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# Differential effects of linolenic acid and methyl jasmonate on the degradation of chlorophylls and carotenoids of senescing barley leaves

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## SUMMARY

This study compares the effects of linolenic acid and methyl jasmonate, applied to isolated thylakoids and sections of senescent barley leaves, on photosynthetic pigment degradation. With regard to total chlorophylls, linolenic acid has little effect on leaf sections, whereas its effect on thylakoids is strong and rapid. Methyl jasmonate, on the other hand, has a great effect on sections and a very weak effect on thylakoids. Whereas in methyl jasmonate-treated sections chlorophyll a is degraded more quickly than chlorophyll b throughout the 3 days of treatment, in linolenic acid-incubated thylakoids the same only occurs during the first 12 hours. With regard to total carotenoid degradation, methyl jasmonate has no effect on sections whereas linolenic acid strongly stimulates such degradation in thylakoids. The general scavenger of free radicals, n-propyl-gallate, only inhibits chlorophyll and carotenoid degradation by linolenic acid in thylakoids. Chlorophyll retention in relation to effector concentration shows different kinetics for linolenic acid and methyl jasmonate. The results suggest different mechanisms for the action of linolenic acid and methyl jasmonate on photosynthetic pigments. Although the action of linolenic acid can be rather complex, one possibility is that it acts principally through free radicals during the last stages of senescence or stress, while it could act most as precursor of methyl jasmonate during the earlier stages.

*Key-words:* carotenoid degradation, chlorophyll degradation, free radicals, linolenic acid, methyl jasmonate, senescence.

## INTRODUCTION

Among the internal factors that regulate leaf senescence are plant growth regulators (Stoddart & Thomas 1982; Noodén & Leopold 1988; Smart 1994). The strongest promoting regulators in this developmental process are abscisic acid (ABA) and jasmonates, which are chemically derived from cyclopentanone. The most active jasmonate seems to be methyl jasmonate (JA-Me) (Sembdner & Gross 1986; Weidhase *et al.* 1987; Gross & Parthier 1994), which was first indicated as stimulating senescence in excised oat leaves (Ueda *et al.* 1981).

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*Abbreviations*: ABA, abscisic acid; JA-Me, methyl jasmonate; LNA, linolenic acid; n-PG, n-propyl-gallate.

Linolenic acid (LNA) has been found to stimulate chlorophyll degradation and senescence, chlorophyll degradation occurring through a LNA-dependent thylakoidal enzyme according to Lüthy *et al.* (1984) and Thomas *et al.* (1985). However, Dupont & Siegenthaler (1986) found no experimental evidence to support the existence of the thylakoidal enzyme and they indicated a non-enzymatic mechanism for chlorophyll a degradation, perhaps involving free radicals. Cuello & Lahora (1993), who also found no evidence of this thylakoidal enzyme, suggested a non-enzymatic chlorophyll degradation process through free radicals derived from LNA. In leaves of 18:3 plants, in which the polyunsaturated fatty acid components of galactolipids are almost exclusively 18:3 acids, LNA represents more than 90% of total esterified fatty acids in thylakoidal galactolipids (Douce & Joyard 1980). It has also been reported that LNA is released from thylakoids in *in vitro* experiments (O'Sullivan *et al.* 1987; O'Sullivan & Dalling 1989). In addition, LNA stimulates the senescence of oat leaf sections both in light and darkness (Ueda & Kato 1982) and the senescence of the pulvinus in bean petiole explants in light (Ueda *et al.* 1991).

Since it has been demonstrated that jasmonic acid is biosynthetized from LNA, which is transformed by lipoxygenase to first give 13-hydroperoxy-linolenic acid (Vick & Zimmerman 1987), it might be expected that JA-Me and LNA would have the same effect in many assays. Nevertheless, to the best of our knowledge, this has only been demonstrated in the induction of tendril coiling (Falkenstein *et al.* 1991) and in the induction of proteinase inhibitor synthesis (Farmer & Ryan 1992).

We have studied the comparative effects of LNA and JA-Me, applied to barley leaf sections and isolated thylakoids, on chlorophyll and carotenoid losses. The results show that LNA has a degradative effect on thylakoids, at least partly through free radicals, and that this effect is independent of its conversion to JA-Me.

