Volume 298, number 2,3, 223–225 FEBS 10749 February 1992

A lipoxygenase is the main lipid body protein in cucumber and soybean cotyledons during the stage of triglyceride mobilization

Ivo Feußner and Helmut Kindl

Biochemie, FB Chemie, Universität Marburg, W-3550 Marburg, Germany

Received 18 December 1991

The 90-kDa lipid body protein characterized earlier by its high expression during the stage of fat degradation was identified as a form of lipoxygenase. This organelle form was compared with lipoxygenase species purified from the cytosol. It is further shown that the antibodies raised against the lipid body membrane lipoxygenase from cucumber cotyledons cross-react with both cytosolic and lipid body lipoxygenase from soybean.

Cucumis sativus; *Glycine max*; Lipid body; Lipoxygenase

1. INTRODUCTION

Nonadienal and other volatile C_9 compounds are characteristic compounds of cucumber fruits and seedlings [1–3]. Their biosynthesis was proposed to take place by cleavage of 9-hydroperoxi-octadecadienoic acid which results from the action of lipoxygenase on linoleate [4]. However, it is likely that within the family of lipoxygenases enzymes may be included which act on esterified polyenoic fatty acids [5,6], e.g. membrane constituents or lipoproteins [7]. It was proposed that vertebrate 15-lipoxygenases may oxygenate the fatty acid moiety of cholesterol esters and may thus change the role of LDLs [8].

We report here, for the stage of maximal fat degradation in plant cells, the identity between a well-described constituent of the lipid body membrane [9] and the predominant form of lipoxygenase. The co-existence of triglycerides with linoleoyl residues and lipoxygenase within a cell compartment implies that lipoxygenase plays a physiological role in triglyceride metabolism in lipid bodies.

2. MATERIALS AND METHODS

Cotyledons of 4-day-old cucumber or soybean seedlings were used as enzyme source. Lipid body fractions were prepared according to [9]. Antiserum was raised against the 90-kDa protein isolated from purified lipid bodies of cucumber cotyledons [9]. Fragments of the 90-kDa protein were prepared by exhaustive tryptic cleavage at pH 7. Peptides were isolated by two consecutive chromatographies on reversed phase material at pH 2.5 and pH 7, respectively. N-terminal sequences were determined by Edman degradation in a gas-phase sequencer.

Correspondence address: H. Kindl, Biochemie, FB Chemie, Hans-Meerwein-Straße, W-3550 Marburg, Germany. Fax: (49) (6421) 285547.

The cytosolic species of the 90-kDa protein was isolated from a 200,000 × *g* supernatant by fractionation with ammonium sulfate and by chromatography on DEAE-cellulose and subsequent hydrophobic-interaction chromatography on Fractogel TSK-butyl. Lipoxygenase activity was assayed according to [10] using linoleate or trilinolein as substrate.

3. RESULTS

3.1. *The 90-kDa protein of lipid bodies at the stage of maximal fat mobilization is a lipoxygenase*

Purified 90-kDa protein from cucumber lipid bodies was subjected to trypsin cleavage, and the amino acid sequences of three peptides were compared with sequences in the EMBL database. It showed homology between the 90-kDa protein and plant lipoxygenases characterized by a size of approximately 95 kDa (Fig. 1).

Our search for a cytosolic precursor form of the lipid body protein resulted in the detection of soluble 90-kDa species which cross-reacted with the antibodies raised against the lipid body 90-kDa protein (Fig. 2). Following chromatographic purification of the cytosolic enzymes, three forms of lipoxygenase differing in size (Fig. 2) and charge (data not shown) were characterized. The purification of the soluble 90-kDa forms led to the isolation of protein with the specific lipoxygenase activity of 600 nkat/mg protein.

3.2. *The germination-related forms of lipoxygenase is also found in lipid bodies of soybean*

As most previous work on plant lipoxygenases has been done with extracts from dry seeds or plants of soybean [11,12], we investigated whether a lipoxygenase is located in the lipid bodies of cotyledons of this plant. Using the polyclonal antibodies raised against the cubumber lipoxygenase we identified, in cotyledons of ger-

	Amino acid sequence	residues	identity (%)
90 kDa	--YFEEELWNL		
Soybean 3	K-YREEELHNLR	178-189	78
Pea	H-YREEELNNL	186-195	78
90 kDa	--LYIVGFHIALMPY		
Soybean	R-LFLLGHHDPIMPY	423-436	46
Pea	K-LFLLDHHDSIMPY	431-444	39
90 kDa	--LFPYNLPYTYAVP		
Soybean	R-CGPVQMPYTLLLP	827-841	39
Pea	R-HGPVEMPYTLLYP	835-849	39

Fig. 1. Alignment of amino acid sequences of peptides obtained by fragmentation of the 90-kDa lipid body protein and of soybean lipoxygenase-3 [20] and pea lipoxygenase [21]. The N-termini of the fragments are preceded, in the sequences compared, by lysine or arginine.

minating seeds, lipoxygenase in the cytosol and in the purified lipid body fraction (Fig. 3). Similar data were obtained for melon seedlings (data not shown).

