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MODIFICATION OF THE BIOSYNTHESIS OF RAPE LIPID MOLECULAR SPECIES BY HEAT SHOCK

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Key Word Index—*Brassica napus*; crucifereae; rape; leaves; heat shock; lipids; molecular species; biosynthesis.

Abstract—Rape, *Brassica napus* is a $C_{16:3}$ plant, the leaves of which contain mainly "prokaryotic" molecular species† of monogalactosyldiacylglycerol (MGDG) (18:3/16:3, 76 mol%) and "eukaryotic" molecular species of digalactosyldiacylglycerol (DGDG) (18:3/18:3, 70 mol%). Phosphatidylglycerol (PG) contains nearly exclusively "prokaryotic" molecular species (18:3/16:1^t, 18:3/16:0 and 18:2/16:1^t), whereas phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are exclusively formed by "eukaryotic" (C_{18}/C_{18}, C_{16}/C_{18}) molecular species. A heat shock provoked a great decrease in [1-^{14}C] acetate incorporation into rape leaf lipids, a decrease in the labelling of polyunsaturated fatty acids and an increase in the labelling of palmitic acid. After a heat shock, more saturated labelled molecular species (16:0/18:2, 16:0/18:3) appeared in PC and DGDG. The biosynthesis of prokaryotic MGDG molecular species was more inhibited by a heat shock than the biosynthesis of those of eukaryotic MGDG. Heat shock provoked an increase of 18:3/16:0 and 18:2/16:0 MGDG molecular species; thus, the chloroplastic palmitoyl MGDG desaturase could be affected.

INTRODUCTION

Rape (*Brassica napus*) is an oilseed plant of great potential economical interest for Algeria. The main problem for growing rape in this country, however, is the high environmental temperature encountered during the summer.

Many organisms respond to a change in environmental temperature by altering the fatty acid composition of their membrane lipids. Growth at high temperature usually results into a decrease in the polyunsaturated fatty acid content; such a decrease is associated with reduced membrane fluidity [1]. On the contrary, growth at low temperature is usually accompanied by an increase in the unsaturation of membrane fatty acids, possibly to maintain a high membrane fluidity [2]. The upper limit of temperature at which most plant species can survive appears to be dependent on the stability of the chloroplast membrane [3].

Brassica napus is a $C_{16:3}$ plant, incorporating *cis*-7,10,13 hexadecatrienoic acid ($C_{16:3}$) into monogalactosyldiacylglycerol (MGDG) [4]. It has been shown, that in such plants, MGDG is synthesized both by chloroplastic (or prokaryotic) and cytoplasmic (or eukaryotic) pathways, the former leading to C_{18}/C_{16} molecular species, the latter to C_{18}/C_{18} and C_{16}/C_{18} molecular species [5]. We reported herein, the consequences of a heat shock on molecular species composition and on the biosynthesis of the main polar lipids in rape leaves.

RESULTS AND DISCUSSION

Composition of rape leaf lipids

The main polar lipid classes found in rape leaves are monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), phosphatidylglycerol (PG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Table 1). The major molecular species of MGDG is the 18:3/16:3 prokaryotic molecular species (77%). MGDG is very rich in molecular species containing six double bonds

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† A molecular species is defined by its fatty acid composition; the fatty acid esterified in position sn1 of glycerol is given first, the fatty acid esterified in position 2 being given after the slash bar.

Table 1. Molecular species composition (mol%) of main polar lipids of rape leaves

Molecular species	MGDG	DGDG	PG	PC	PE
18:3/16:3	77.1	5.3	—	—	—
18:3/18:3	18.2	71.2	—	24.4	11.6
18:3/16:2	1.1				
18:2/18:3	1.1	1.5	—	25.8	25.3
18:2/16:3	0.4				
18:2/18:2	—	—	—	10.0	14.5
18:2/16:2	1.2				
18:3/16:1[t]	—	—	20.0	—	—
18:3/16:0	—	—	53.0	—	—
16:0/18:3	0.8	17.8	—	17.7	23.3
18:1/18:3				5.0	
18:2/16:1[t]	—	—	4.0	—	—
18:2/16:0	—	—	21.0	—	—
16:0/18:2	—	4.2	—	11.4	21.1
18:1/18:2	—		—	4.0	—
18:0/18:3	—	—	—	tr	4.2
18:1/16:0	—	—	2.0	—	—
16:0/18:1	—	—	—	1.8	—

