl, then cooled, centrifuged, and an aliquot of the supernatant representing the dioxane/HCl-subfraction was counted for radioactivity. For HPLC-analysis the dioxane/HCl-subfraction (5 ml) was extracted three times with 2 ml *n*-hexane after adding 5 ml water. The hexane extracts were dried under nitrogen, dissolved in 20 µl *n*-hexane, and injected into the HPLC.

HPLC analysis

HPLC was performed with a Kontron Analytical Constant Flow Pump (Neufahrn, Germany) using a C_{18} reversed-phase column (4 µm particle size, 25 cm × 4.6 mm i.d., Macherey-Nagel, Oensingen, Switzerland) and a low-pressure gradient controller (CIM Autochrom; ERC, Alteglofsheim, Germany). The three mobile phase solvents were acetonitrile, methanol, and water containing 0.1% trifluoroacetic acid. The metabolites were separated and eluted with a linear gradient from methanol/acetonitrile/water (10/80/10, v/v/v) to methanol/acetonitrile (10/90, v/v) over 10 min. Another linear gradient changed the solvent to 100% methanol within 20 min, after a 30 min isocratic period in the latter solvent mixture. Before reequilibrating, the column was rinsed for ten minutes with methanol (system I). In some experiments a system (II) was used starting with acetonitrile/water (90/10, v/v) and changing within 10 min to 100% acetonitrile. Then, a linear gradient changed the solvent to 100% methanol within another 15 min. With this solvent the column was rinsed another 35 min. The HPLC flow rate was 1 ml/min. Radiolabelled metabolites were detected with an on-line radioactivity detector (Ramona, Raytest, Straubenhardt, Germany) using the scintillation cocktail Flo-Scint III (Packard, Groningen, The Netherlands). In case of MS-detection, an on-line coupled Sciex API III electrospray mass spectrometer was used, which was connected parallel to a radioactivity detector. Ionization was performed in the negative mode.

Extraction of endogenous fatty acids

Freshly subcultured cells of the mutant Mz-1 or of the wild-type of *S. acutus* were grown under the above described conditions for 48 h in a volume of 220 ml. To extract the lipids and pigments of the algae, the suspension was centrifuged (4000 × *g*, 12 min), the growth medium discarded, and the cells were boiled in 100 ml methanol under reflux for 30 min. The cell debris was washed with 50 ml chloroform/methanol (2/1, v/v), 50 ml methanol, and then boiled under reflux in 100 ml phosphate buffer (50 mM, pH 7) for 30 min. The cooled suspension was filtered and the solid residue boiled under nitrogen in 50 ml dioxane/2 M HCl (9/1, v/v) for further three hours. After cooling, 50 ml water

and 20 µg *n*-undecanoic acid (Sigma, Deisenhofen, Germany) dissolved in 20 µl *n*-hexane were added. This acid was used as internal standard. The dioxane/HCl-solution was extracted three times with 20 ml *n*-hexane. From the combined extracts the hexane was evaporated and 400 µl boron trifluoride in methanol (14%) were added to the residue. The closed reaction vessel was heated at 100 °C for 15 min and the fatty acid methyl esters were extracted with 400 µl *n*-hexane after adding 400 µl of water.

GC-Analysis

Fatty acid methyl esters were separated using a Shimadzu GC-15A gas chromatograph (Duisburg, Germany) fitted with a fused silica capillary column (SP-2330, 30 m × 0.25 mm i.d., Supelco, Deisenhofen, Germany), and equipped with a flame ionization detector (FID); carrier gas (helium) was supplied at 25 ml/min. The GC conditions were: injector and detector temperature, 250 °C; running temperature program, 50 °C for 1 min, then increasing at 10 °C/min to 190 °C and holding this temperature for 3 min, finally increasing at the same rate to 230 °C and holding this temperature for another 18 min. Injection volume was 1 µl using a split of 50 ml/min and a purge of 10 ml/min. Identification was based on the retention times of commercially available reference methyl esters (Sigma, Deisenhofen, Germany) and confirmed by mass spectrometry.