4. DISCUSSION

Lipid bodies which are formed during seed development [13,14] receive a new set of proteins [9] during germination, i.e. during fat mobilization. The most highly expressed species among these newly synthesized proteins is the 90-kDa protein, now identified as lipoxygenase. A possible model supposes that lipid bodies constitute the compartment where lipid degradation starts, not only by the formation of odorant aldehyds representing the characteristic smell of cucumber fruits, but mainly by yielding a C_9 acyl moiety. Subsequent to the formation of the 9-hydroperoxi derivative of linoleoyl groups, cleavage may yield a C_9 compound originating from C-atoms 10–18 of the unsaturated fatty

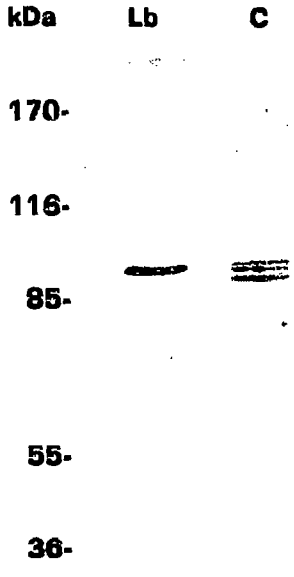

Fig. 2. Denaturing electrophoresis of lipoxygenase preparations and immunoblotting with antibodies raised against the 90-kDa protein. Lipid body membrane proteins (Lb); purified cytosolic lipoxygenase of cucumber cotyledons (C).

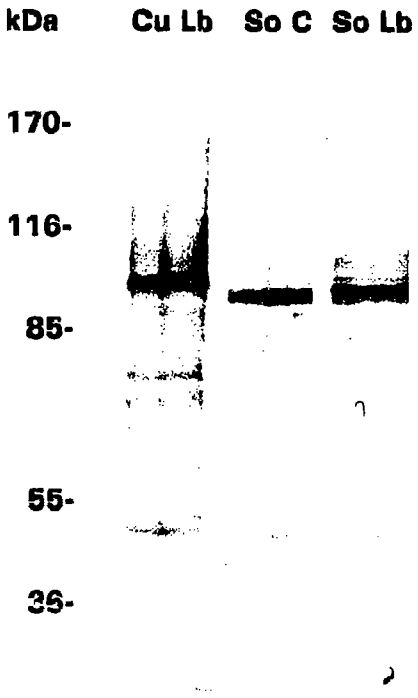

Fig. 3. Occurrence in soybean lipid bodies of a 90-kDa protein immunologically related to the cucumber 90-kDa protein. Samples of lipid body proteins from cucumber cotyledons (Cu Lb) or soybean cotyledons (So Lb), and from soybean cytosol (So C) were separated by denaturing electrophoresis, blotted on nitrocellulose and immunodecorated with antibodies against the cucumber 90-kDa protein.

acid and another C_9 compound bound as triglyceride intermediate. Thus, an oxidative modification of triglycerides, including the hydroperoxide formation, may precede the hydrolysis leading to fatty acids and their further degradation on glyoxysomes [15,16]. Together with several pathways leading to jasmonate [17] and C_{18} oxoderivatives, a number of metabolic sequences may start out by the action of lipoxygenases [4]. Our search for a pathway of triglyceride mobilization not initiated by lipase activity resulted from the fact that cucumber and soybean lipid bodies lack lipase activities ([15]; Kindl, unpublished results).

Irrespective of a lipoxygenase function in triglyceride metabolism, lipoxygenase is implied in the O_2-dependent elicitation of a signal transduction chain which acts in response to the attack by an pathogen [18].

We included lipid bodies from soybean where most work with plant lipoxygenases has been done. It became evident that a lipoxygenase similar to the form occurring in various cucurbitaceae is also localized on soybean lipid bodies. Earlier immunochemical investigations with soybean cotyledons and excluded such a possibility [19].

Acknowledgements: We thank the Friedrich-Ebert-Stiftung and the Deutsche Forschungsgemeinschaft (SFB 286) for the support of this work.

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