INVESTIGATION OF LIPOXYGENASE FUNCTIONS IN CHLOROPLASTS AND MITOCHONDRIA FROM *PISUM SATIVUM* SEEDLINGS

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1. Introduction

Membranes are considered at present to be the most important structural and biochemically active components of the cell responsible for its metabolic integrity. Therefore the study of lipid catalytic oxidation in the membranes of subcellular particles is one of the important problems in modern biochemistry [1–4].

The biological role of lipid peroxides in living organisms has not been clarified yet. It is known, however, that peroxides of unsaturated higher fatty acids inactivate a number of most important enzymes and cause destruction of intracellular membranes [5–10].

The processes of peroxide oxidation of lipids arising in the lipid phase of a cell were shown to be greatly activated by the application of ionizing radiation to the organisms [11–13]. Earlier we observed accumulation of lipid peroxides together with a considerable activation of lipoxygenase and variations in permeability and electrical parameters, which we supposed to be due to some changes in the structural organization of irradiated tissues [14, 15]. This necessitated a study of the relation between oxidation of unsaturated higher fatty acids and changes in the localization of intracellular lipoxygenase in plant tissues.

The literature on lipoxygenase localization is scarce. The presence of lipoxygenase in animals has not been established yet but it is found in all plant tissues. Lipoxygenase occurs not only in the soluble part of cytoplasm, as was suggested earlier, but activity has also been found in chloroplasts, mitochondria, and other structures isolated from plants [16–18].

This study deals with intracellular localization of lipoxygenase in pea seedling leaves and variations in its localization caused by destructive processes in tissues, such as refrigeration, hypotonic shock, X-irradiation.

2. Materials and methods

Chloroplasts and mitochondria from 10–12 day pea seedlings were obtained by differential centrifuging as described earlier [19, 20]. The composition of the medium used for isolation of chloroplasts was as follows: sucrose, 0.4 M; KCl, 0.01 M; $MgCl_2$, 0.01 M; Tris-HCl, 0.01 M, pH 7.5–7.9. A solution containing sucrose, 0.4 M; human serum albumin, 0.1%; EDTA, 0.005 M; phosphate buffer, 0.006 M or Tris-HCl, 0.01 M, pH 7.2–7.3 was used to isolate mitochondria.

The pellets of chloroplasts (1000 *g*, 15 min) and mitochondria (10 000 *g*, 15 min) were washed with the isolation medium and resedimented repeatedly by centrifugation under the same conditions. The washed structures were resuspended in a 0.25 M sucrose solution or in bidistilled water for the determination of lipoxygenase activity.

Lipoxygenase activity was determined in an isotonic reaction medium (0.25 M sucrose, 0.1% sodium salt solution of linoleic acid, phosphate buffer 0.006 M, pH 7.2–7.3), or hypotonic medium, without the sucrose. The Warburg gasometric method [14] and a spectrophotometric one [17] were used. Measurements were made 3–6 min after the onset of the reaction.

A RYP-200 unit, with a power of 258 r/min, was used for irradiation.

Table 1
Specific lipoxygenase activity of subcellular fractions from pea seedling leaves of "Neistoshchimy" ("inexhaustible") variety*.

Fraction	Absorption of O_2 (μl O_2/mg of protein)
Chloroplasts 1,000 *g*	7.14 + 0.23
Mitochondria 10,000 *g*	7.60 + 0.40
Supernatant 10,000 *g*	10.26 + 0.57

* Manometric method.

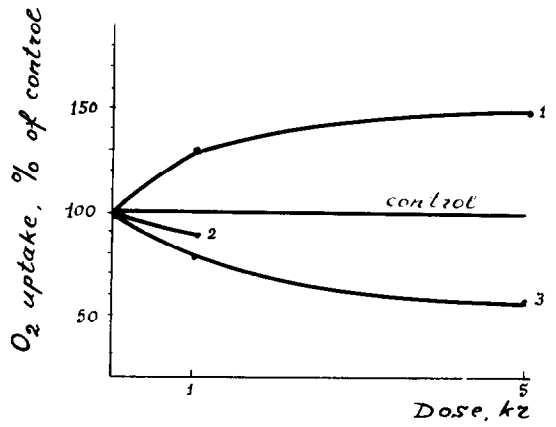

Fig. 1. Variation in lipoxygenase activity of subcellular structures 60 min after irradiation *in vitro*. 1) Lipoxygenase of the supernatant fraction; 2) lipoxygenase of mitochondria; 3) lipoxygenase of chloroplasts.