Data reliability

The experiments were repeated at least three times; the MS-data are based on two independent experiments. The data from representative experiments are shown here. The deviation from the mean within the repetitions did not exceed 10%.

Results

The cells of the control samples incorporated 17% stearic acid and 6% of the monounsaturated oleic acid applied. The inhibition of fatty acid incorporation into the dioxane/HCl-subfraction caused by 1 µM metazachlor was strongest with oleic acid, since the radioactivity in that subfraction decreased by 92%, the incorporation of acetate, palmitic acid and stearic acid decreased by 38–52%. The corresponding subfraction of the Mz-1 mutant cells showed a 4.5- fold decrease of radioactivity incorporated from the applied [^{14}C]oleic acid compared with the wild-type. Moreover, 1 µM metazachlor did not influence the amount of incorporation (Fig. 1).

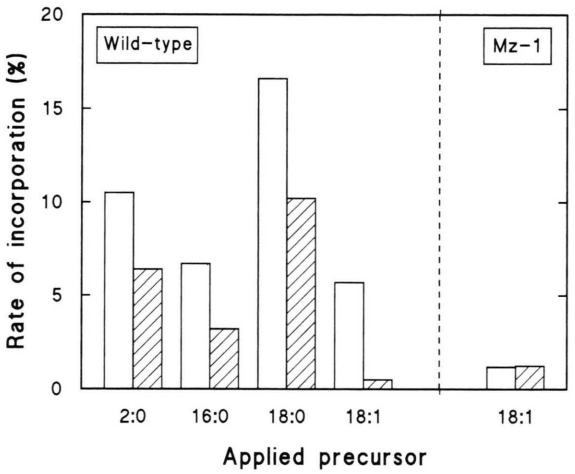

Fig. 1. Rate of incorporation of the applied ^{14}C-labelled precursors into the dioxane/HCl-subfraction of wild-type and metazachlor-resistant mutant (Mz-1) cells of *Scenedesmus acutus*. After preincubation without herbicide (open bars) or with 1 µM metazachlor (hatched bars) the algae was incubated with 16.7 kBq ^{14}C-labelled precursor per sample as indicated. For the organic acid *x:y*, *x* represents the number of C atoms and *y* the number of double bonds.

About 40% of the radioactivity recovered in the dioxane/HCl-subfraction could be extracted with *n*-hexane regardless of incubation with herbicide (data not shown). In the control these extracts by HPLC indicated various radioactivity peaks *less polar* than the applied oleic acid (Fig. 2A). In samples treated with metazachlor these metabolites could not be detected (Fig. 2B). Without herbicide treatment the chromatogram of the corresponding extract from the metazachlor-resistant mutant showed a single peak only representing the applied oleic acid (Fig. 2C), a finding similar to the inhibited wild-type.

The compounds less polar than oleic acid whose formation was inhibited by metazachlor and therefore present in control samples of the wild-type only were characterized by radio-HPLC/MS. Parallel to on-line detection by mass spectrometry the metabolites were detected by radioactivity which gave the masses of the molecular ions of the radio-