(18:3/16:3 and 18:3/18:3), which represent 95% of the total MGDG. In marked contrast to MGDG, the 18:3/18:3 eukaryotic molecular species is the most abundant (71%) in DGDG. Three kinds of molecular species, all of "prokaryotic type", are abundant in PG: 18:3/16:0, 18:3/16:1[t], and 18:2/16:0. In PC and PE, the eukaryotic molecular species, 18:3/18:3, 18:2/18:3, 18:2/18:2, 16:0/18:3 and 16:0/18:2, are predominant.

Effect of heat shock on composition of molecular species

Applying a heat shock (36° for 24 h) to rape plants caused small changes in the molecular species composition of MGDG (Table 2). The percentage of the 18:3/16:3 prokaryotic molecular species decreased in MGDG from 77% to 72%, but those of all the eukaryotic molecular species (18:3/18:3, 18:2/18:3 and 16:0/18:3) increased in MGDG. The ratio of C_{18}/C_{16} (prokaryotic) MGDG molecular species to C_{18}/C_{18} (eukaryotic) MGDG molecular species was reduced from 4.0 in control plants to 3.0 in stressed plants. This result shows that the chloroplastic pathway of MGDG biosynthesis was more sensitive to a heat shock than the extrachloroplastic pathway, suggesting that the non-chloroplastic biosynthesis pathway contributed to maintain a correct amount of MGDG in stressed plants. There were also significant changes in PC and DGDG molecular species. 16:0/18:3 PC and 16:0/18:3 DGDG increasing from 18% to 36% and 18% to 30%, respectively, with 18:2/18:3 PC decreasing from 25% to 6% (Table 2).

After heat shock, the appearance or the increase of content more saturated PC, MGDG and DGDG molecular species, like 16:0/18:1 PC, 18:0/18:3 PC, 18:1/18:2 PC and 16:0/18:3 MGDG were noted. 16:0/18:3 eukaryotic molecular species increased noticeably in the three lipid classes.

Effect of heat shock on incorporation of [1-^{14}C] acetate into polar lipids

Microdroplets of [1-^{14}C] acetate were deposited on the leaf surface of control and stressed plants. After 24 h of labelling, a marked decrease in the labelling of leaf lipids was observed in heat stressed plants cultivated at 36° ([^{14}C] incorporation decreased from 43×10^6 dpm g^{-1} FW to 13×10^6 dpm g^{-1} FW). Because acetate is a direct precursor of fatty acid synthesis, the decrease in labelling probably reflected inhibition of fatty acid synthetase activity. The distribution of label in polar lipids after 24 h in control and stressed plants is shown in Fig. 1. The [1-^{14}C] radioactivity was recovered mainly in MGDG (40%) and PC (30%) in control plants.

Table 2. Effects of heat shock on composition (mol%) of main molecular species of MGDG, DGDG and PC of rape leaf lipids

Molecular species	MGDG		DGDG		PC	
	Control	Stressed	Control	Stressed	Control	Stressed
18:3/16:3	77.1	72.0	5.3	—	—	—
18:3/18:3	18.2	19.8	71.2	69.0	24.4	15.0
18:2/18:3	1.1	3.3	1.5	t	25.8	6.2
16:0/18:3	0.8	4.5	17.8	30.0	17.7	36.5
18:2/18:2	—	—	—	—	10.0	10.4
18:1/18:3	—	—	—	—	5.0	5.2
16:0/18:2			4.2		11.4	10.2
18:1/18:2	—	—	—	—	4.0	2.6
18:0/18:3	—	—	—	—	—	7.0
16:0/18:1						6.2
Others	2.8	0.4		1	1.7	0.7