3. Results and discussion

During determination of lipoxygenase localization it was found that chloroplasts and mitochondria isolated from seedling leaves show a high lipoxygenase activity (table 1).

It should be noted that lipoxygenase activity from chloroplasts of seedling leaves was found to vary significantly with the pea variety studied. The specific activity of the lipoxygenase of "Pobeditel" chloroplasts is 7.93 μl of O_2/mg of protein, and that of "Beladonna" is 15.77 μl of O_2/mg of protein.

Lipoxygenase activity in the mitochondrial and supernatant fractions was determined in conditions leading to destruction of mitochondria. This showed that the lipoxygenase activity of subcellular structures depended on their integrity and intactness. Thus, in a hypotonic reaction medium the activity of mitochondrial lipoxygenase was found to be higher than that in isotonic medium (table 2).

Moreover, refrigeration and thawing of seedlings repeated three times was found to cause an increase in the lipoxygenase activity in the supernatant fraction, and a decrease in that of the mitochondria (table 3).

These data lead to the conclusion that a structurally bound form of lipoxygenase is released into the soluble part of the cytoplasm as a result of destructive processes in cells.

Ionizing radiation is known to cause structural disturbances in irradiated organisms. Therefore, in

Table 2
Lipoxygenase activity in isotonic and hypotonic media*.

Fraction	Optical density 232 nm		Percentage of control	*P*
	Control-isotonic	Hypotonic		
Mitochondria 10,000 *g*	0.106	0.187	176.4	< 0.05
Supernatant 10,000 *g*	1.07	1.30	120.5	< 0.01

* Spectrophotometric method.

order to find what is responsible for lipoxygenase activation studies were made of the effect of X-radiation on the activity of chloroplast and mitochondrial lipoxygenases.

In vivo, irradiation in doses of 1–10 kr resulted in an increased lipoxygenase activity in the supernatant fraction, whereas the activity of chloroplasts and mitochondria decreased by over 40% (table 4).

Irradiation of chloroplast and mitochondrial suspensions *in vitro* also led to a redistribution of lipoxygenase, that is to a reduction in activity in the particulate fractions and an increase in the supernatant liquid obtained from washing the irradiated structures (fig. 1).

Table 3
Effect of refrigeration on lipoxygenase activity of subcellular fractions*.

Object	Fraction	Optical density 232 nm		Percentage of control	P
		Control	Experiment		
Roots	Mitochondria 10,000 *g*	0.098	0.073	86.9	< 0.01
	Supernatant 10,000 *g*	0.115	0.154	133.8	< 0.05
Leaves	Supernatant 10,000 *g*	0.423	0.495	117.9	< 0.05

* Spectrophotometric method.

Table 4
Activity of subcellular fractions 60 min after irradiation *in vivo**.

Fraction	Dose (kr)	Absorption of O_2 (μl of O_2/ml)		Percentage of control	P
		Control	Irradiation		
Mitochondria 10,000 *g*	1	6.57	4.56	69.40	< 0.01
Supernatant 10,000 *g*	1	39.27	42.51	108.2	< 0.02
Supernatant 10,000 *g*	10	39.27	43.05	109.62	< 0.02
Chloroplasts 1,000 *g*	5	10.44	4.93	57.22	< 0.01
Supernatant 10,000 *g*	5	18.91	22.16	117.2	< 0.02

*Manometric method.

Thus it has been shown that activation of the lipoxygenase of the supernatant fraction is a result of the irradiation damage caused to the chloroplast and mitochondrial structures and the subsequent release of the enzyme into the soluble part of cytoplasm.

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