## MATERIALS AND METHODS

### *Plant material*

Barley seedlings (*Hordeum vulgare* L. var. Hassan) were grown for 14 days as described by Cuello *et al.* (1987). All the experiments were carried out with 4 cm apical sections (containing 1·52 mg chlorophyll $g^{-1}$, measured by the formula of Lichtenthaler & Wellburn (1983)) of the oldest leaf, discarding 0·5 cm of the tip.

### *Isolation of chloroplasts*

For the isolation of chloroplasts, 6 g of recently detached leaf sections were homogenized at 0–5°C in a Sorvall Omni-mixer (with four pulses of 4 s at intensity 6) with 37·5 ml of a buffer (E) containing 0·35 M sucrose, 25 mM Na-HEPES, 2 mM $Na_2$-EDTA and 2 mM ascorbic acid (pH 7·6). The homogenate was strained through four layers of muslin and centrifuged at 200 *g* for 5 min. The supernatant was centrifuged at 2500 *g* for 10 min and the pellet was washed with 10 ml of buffer E and newly precipitated. The washed chloroplast pellet was finally resuspended in 20 mM tricine hypotonic buffer, pH 7·5, to 250 mg chlorophyll $l^{-1}$(thylakoid suspension).

### *Incubation of leaf sections and isolated thylakoids*

Samples of five sections (0·23 g) or 0·5 g of these sections were incubated for 24–72 h in darkness at 25°C in Petri dishes containing one circle of filter paper soaked with

4 ml of assayed regulator dissolved in 0·025% (w/v) Tween-20+Span-60 (7:3, w/w) (TS) in water.

The thylakoids were incubated in graduated test tubes for 1–72 h in darkness at 25°C with gentle shaking. The reaction medium consisted of: 0·025% (w/v) TS, 4 mM tricine, thylakoid suspension (35 mg chlorophyll $l^{-1}$, measured according to Lichtenthaler & Wellburn (1983)) and the regulator at the assayed concentration, pH 7·5. Control media (without regulator) were made in all cases. The degree of LNA peroxidation was taken to be that at the beginning of the reaction, because in the reaction medium (without thylakoids) there was no absorbance change at 234 nm at any time during incubation. This indicated a negligible transformation of LNA to its hydroperoxide in the reaction medium (Ueda *et al.* 1991).

Membrane permeability changes (Cuello *et al.* 1994) were followed by measuring conductivity in 25 ml of solution of senescence effector in 0·025% (w/v) TS floating 10 similar 4-cm blade sections (about 0·46 g) for different incubation periods.

*Measure of LNA peroxidation degree*

The content of peroxides in the initial 2·8 mM LNA solutions used, prepared by shaking in 0·05% (w/v) TS, was determined by the method of Asakawa & Matsushita (1978), with minor modifications. The KI-silica gel reagent was prepared with 50 g of silica gel (Merck no. 60, 70–230 mesh) soaked with 100 ml of 10% (w/v) KI solution, as described by Asakawa & Matsushita (1978). The reaction medium consisted of a mixture of 0·15 ml LNA solution, 0·85 ml 0·05% (w/v) TS and 0·75 g KI-silica gel, which was incubated for 5 min at 30°C. To this, 2 ml 50% (v/v) ethylic alcohol were immediately added and, after shaking, 15 ml 0·01 N HCl and 0·5 ml 1% (w/v) starch. The medium was immediately centrifuged for 10 min at 2000 ***g*** and the optical density at 560 nm in the supernatant measured. A standard curve with $H_2O_2$ (Merck) was made.

*Chlorophyll measurements and other determinations*

The chlorophyll contents were determined by various methods. Relative chlorophyll changes (after leaf section incubations) were determined according to Schistad & Nissen (1984). The absolute contents of total (and chlorophylls a and b) chlorophylls in sections or thylakoidal suspensions were determined, both by Arnon (1949) and by Lichtenthaler & Wellburn's (1983) methods, in both cases from its 80% acetone solutions. For total chlorophylls, the value obtained by Arnon's formula was higher than that obtained by Lichtenthaler & Wellburn's formula by a factor of 1·11. The latter method was also used to measure the total carotenoid contents. Conductivity measurements were obtained with a Crison Micro CM 2100 conductimeter.