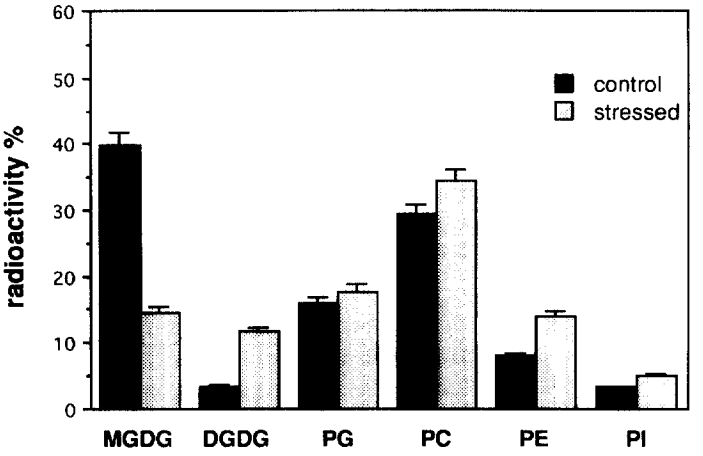

Fig. 1. Distribution of radioactivity in polar lipids from control or stressed plants after 24 h labelling by [1-^{14}C] acetate. Results are expressed as % of total lipid radioactivity and each value is the average of two independent assays.

After a heat shock, the incorporation of radioactivity into DGDG and MGDG decreased by 10% and 90%, respectively; the relative radioactivity decreased markedly in MGDG but remained quite constant or slightly increased in all other lipid classes (Fig. 1). Thus, heat treatment decreased the labelled MGDG to labelled DGDG ratio from 11.4 to 1.5, but did not affect so much DGDG synthesis and decreased the MGDG/DGDG ratio. These fluctuations have already been correlated with the heat tolerance of the photosynthetic apparatus of chloroplasts *in vivo* [6]. In contrast, the labelled phospholipid/galactolipid ratio (PL/GL) increased from 1.3 to 2.6 MGDG biosynthesis was thus apparently more sensitive to heat shock than that of DGDG and phospholipid.

Effects of heat shock on incorporation of [1-^{14}C] *acetate into fatty acids*

Table 3 shows the labelling of fatty acids in the main polar lipids of rape leaves 24 h following the deposition of [^{14}C]-acetate microdroplets onto control or stressed plants. In control plants, the main labelled fatty acids synthesized in leaf total lipids were palmitic acid ($C_{16:0}$) (19.5% of total fatty acid radioactivity), linoleic acid ($C_{18:2}$) (32.3%), linolenic acid ($C_{18:3}$) (26%). In MGDG, $C_{16:3}$ (32%) and $C_{18:3}$ (48.5%) were the most labelled fatty acids (Table 3). In DGDG, PG and PC, the fatty acids labelled the most were palmitic, linoleic and linolenic.

After a heat shock, there was a marked decrease in the amount of labelling of unsaturated fatty acid in total, as well as in polar lipids. For example, labelling of linolenic and hexadecatrienoic acids in MGDG decreased from 48% to 23% and from 32% to 13%, respectively (Table 3). The relative labelling of palmitic acid increased in DGDG, PG and PC and that of stearic acid increased in MGDG, DGDG and PC.

A 24 h heat shock produced a drastic decrease of labelled polyunsaturated fatty acids in phospholipids, as well as in galactolipids. On the other hand, the same heat shock increased the percentage of labelled palmitic acid in phospholipids and in galactolipids. To explain these modifications, it can be supposed

Table 3. Effect of heat shock on incorporation of sodium [1-^{14}C] acetate into rape polar lipid fatty acids (results expressed as % of total fatty acid radioactivity within a lipid class)

	MGDG		DGDG		PG		PC	
Lipids	Control	Stressed	Control	Stressed	Control	Stressed	Control	Stressed
$C_{16:0}$	2.0	27.1	31.6	63.8	52.0	74.7	17.6	45.2
$C_{16:1t}$	—	—	—	—	4.1	4.2	—	—
$C_{16:2}$	8.9	12.5	4.1	1.0	—	—	—	—
$C_{16:3}$	31.7	12.5	2.1	—	—	—	—	—
$C_{18:0}$	—	4.1	—	4.2	—	2.1	3.0	16.8
$C_{18:1}$	—	7.3	6.1	9.6	5.1	11.5	10.0	18.9
$C_{18:2}$	8.9	13.5	25.5	12.8	23.5	7.4	57.3	13.7
$C_{18:3}$	48.5	22.9	30.6	8.5	15.3	—	12.1	5.3

Mean values from four experiments.

that the heat shock partially inhibited chloroplastic palmitoyl-ACP elongase in chloroplasts. However, we also observed that the labelled stearic acid content increased in all polar lipids; thus, a simultaneous inhibition of chloroplastic stearoyl-ACP desaturase and an increased export of stearic acid towards cytoplasm can also be postulated. In good agreement with this hypothesis, Cheesbrough [7] has demonstrated the regulation of stearate biosynthesis by growth temperature.