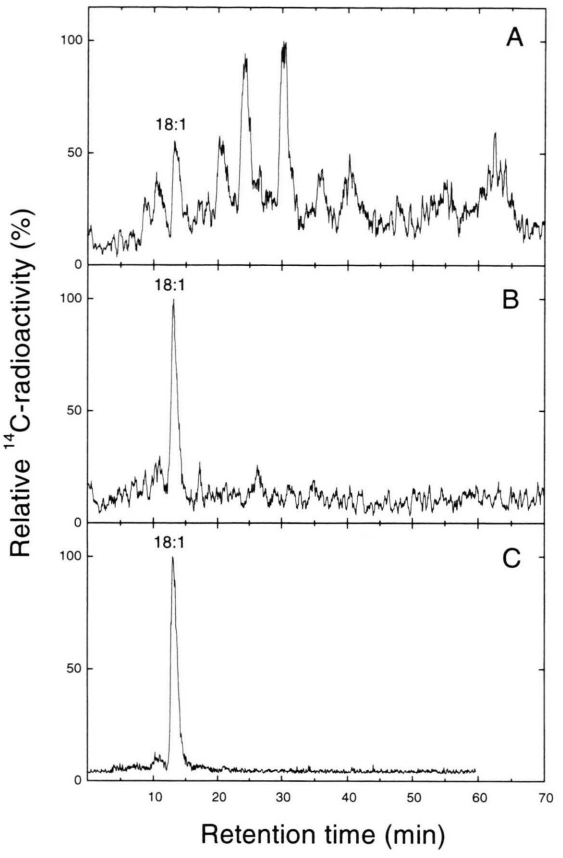

Fig. 2. HPLC chromatogram of the hexane extract of the dioxane/HCl-subfraction (on-line radioactivity detection, see system I in Materials and Methods). Before incubation with [1-^{14}C]oleic acid (18:1) *Scenedesmus acutus* wild-type cells were preincubated without herbicide (**A**) or with 1 µM metazachlor (**B**). The metazachlor-resistant mutant (Mz-1) was preincubated without herbicide (**C**).

detected metabolites (see Fig. 3 and Table I). Since a negative ionization mode was used, the compounds were deprotonized in the ion source and converted into molecular ions of structure $[M\text{-}H]^-$. The masses of these molecular ions detected at the retention times of the ^{14}C-peaks agreed with the theoretical masses of unlabelled deprotonized *monounsaturated* very long chain fatty acids (Table I). The masses of molecular ions detected probably originated from saturated very long chain fatty acids such as arachidic acid (20:0), behenic acid (22:0) and lignoceric acid (24:0). The saturated fatty acid and the monounsaturated homologue prolonged by two methylene groups could only be

Table I. Molecular ions $[M\text{-}H]^-$ measured using the negative ionisation mode at the retention times indicated (see Fig. 3) and suggested structures of the corresponding fatty acids. For the fatty acid in the third column *x:y*, *x* represents the number of C atoms and *y* the number of double bonds.

Retention time [min]	$[M\text{-}H]^-$ (*m/z*)	Suggested structure
8.7	253	16:1
8.7	279	18:2
11.0	255	16:0
11.0	281	18:1
14.2	283	18:0
14.2	309	20:1
18.5	311	20:0
18.5	337	22:1
22.6	365	24:1
23.6	339	22:0
30.0	393	26:1
31.1	367	24:0

separated by our system when the carbon number was greater than 20 or 22, respectively. Only monounsaturated fatty acids could be identified as metabolites of the applied oleic acid, since the separated saturated fatty acids did not show any radioactivity peaks (see Table I and Fig. 3). According to the intensity of the different peaks in the radio-chromatogram, oleic acid seemed to be converted mainly to nervonic acid (24:1) and ximenic acid (26:1).

A reduced VLCFA formation could also be found when incubating the cells with metolachlor (Fig. 4). Furthermore, a stereospecifity showed up since the *R*-enantiomer caused only a slight inhibition of the VLCFAs (Fig 4B) while incubation with 1 µM *S*-metolachlor led to an almost total decrease of these metabolites (Fig 4C).