## RESULTS

The effects of JA-Me and LNA, the latter in the presence and absence of n-propyl-gallate (n-PG), on chlorophyll retention by incubated leaf sections are shown in Fig. 1, which also shows the effects of kinetin and abscisic acid (ABA), hormones which are known to retard and stimulate leaf senescence, respectively. JA-Me had a very strong stimulatory effect on chlorophyll degradation, in contrast to the very weak effect of LNA despite the high concentration of LNA (1·4 mM) compared with that of JA-Me (45 μM). The weak stimulation of the chlorophyll degradation by LNA is inhibited, at least partly, by n-PG, suggesting that the effect of LNA may to be due to free radicals

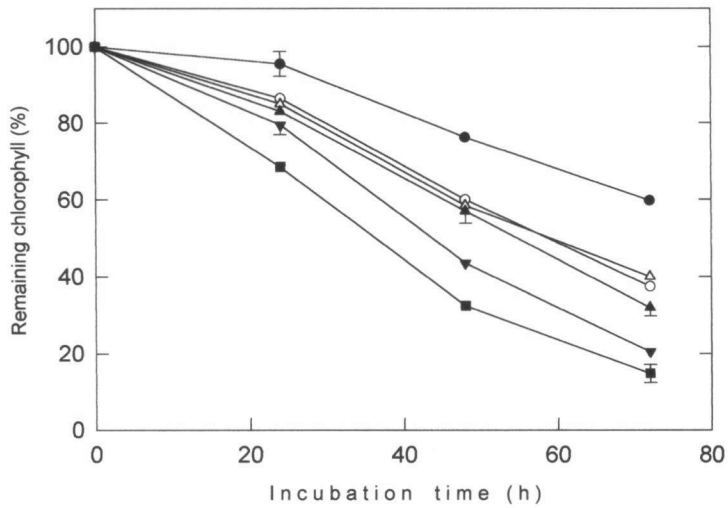


**Fig. 1.** Influence of different senescence effectors on chlorophyll retention in leaf sections incubated for 72 h. The sections were incubated with: 47 μM kinetin (●), 34 μM ABA (▼), 45 μM JA-Me (■), 1·4 mM LNA (▲), 1·4 mM LNA+0·14 mM n-PG (△) and control medium (○). Chlorophyll was measured by the method of Schistad & Nissen. The values are means ± SE of 3–9 independent determinations. Only SE higher than 2 are represented. The peroxidation degree of the LNA used was 0·65 μequivalents $H_2O_2$ $ml^{-1}$.

derived from LNA. However, n-PG alone had an insignificant effect on chlorophyll degradation in sections and no effect on the stimulation of the chlorophyll degradation by JA-Me (results not shown). Kinetin retarded and ABA accelerated (although less than JA-Me) the loss of chlorophyll (Fig. 1), as is to be expected from its effects on leaf senescence.

The effects of LNA and JA-Me on membrane permeability can be deduced from Fig. 2, in which are represented the conductivities of the incubation media of the sections versus the incubation time. Both LNA and JA-Me strongly increased the conductivity. However, the effect of LNA was faster than that of JA-Me since after 24 h of incubation the former showed near maximum values, whereas the latter still did not show any effect. n-PG slightly intensified the LNA effect while ABA had a less pronounced stimulating effect on conductivity than JA-Me (Fig. 2). The strong and rapid increase in conductivity caused by LNA indicates increased permeability of the cellular membranes and suggests, therefore, that it can penetrate the membranes. According to this, the weak effect of LNA on chlorophyll degradation in leaf sections (Fig. 1) is unlikely to be due to the LNA oxidation products not having access to the chlorophylls. In any case, the weak effect of LNA on chlorophyll degradation in sections (Fig. 1) and its strong stimulation of membrane permeability (Fig. 2) indicate that the increases of these two parameters may not occur synchronously in certain conditions of section senescence.