Effects of heat shock on incorporation of [1-^{14}C] *acetate into molecular species*

In PC and DGDG, we observed, after a heat shock, a decrease in the relative labelling of polyunsaturated molecular species, such as 18:3/18:3, 18:2/18:3 and 18:2/18:2, and an enhancement of the relative labelling of more saturated molecular species, such as 16:0/18:3 and 18:0/18:3, 16:0/18:1 and 18:0/18:2 (Fig. 2). In PC, the absolute lowering of the biosynthesis of the 18:3/18:3 molecular species is confirmed by the decrease in the specific activity of $C_{18:3}$ in this molecular species (1702 dpm μg^{-1} FA in controls; 171 dpm μg^{-1} FA in stressed plants). On the other hand, the specific activities of the fatty acids in more saturated molecular species increased after heat shock.

The heat shock caused a decrease in the labelling of prokaryotic MGDG molecular species from 93.6% to 77.7% and the percentage of labelled eukaryotic MGDG molecular species increased from 6.4% to 22.3%. The ratio of labelled prokaryotic molecular species vs eukaryotic molecular species varied from 14.6 in control plants to 3.4 in stressed plants. The relative labelling of prokaryotic MGDG containing more unsaturated molecular species (18:3/16:3 and 18:3/16:2) decreased, while the prokaryotic MGDG comprising more saturated molecular species slightly increased (18:3/16:1, 18:2/16:2, 18:3/16:0 and 18:2/16:0). On the other hand, the percentage of more saturated MGDG molecular species, both of eukaryotic (16:0/18:3 and 16:0/18:2) and prokaryotic (18:3/16:0 and 18:2/16:0) types, increased. Thus, considering the prokaryotic molecular species, 18:3/16:0 MGDG increased from 0.5% to 13.1% and 18:2/16:0 MGDG increased from 2.3% to 10.5% (Fig. 2). In marked contrast, 18:3/16:3 MGDG decreased from 65.7% to 20.2% after heat shock.

The quantitative variations of molecular species constituting MGDG in rape leaves showed that the unsaturation of this lipid class did not vary so much after a 48 h heat shock. To explain these results we suppose that an increase in the saturation of unlabelled lipids could not be detected after 48 h of heat shock, whereas the decrease in unsaturation of newly synthesized labelled lipids became fully apparent after 48 h of heat shock. On the other hand, the mass of DGDG was not significantly altered by the heat shock and the incorporation of [1-^{14}C] acetate into this particular lipid decreased only by ca 10%: thus the biosynthesis of DGDG could be relatively insensitive to temperature. This is in good agreement with out quantitative data.

Considering the reaction sequence known for the prokaryotic MGDG species [8]:

$$18:1/16:0 \rightarrow 18:1/16:1 \rightarrow 18:2/16:2 \rightarrow 18:3/16:3$$

we propose that the palmitoyl MGDG desaturase could be specifically inhibited by the heat shock treatment, thus giving the following reaction sequence:

$$18:1/16:0 \rightarrow 18:2/16:0 \rightarrow 18:3/16:0$$

which would explain the relative accumulation of 18:3/16:0 and 18:2/16:0 molecular species after a heat shock.

In conclusion, a heat treatment reduced the activity of several enzymes involved in the chloroplastic pathway of MGDG biosynthesis. Chloroplastic (prokaryotic) molecular species of MGDG represented 93.6% of total MGDG in control plants and 77.7% in plants shocked at 36°. The biosynthesis of eukaryotic molecular species of MGDG (18:3/18:3 MGDG and 16:0/18:3 MGDG) was less affected by a heat shock than the synthesis of prokaryotic molecular species. Thus, a 48 h heat shock at 36° had the same consequences as growth at a constant high temperature [9]. These results could be explained by the presence of different types of fatty acid desaturases in plant cells as suggested by Williams *et al.* [10] and Gibson *et al.* [11]; the synthesis of certain desaturases would be controlled by temperature and other desaturases would function equally well at all temperatures.