Investigations of the endogenous fatty acid content of the dioxane/HCl-subfraction by gas chromatography confirmed the tracing experiments of above giving evidence that VLCFAs were hydrolyzed off with dioxane/HCl during the solubilization procedure of the algae. The wild-type contains mainly fatty acids with a carbon number of 16 and 18, in particular, palmitic acid (16:0) with a relative amount of 39%, and also saturated and monounsaturated VLCFAs with a chain length of 24 and 26 carbon atoms (Fig. 5). The relative amount of VLCFAs in the fatty acid content of that subfraction was about 24%. In contrast in the metazachlor-resistant cell line Mz-1 no monounsa-

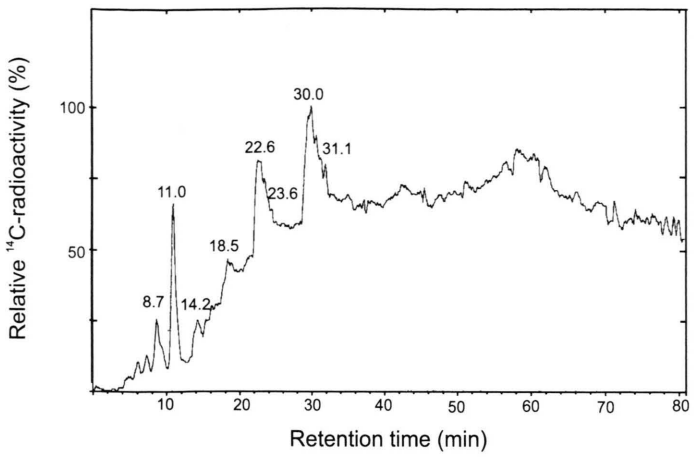

Fig. 3. HPLC chromatogram of the hexane extract of the dioxane/HCl-subfraction (on-line radioactivity detection, see system I in Materials and Methods). Before incubation with [1-^{14}C]oleic acid *Scenedesmus acutus* wild-type cells were preincubated without herbicide. Parallel to radioactivity detection a mass spectrometric measurement was performed to characterize the unknown peaks (see Table I).

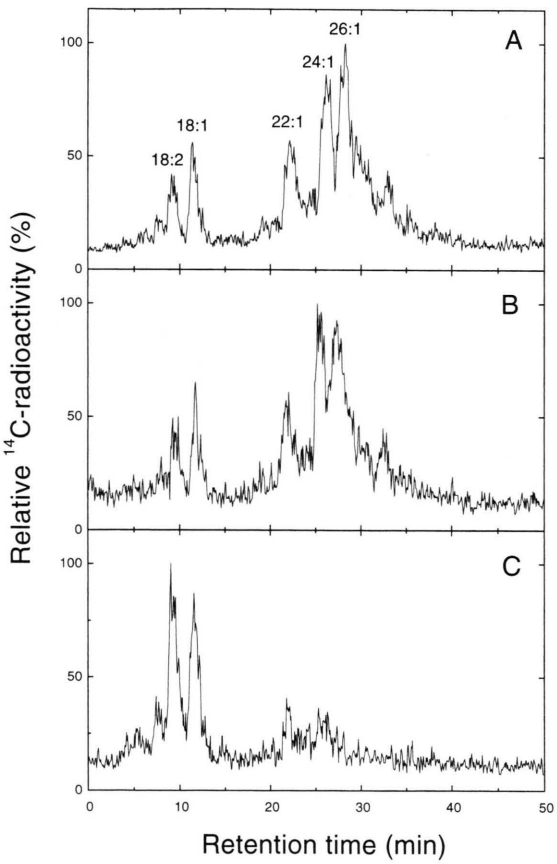

Fig. 4. HPLC chromatogram of the hexane extract of the dioxane/HCl-subfraction (on-line radioactivity detection, see system II in Materials and Methods). Before incubation with [1-^{14}C]oleic acid *Scenedesmus acutus* wild-type cells were either preincubated without herbicide (**A**), with 1 µM *R*-metolachlor (**B**), or with 1 µM *S*-metolachlor (**C**). For the fatty acid *x*:*y*, *x* represents the number of C atoms and *y* the number of double bonds.

turated VLCFA could be detected and only traces of saturated VLCFAs added up to a relative amount of about 4%. Compared with the wild-type the amount of long chain fatty acids in the mutant was more than twice as high mainly due to an 11-fold increase of oleic acid (18:1). In addition to a drastic change of the fatty acid profile, this resulted in a 1.9 fold higher level of the total fatty acid amount in the dioxane/HCl-subfraction.