When LNA and JA-Me were applied to isolated thylakoids, as opposed to leaf sections, contrary quantitative effects were found (Fig. 3). Whereas JA-Me stimulated chlorophyll loss to a very small degree, LNA had a comparatively strong effect. Moreover, this effect was very fast (during the first hour of incubation the chlorophyll decreased to 63%) although after 5 h of incubation the diminution was relatively small (Fig. 3). The effect of LNA was partially inhibited by n-PG (Fig. 3), which did not seem

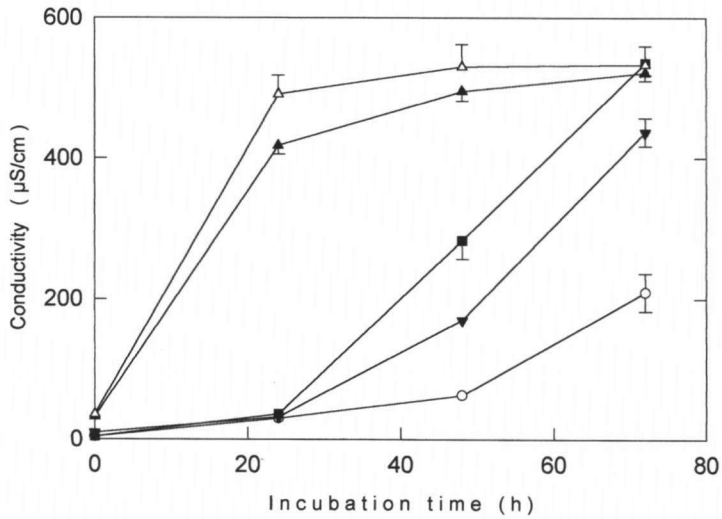


**Fig. 2.** Effects of various senescence stimulators on the electrical conductivity of the floating medium used for leaf sections in the dark. The sections were incubated with 34 μM ABA (▼), 45 μM JA-Me (■), 1·4 mM LNA (▲), 1·4 mM LNA+0·14 mM n-propyl-gallate (△) and control medium (○). Each indicated value is the mean ± SE of 8–12 independent experiments. Only SE greater than 10 are represented. The peroxidation degree of the LNA used was 1·30 μequivalents $H_2O_2$ $ml^{-1}$ (S: Siemens).

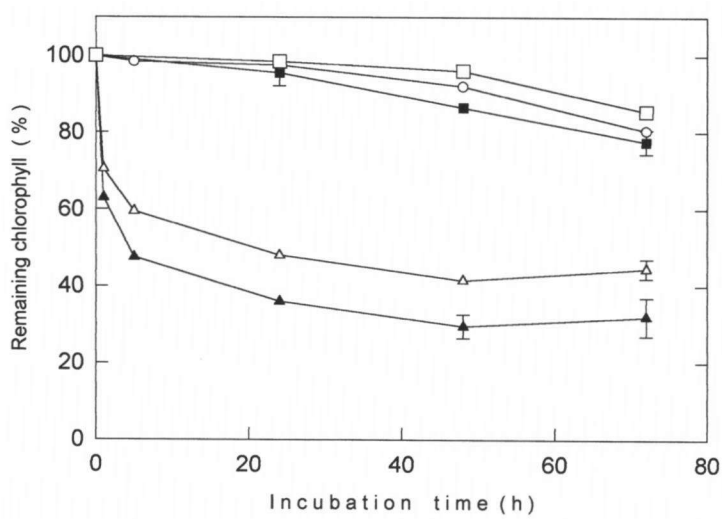


**Fig. 3.** Effects of LNA and JA-Me on chlorophyll retention by thylakoids incubated for 72 h. The thylakoids were incubated with 45 μM JA-Me (■), 1·4 mM LNA (▲), 1·4 mM LNA+0·14 mM n-PG (△), 0·14 mM n-PG (□) and control medium (○). Chlorophyll was measured by Arnon's method. The values are means ± SE of 3–7 independent determinations. Only SE higher than 2 are represented. The peroxidation degree of the LNA used was as in Fig. 1.

to be at a limiting concentration in the reaction because the retention values obtained with 0·14 mM n-PG (shown in Fig. 3) were identical to those obtained with 1·4 mM n-PG (results not shown). n-PG (0·14 mM) alone also inhibits the small chlorophyll loss in isolated thylakoids after 24 h of incubation (Fig. 3).