In good agreement with these proposals, we observed that a heat shock strongly inhibited the chloroplastic palmitoyl MGDG desaturase but only partially the chloroplastic stearoyl ACP desaturase.

However, it must be noted that Brockman *et al.* [12] found that in *Arabidopsis thaliana* calli, the prokaryotic pathway of linoleate desaturation predominated in tissue grown at 28°, while at 18°, the eukaryotic pathway predominated. Thus, in different species and under different culture conditions one or the other pathway of fatty acid desaturation (chloroplastic or extrachloroplastic) could be induced by low temperature or be inhibited by a heat shock.

EXPERIMENTAL

Plant material

Brassica napus L. var. Bienvenue, was grown in a growth chamber with a 16 h photoperiod at 30°/21° light/dark. After one week, plants were transferred to a greenhouse at 12°. For thermic stress expts, 4 to 5-week-old plants were transferred from 12° to 36° during 48 h.

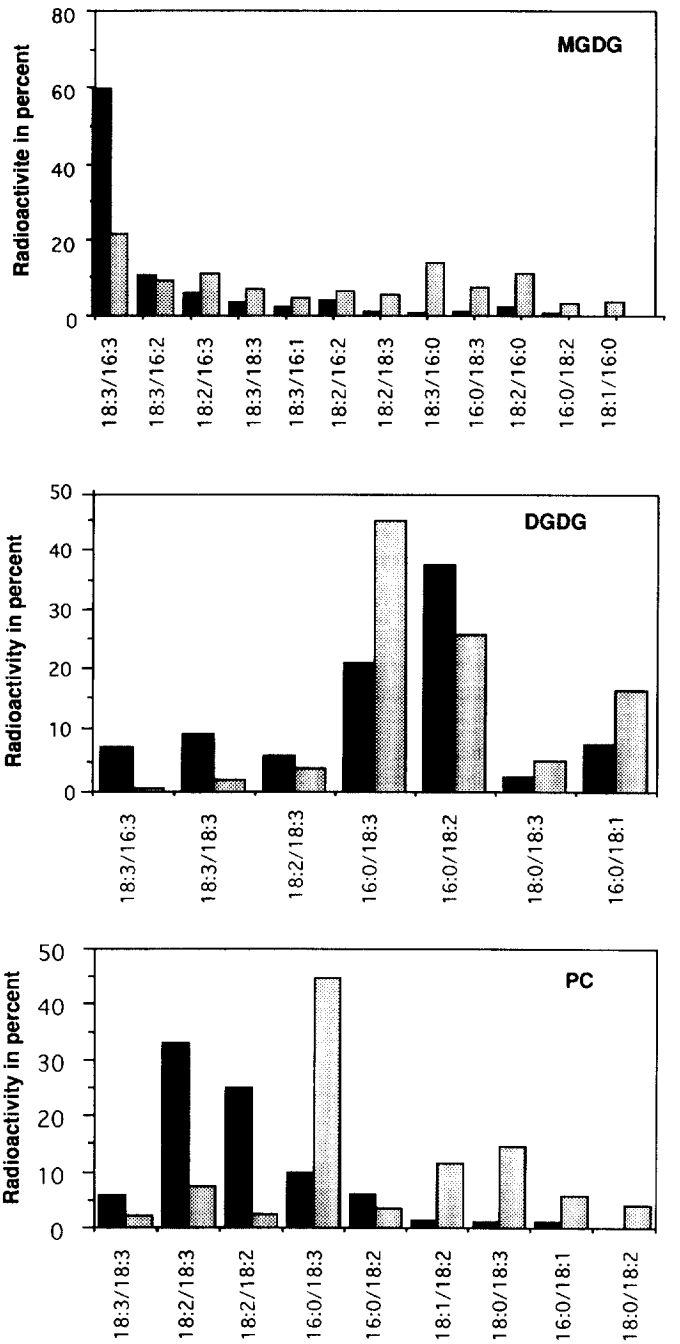

Fig. 2. Labelling of MGDG, DGDG and PC molecular species 24 h after treatment with [1-^{14}C] acetate. ■ control and ▨ stressed.