Discussion

The extraordinary strong inhibition of oleic acid incorporation into an insoluble non-lipid fraction (NLF) of the cell wall caused by chloroacetamides is due to its inhibited incorporation into an insoluble subfraction remaining after stepwise solubilization of the NLF. This subfraction could be partly hydrolyzed by dioxane/HCl leading to a *soluble* subfraction also showing this strong inhibition of oleic acid incorporation (Couderchet *et al.*, 1996). While the incorporation of palmitic acid into the NLF was not inhibited by chloroacetamides (Kring *et al.*, 1995), the incorporation of oleic acid, acetate, and stearic acid into the dioxane/HCl-subfraction was markedly reduced by 1 µM metazachlor (Fig. 1), although less than that of oleic acid. This finding indicates that the target enzyme of chloroacetamides may transform oleic acid to further intermediates. These intermediates, whose formation was completely inhibited by 1 µM metazachlor, are less polar than the applied oleic acid (Fig. 2A and B). A less polar peak in this subfraction was reported previously but could not be further separated or identified as chloroacetamide-

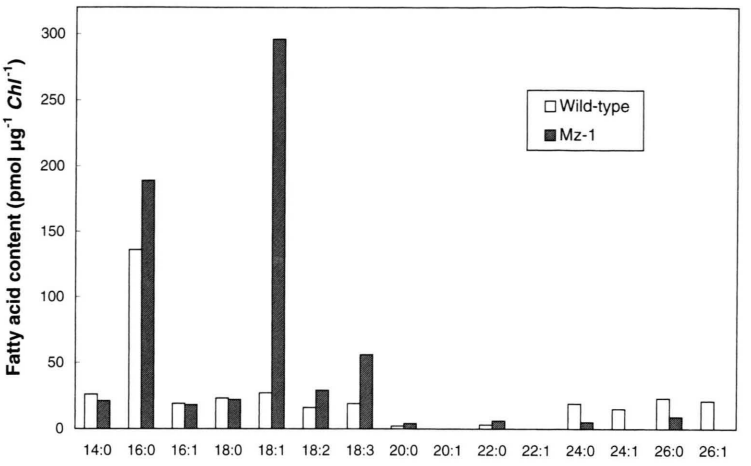

Fig. 5. Content of endogenous fatty acids dissolved by acid hydrolysis in dioxane of wild-type and metazachlor-resistant mutant (Mz-1) cells of *Scenedesmus acutus*. For the fatty acid *x*:*y*, *x* represents the number of C atoms and *y* the number of double bonds.

sensitive intermediates at that time (Couderchet *et al.*, 1996).

Apparently the characterization of the intermediates originated from oleic acid discloses the biosynthetic step in fatty acid metabolism which is inhibited by herbicidal chloroacetamides. Hence, mass spectrometric investigations were performed using HPLC/MS with a radioactivity detector connected in parallel, leading to the chemical structures of the inhibited metabolites. These could be identified as VLCFAs (Table I and Fig. 3). Interestingly, these compounds were not found in the methanolic solution of *alkaline-saponified* algal cells (Couderchet *et al.*, 1995) providing evidence, that in *S. acutus* the VLCFAs are not present as triacylglycerides and probably may have a particular physiological role in membranes or cell wall components. Obviously the inhibition of the VLCFA synthesis by chloroacetamides is responsible for the phytotoxic activity of these compounds in *S. acutus*, since herbicidally active amides inhibit the growth of *S. acutus* according to the inhibition of the oleic acid incorporation into the NLF (Couderchet *et al.*, 1998) which is due to inhibited fatty acid elongation.