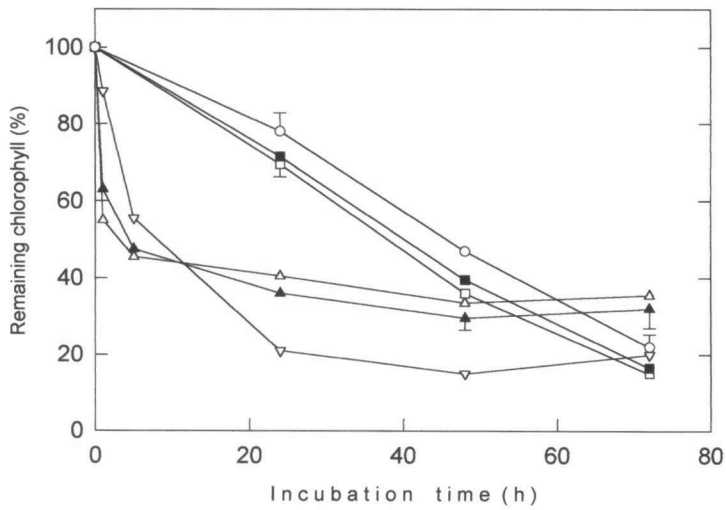


**Fig. 4.** Effects of JA-Me applied to sections and LNA applied to isolated thylakoids on the retention of chlorophylls a and b during 72 h. Total chlorophylls, chlorophyll a and chlorophyll b, were measured in sections treated with 45 μM JA-Me (■, □ and ○, respectively) and in thylakoids incubated with 1·4 mM LNA (▲, △ and ▽, respectively). The values are means ± SE of 3–4 independent determinations. Only SE >2 are represented. The peroxidation degree of the LNA used was as in Fig. 1. The initial chlorophyll contents measured by the method of Lichtenthaler & Wellburn were: 1·23 mg chlorophyll a $g^{-1}$ leaf and 0·29 mg chlorophyll b $g^{-1}$ leaf in sections, and 28·34 mg chlorophyll a $l^{-1}$ and 6·66 mg chlorophyll b $l^{-1}$ in the thylakoid medium.

LNA and JA-Me also had different effects on the relative degradative rates of the chlorophylls a and b (Fig. 4). Whereas the application of JA-Me to sections resulted in greater degradation of chlorophyll a than chlorophyll b for all the incubation times assayed, the inverse result occurred in thylakoids incubated with LNA after 12 h of incubation. Until 12 h of incubation, as with JA-Me applied to sections, chlorophyll a was degraded more intensively than chlorophyll b (Fig. 4). For the purpose of this work, the slightly different values of remaining total chlorophyll percentages in sections treated with JA-Me (Figs 1 and 4), which were measured by Schistad & Nissen (1984) and Arnon (1949), respectively, are irrelevant. On the other hand, according to results which are not shown, in sections treated with ABA and in attached leaves that senesce in the dark, the relative degradation velocities of chlorophylls a and b are similar to those found after application of JA-Me to sections (Fig. 4).

The differential behaviour of LNA and JA-Me was particularly evident when chlorophyll retention was studied as a function of effector concentration (Fig. 5). LNA began to affect isolated thylakoids (95% chlorophyll retention) at $4{\cdot}2\ 10^{-4}$ M, a concentration at which JA-Me has maximum effect on sections (only 18% of chlorophyll retained). In addition, whereas JA-Me showed approximate proportionality between chlorophyll retention and the logarithm of its concentration for $4{\cdot}5\ 10^{-8}$–$4{\cdot}5\ 10^{-4}$ M, the retention obtained with LNA was proportional to concentration in the relatively very short range in which it was effective ($4{\cdot}2\ 10^{-4}$–$2{\cdot}8\ 10^{-3}$ M) (Fig. 5).

When the effects of JA-Me and LNA on total carotenoid degradation were studied, the results were very significant (Fig. 6). JA-Me had no, or negligible, effect on carotenoid loss in sections, whereas LNA degraded them at a very fast rate in isolated