In vivo-*labelling experiments*

Microdroplets of [1-^{14}C] Na acetate (2.1 GBq mmol^{-1}) deposited on the leaf surface of 24 h stressed and control plants were incorporated during 24 h at 36° or 12°, respectively.

Lipid analysis

Lipids were extracted according to Ref. [13]. Polar lipids were separated by silica gel TLC [14]. After spraying with primuline and observation under UV light, lipid spots were scraped off the plates, eluted and counted in a liquid scintillation counter or fatty acids were transmethylated according to Ref. [15] and analysed by radio-GC [16]. Polar lipids were separated by HPLC on a column packed with silica acid (7.8 × 300 mm Waters S.A.) [17]. Separation of molecular species was performed by HPLC [18] on a 5 μm ODS ultraphere HPLC column (Beckman) and collected. For radioactive samples, radioactivity in

each compound eluted from the HPLC column was measured using a continuous flow apparatus. Lipids from each HPLC fr. were extracted [13]. For quantification on a mass basis, an appropriate amount of int. standard (heptadecanoic acid) was added to the pooled eluate representing each peak. Fatty acid Me esters from each lipid class were prepd by transmethylation [19] and analysed by radio-GC [16]. To determine fatty acid positions, each molecular species was hydrolysed with purified lipase A_1 [20]. Products of hydrolysis, 2-acyl lysoMGDG and free fatty acids were separated by TLC [14] and then counted for radioactivity.

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REFERENCES

1. Santarius, K. A. and Weiss, E., in *Plant Membranes: Structure, Assembly and Function*, ed. J. L. Harwood and T. J. Walton. Biochemical Society, London, 1988, p. 97.
2. Pearcy, R. W., *Plant Physiol.*, 1978, **61**, 484.
3. Quinn, P. J., in *Biological role of plant lipids*, ed. P. A. Biacs, K. Gruiz, T. Kremmer and H. K. Lichtenthaler. 1989, p. 511.
4. Williams, J. P. and Khan, M. U., *Biochim. Biophys. Acta*, 1982, **713**, 177.
5. Roughan, P. G. and Slack, C. R., *Ann. Rev. Plant Physiol.*, 1982, **33**, 97.
6. Suss, K. H. and Yordanov, I. T., *Plant Physiol.*, 1986, **81**, 192.
7. Cheesbrough, T. M., *Plant Physiol.*, 1990, **93**, 555.
8. Browse, J. and Somerville, C. R., *Ann. Rev. Plant Physiol. Mol. Biol.*, 1991, **42**, 467.
9. Williams, J. P., Mitchell, K. and Khan, M., in *Metabolism, Structure and Function of Plant Lipids*, ed. P. K. Stumpf, J. B. Mudd and W. D. Nes. Plenum Press, New York, 1987, p. 433.
10. Williams, J. P., Mitchell, K. and Khan, M. U., *Biochim. Biophys. Acta*, 1992, **1125**, 62.
11. Gibson, S., Arondel, V., Iba, K. and Somerville, C., *Plant Physiol.*, 1994, **106**, 1615.
12. Brockman, J. A., Norman, H. A. and Hildebrand, D. F., *Phytochem.*, 1990, **29**(5), 1447.
13. Bligh, E. G. and Dyer, W. J., *Can. J. Biochem. Physiol.*, 1959, **37**, 103.
14. Lepage, M., *Lipids*, 1967, **2**, 244.
15. Metcalfe, L. D., Schmitz, A. A. and Palka, J. R., *Anal. Chem.*, 1966, **38**, 514.
16. Galle, A. M., Joseph, M., Demandre, C., Guerche, P., Dubacq, J. P., Oursel, A., Mazliak, P., Pelletier, G. and Kader, J. C., *Biochim. Biophys. Acta*, 1993, **1158**, 52.
17. Demandre, C., Trémolières, A., Justin, A. M. and Mazliak, P., *Phytochemistry*, 1985, **24**, 481.
18. Demandre, C., Trémolière, A., Justin, A. M. and Mazliak, P., *Biochim. Biophys. Acta*, 1986, **877**, 380.
19. Carreau, J. P. and Dubacq, J. P., *J. Chromato.*, 1978, **151**, 384.
20. Fisher, W. E., Heinz, E. and Zeus, H., *Hoppe-Seyler's Z. Physiol. Chem.*, 1973, **354**, 1115.