VLCFAs are essential components of the plasma membrane in higher plants (Cassagne *et al.*, 1994). A decreased VLCFA synthesis apparently results in a change of membrane properties as reported by Mellis *et al.* (1982), who found an increased membrane permeability in onion roots caused by 10 µM metolachlor. The VLCFA content of *in-vitro* cultured microspore-derived embryoids of *Brassica napus* was found reduced by micromolar concentrations of either alachlor, metolachlor or metazachlor (Möllers and Albrecht, 1994). Moreover, VLCFAs esterified with wax alcohols or reduced to aldehydes, alcohols and alkanes represent the (external) lipids of plant waxes. Inhibition of fatty acid elongases could explain the results of Ebert and Ramsteiner (1983), who reported a reduced epicuticular wax synthesis in the primary leaf of *Sorghum bicolor* seedlings treated with 18 µM metolachlor. In cucumber seedlings the same high concentration of metolachlor inhibited the elongation beyond a carbon number of more than 30 seen by a reduced content of corresponding alkane homologues in the leaf surface waxes (Tevini and Steinmüller, 1987). High inhibitory concentrations, have been criticized as being due to additional side effects not to the influence of an inhibited primary target. In contrast, our experimental system exhibits half-inhibition values in the 10–100 nM range (Kring *et al.*, 1995; Couderchet *et al.*, 1998).

The role of the VLCFAs in green algae has not been elucidated until now since in most (algal) species VLCFAs have not even been found (Thompson, 1996) which may be explained by the analysis methods applied. The lipids are usually only alkaline-saponified, leading to the disadvantage that presence of acid-saponifiable fatty acids are overlooked. Most likely these fatty acids are bound to resistant biopolymers in the cell wall which can partly be solubilized by dioxane/HCl. This solubilizing mixture was originally designed

to dissolve lignin by cleaving ether bonds and depolymerizing the lignin network (Monties, 1988). The cell wall of *S. acutus* consists in part of the lignin-like polymer sporopollenin (Couderchet *et al.*, 1996) which is a substantial component of the outer walls of spores and pollen in higher plants. After metabolic activation via enzymatic sulfoxidation the thiocarbamate EPTC decreased the level of VLCFAs (Harwood *et al.*, 1989; Kern *et al.*, 1997). Accordingly, Wilmesmeier and Wiermann (1995) found a shift to shorter chain length in the fatty acid composition of external pollen lipids, which could explain a change in their IR-band pattern by a reduced chain length of the aliphatic compounds incorporated into their sporopollenin sample.

Moser *et al.* (1982) reported that the herbicidal activity of the chloroacetamide metolachlor is dependent on the configuration of the chiral center located in one of the amide substituents of the molecule. Thus, the *S*-enantiomer represents the phytotoxic (herbicidal) stereoisomer. Accordingly, 1 μM *S*-metolachlor inhibited VLCFA synthesis in *S. acutus* almost completely, while the same concentration of *R*-metolachlor had no influence (Fig. 4).

Furthermore, the metazachlor-resistant mutant Mz-1, gives strong evidence that elongation to VLCFAs is the biosynthetic step inhibited by the chloroacetamides. The mutant does not elongate applied oleic acid (Fig. 2C), no monounsaturated VLCFA could be detected, and the content of 24:0 and 26:0 was comparatively low while the 20:0 and 22:0 species seemed to be slightly increased (Fig. 5). The levels of palmitic acid, oleic acid, and its desaturated products are markedly higher. The 11-fold higher oleic acid level and the precence of other long chain fatty acids apparently replacing VLCFAs in essential membranes or cell wall components may be the reason for the resistance of the mutant to micromolar concentrations of metazachlor.

Considering the results of this study, our previous investigations, and reports by other laboratories, we suggest that the phytotoxic activity of chloroacetamides and related compounds has its start at the elongase system leading to the inhibited formation of VLCFAs resulting in their depletion in biomembranes and other vital cellular constituents. Further support is presented by Matthes *et al.* (1998), who could essentially verify the results with the model alga by corresponding findings in higher plants. In order to characterize the primary target enzyme of these herbicides *in-vitro* investigations are in progress.

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