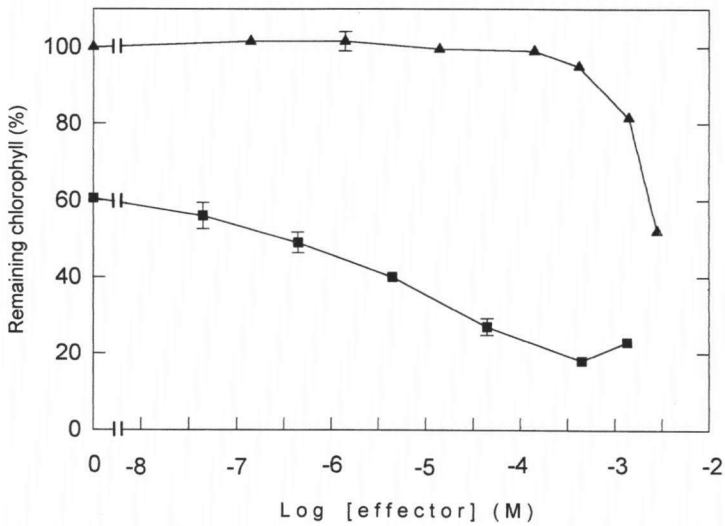


**Fig. 5.** Effects of variable concentrations of JA-Me applied to sections (■) and LNA applied to isolated thylakoids (▲) on chlorophyll retentions. The sections were incubated for 48 h and the thylakoids for 5 h. The values are means ± SE of 5–8 independent assays. Only SE >2 are represented. The peroxidation degree of the LNA used was 1·75 μequivalents $H_2O_2$ $ml^{-1}$. In comparison, the effects on chlorophyll retentions, of LNA applied to isolated thylakoids, were insignificant. The total chlorophyll in sections was measured by the method of Schistad & Nissen, and in thylakoids by Arnon's method.

thylakoids (after 1 h of incubation only 36% was retained, compared with almost 100% in the control) (Fig. 6). Moreover, n-PG strongly inhibited the carotenoid loss produced by LNA, particularly over short times and, in contrast to that occurring with chlorophylls, the inhibition increased with the concentration of n-PG (1·4 mM n-PG was more effective than 0·14 mM n-PG) (Fig. 6). Besides, n-PG inhibited the stimulation of carotenoid loss by LNA to a greater extent than that of chlorophyll loss, particularly at short times (Figs 3 and 6). n-PG alone had no significant effects, at least for the first 5 h, on the carotenoid loss, either in sections or isolated thylakoids (results not shown). Note that, according to Lichtenthaler & Wellburn's formulae, the ratio of total chlorophyll (1·52 mg $g^{-1}$) and carotenoid (0·26 mg $g^{-1}$) contents in recently detached leaves is almost the 4·3:0·7 described for leaf pigments by several authors, including Barceló *et al.* (1992).

A relatively intense stimulation of carotenoid degradation is also produced in thylakoids incubated with 45 μM LNA (the same concentration of JA-Me used). In these conditions the carotenoid levels at 24 h and 48 h were 90% and 78%, respectively (mean values of three independent assays, with SE <4) of the control values without LNA.

It is interesting that an inverse relation was found between the remaining chlorophyll (or carotenoid) percentage in thylakoids incubated for 1 h and the degree of peroxidation of the LNA used (Fig. 7). The greatest chlorophyll (and carotenoid) degradation rates were found at the lowest peroxide concentrations which contrasts with the direct relation observed in a previous paper (Cuello & Lahora 1993) between chlorophyll degradation in thylakoidal medium and the malondialdehyde concentration of the LNA solution used.

 

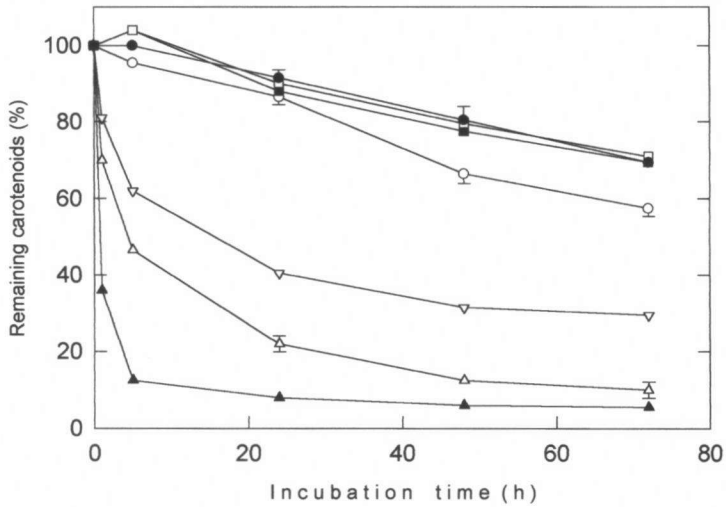


**Fig. 6.** Effects of JA-Me applied to sections and LNA applied to isolated thylakoids on the total carotenoid retentions. The sections were incubated with control medium (●) and 45 μM JA-Me, without (■) and with (□) 1·4 mM n-PG. The thylakoids were incubated with control medium (○) and 1·4 mM LNA, without (▲) and with 1·4 mM (▽) or 0·14 mM (△) n-PG. The values are means ± SE of 4–6 independent determinations. Only SE >2 are represented. The peroxidation degree of the LNA was as in Fig. 1. The initial carotenoid content in the thylakoidal medium was 6·42 mg $l^{-1}$, and in the leaf sections 0·26 mg $g^{-1}$ leaf.

## DISCUSSION

Although chlorophyll degradation is relatively fast in leaf sections incubated without effector (control), Figs 1 and 3 show the differential effects of JA-Me and LNA on chlorophyll retention in sections and isolated thylakoids from senescing leaves. The concentration of LNA is relatively high, but this can also be the case in *in vivo* conditions, e.g. in chloroplasts of cold and dark-treated plants (Kaniuga & Michalski 1978). LNA at 1·4 mM concentration was used when the compound was assayed as cofactor of the hypothetic thylakoidal enzyme 'chlorophyll oxidase' (Lüthy *et al.* 1984; Thomas *et al.* 1985), and at an even higher concentration when it was used as leaf senescence factor (Ueda & Kato 1982). The increase in conductivity, produced by the addition of LNA to the incubation medium of the sections, but not inhibited by n-PG (Fig. 2), suggests that free radicals do not participate in the process and that LNA and its peroxidation products must have penetrated the cells. However, it is possible that the free radicals are inactivated by natural scavengers (such as phenolic compounds) which would explain the strong effect of LNA on isolated thylakoids but not on leaf sections. Nevertheless, the rapid lowering of thylakoidal chlorophyll levels by LNA in the presence of n-PG at a non-limiting concentration (Fig. 3) indicates that products other than free radicals participate in the reaction. In any case, the rapid effect of LNA is in accordance with the operation of an exclusively chemical reaction between product(s) of LNA peroxidation and chlorophylls (Cuello & Lahora 1993).

It is known that free LNA is toxic to cells, because of its action as detergent on the cellular membranes (Thomas 1986). This is evident from the rapid increases in conductivity (Fig. 2), which are not related to senescence.

During JA-Me stimulation of chlorophyll degradation in sections, chlorophyll a is degraded more intensively than chlorophyll b, which leads to a diminution in the

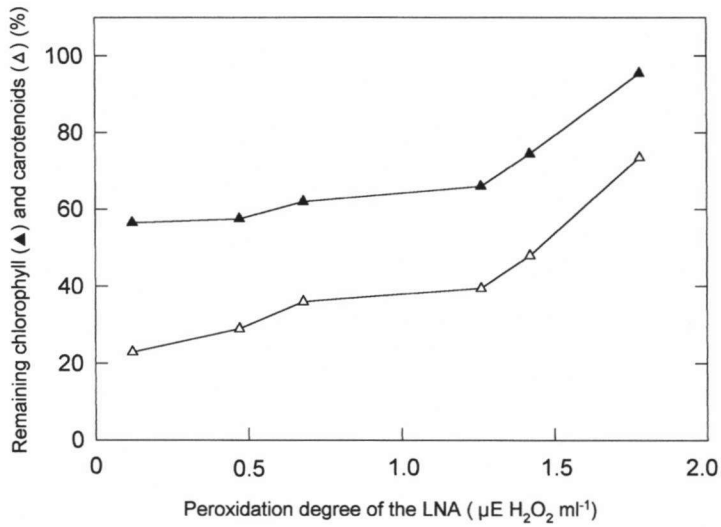


**Fig. 7.** Effect of the peroxidation degree of the LNA, applied to isolated thylakoids, on the total chlorophyll and carotenoid retentions. The thylakoids were incubated for 1 h with 1·4 mM LNA, and the values are the means of two independent experiments. No individual value deviates more than 10% from the mean. The initial pigment contents, measured by the method of Lichtenthaler & Wellburn, were: 35·00 mg chlorophyll $l^{-1}$ and 6·42 mg carotenoids $l^{-1}$ (E: equivalent).

chlorophyll a/chlorophyll b ratio as occurs in natural senescence (Hendry *et al.* 1987). However, when LNA is applied to thylakoids, the relative retention of chlorophylls a and b is reversed after 12 h of incubation (Fig. 4). This is probably due, at least in part, to secondary reactions in which the peroxidation products of LNA react with thylakoid chlorophylls producing absorbance changes at 645, 646 and 663 nm (Cuello & Lahora 1993), the wavelengths used for chlorophyll determinations.

The effects of variable concentrations of JA-Me and LNA on chlorophyll retention (Fig. 5) also indicate that these effectors act through different action mechanisms. The proportionality between the effect of JA-Me and its concentration logarithm for four orders of magnitude suggests a hormonal behaviour, whereas the proportionality between the effect of LNA and its concentration suggests the operation of a chemical reaction. On the other hand, the ineffectiveness of JA-Me on chlorophyll degradation in isolated thylakoids is in accordance with the action of JA-Me through extrachloroplastic receptors. In this respect it should be indicated that jasmonate-induced proteins are synthetized in the cytoplasm (Sembdner & Parthier 1993).

LNA (but not JA-Me) strongly stimulates carotenoid degradation, which depends, at least in part, on the free radicals produced by LNA peroxidation (Fig. 6). This effect is in accordance with the spectral changes of LNA treated thylakoidal suspensions (the shoulder at 470 nm disappears) and with the results of the chromatographic analysis of these media (the xanthophylls disappear) (Cuello & Lahora 1993). Dupont & Siegenthaler (1986) described the inhibition of chlorophyll a and β-carotene degradation by free radical scavengers. On the other hand, the ineffectiveness of JA-Me on the diminution of carotenoid levels in senescent leaf sections is in accordance with the intense yellow colour of the leaf sections after several days of treatment. The possible stimulation of carotenoid biosynthesis by JA-Me, which stimulates β-carotene synthesis, cannot be ruled out (Perez *et al.* 1993; Saniewski & Czapski 1983).

The results of Fig. 7 suggest that LNA hydroperoxides themselves are not decisive agents in pigment degradation in isolated thylakoids, at least during the first stages. Instead, free radicals can be important reactives, because of the direct relation between chlorophyll degradation and the malondialdehyde concentration of the LNA solution used (Cuello & Lahora 1993). It is possible that during senescence or stress LNA acts, at least in part, by producing free radicals which react chemically with chlorophylls and carotenoids. It is therefore important to know the free radical content (e.g. superoxide anion), rather than the degree of peroxidation of the LNA used in the assays. It would be also inadequate to characterize the initial LNA preparation used with regard to its malondialdehyde content, because it is not generally accepted that the amount of malondialdehyde in the LNA-solution is an indication of the concentration of free radicals.

Although the application of LNA to isolated thylakoids in this work does not faithfully reproduce the *in vivo* situation, it permits us to study the more direct effects of LNA on the thylakoidal pigment degradation. The results, although confirming that the free radicals participate (Cuello & Lahora 1993), indicate that they are not unique agents because high n-PG concentrations do not abolish the effects of LNA. Moreover, the free radicals have a more intense effect in stimulating carotenoid than chlorophyll degradation. The chemical degradation of pigments by LNA seems to be a relatively complex process carried out by LNA oxidation products, among which free radicals are only a part.

There is evidence to show that JA-Me participates in defence responses during stress situations (Gross & Parthier 1994). However, when LNA acts as JA-Me precursor, it may not usually be at a limiting level, since water stress induces the diminution of the LNA content in cotton chloroplasts (Ferrari-Iliou *et al.* 1984) and stressed cherry leaves contain significantly reduced levels of LNA compared with those of healthy leaves (Schmitt & Feucht 1993). It is possible that during senescence and stress LNA acts through two sequential mechanisms, first as JA-Me precursor and, later, on the threshold of leaf cell disorganization, by producing free radicals (among other products) which would strongly degrade the carotenoids (Fig. 6). It would explain why the carotenoids are retained in leaves until the terminal stages of the senescence process (Woolhouse 1984; Hendry *et al.* 1987).

It cannot be ruled out that the two LNA action mechanisms proposed are related in natural conditions. JA-Me induces the enzyme lipoxygenase (Grimes *et al.* 1992) and increases the LNA content (Czapski *et al.* 1992), which may result in the production of free radicals since the catabolism of polyunsaturated fatty acids in plant cells is catalysed by lipoxygenase (Siedow 1991).

## ACKNOWLEDGEMENTS

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