Phytochemistry, 1975, Vol. 14, pp. 703–706. Pergamon Press. Printed in England.

BIOSYNTHESIS OF α-LINOLENIC ACID BY DESATURATION OF OLEIC AND LINOLEIC ACIDS IN SEVERAL ORGANS OF HIGHER AND LOWER PLANTS AND IN ALGAE

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(*Received* 30 *July* 1974)

Key Word Index—Higher plants; algae; α-linolenic acid; biosynthesis; oleic and linoleic acid desaturation; age and state of plant organs.

Abstract—The biosynthesis of α-linolenic acid by successive desaturations of oleic and linoleic acids has been shown to occur in the leaves, roots and seeds of many higher plants. The age and the physiological state of the plant organs are extremely important. This route occurs also in several lower plants including algae. It is concluded that the desaturation pathway is the major route for the biosynthesis of α-linolenic acid in plants.

INTRODUCTION

The nature of the biosynthetic pathway of α-linolenic acid ($C_{18:3}\Delta^{9,12,15}$) in plant tissues remains an important biochemical and physiological problem. Ten years ago, Harris and James [1] proposed a pathway involving successive desaturations of oleic acid ($C_{18:1}\Delta^{9}$) to linoleic ($C_{18:2}\Delta^{9,12}$) and then to linolenic acid, but this scheme could not be completely confirmed at that time, by either *in vivo* or *in vitro* experiments. On the other hand, Kannangara *et al.* [2–4] have recently proposed an entirely different pathway in spinach chloroplasts for the biosynthesis of linolenic acid by elongation of a dodecatrienoic acid; only the last elongation step from hexadecatrienoic acid to linolenic acid was demonstrated to occur *in vitro* in isolated plastids. In young growing pea leaves, we have previously demonstrated [5–8] that the two successive desaturation steps postulated by James and Harris do in fact occur and that 1-[^{14}C]oleate and 1-[^{14}C]linoleate or U-[^{14}C]linoleate are converted to α-linolenic acid by the whole leaf. Moreover, we have presented evidence for the existence of an oleyl-CoA desaturase (in microsomes) and of a linoleate desaturase (in plastids) in young pea leaves [7, 8].

In this paper, we present data on the occurrence of both desaturation reactions in several plant organs which confirms the general occurrence of this biosynthetic pathway to α-linolenic acid in plants.

RESULTS AND DISCUSSION

The results shown in Table 1 clearly demonstrate that in all tissues examined α-linolenic acid is synthesized via the two-step desaturation pathway. This is most clearly seen by the results on the leaves of higher plants, where 1-[^{14}C]oléate, 1-[^{14}C]linoleate and U-[^{14}C]linoleate are readily converted in good yield to α-linolenic acid. There is an inverse relationship between the age of the leaves and the overall rate of desaturation. The desaturases are obviously far more active in young leaves. (Compare young *Spinacea oleracea* leaves with adult leaves, Table 1.)

Roots of higher plants which are rich in linolenic acid and which are actively synthesizing it (e.g. those of 7-day-old seedling of *Lupinus luteus*) con-

Table 1. Incorporation of acetate, oleate and linoleate into polyunsaturated fatty acids of plant tissues

Species and plant part examined	^{14}C Precursor added*	% Recovered total radioactivity in fatty acids 16:0	16:1 *cis* + *trans*	18:0	18:1	18:2	18:3 α
ANGIOSPERMS							
Pisum sativum, leaf	Ac	31	Trace	Trace	3	34	38
7-Day-old	Ol				5	55	40
	Lin*u					70	30
18-Day-old	Ac	29	Trace	Trace	5	53	19
Spinacia oleracea, leaf	Ol				78	20	2
1 cm Long	Lin					78	22
5 cm Long	Ol				85	15	Trace
	Lin					82	18
Adult	Ac	42	6	Trace	14	26	7
	Ol	30			58	11	
	Lin	100				0	0
Lupinus luteus							
7-Day-old seedlings	Ol				81	11	8
Leaves	Lin					58	42
Roots	Ac	26	41	10	18	5	Trace
	Ol				71	29	Trace
	Lin					82	18
Vicia faba							
7-Day-old seedlings	Ol				53	24	22
Leaves	Lin					60	39
Roots	Ac	16	14	14	15	31	—
	Ol				53	67	0
	Lin					100	0
Linum usitatissimum							
Young seedling	Lin					85	15
Flowers	Ol				51	27	22
Seeds 2 weeks after flowering	Ol				56	38	6
Brassica napus							
Flowers	Ol				61	32	7
Seeds 4 weeks after flowering	Ol				12	79	10
Helianthus annuus							
Flowers	Ol				50	48	Trace
Seeds 1 week after flowering	Ol				38	60	Trace
GYMNOSPERMS							
Ginkgo biloba							
Young leaves	Ac	37	†	—	9	32	16
	Ol				50	50	Trace
	Lin					87	13
PTERIDOPHYTES							
Athyrium filix femina							
5 cm Prothallus	Ac	30	—	Trace	34	28	7
	Ol				30	64	6
	Lin					95	5
Polytricum commune	Ac	32	—	Trace	48	20	Trace
Fronds	Ol				81	11	7
	Lin					100	Trace
BRYOPHYTES							
Marchantia polymorpha	Ac	31	6‡	1	17	11	21‡
Growing thallus	Ol				24	55	21
	Lin					70	30
ALGAE							
Dunaliella tertiolecta	Ac	+ + +			+ + +	+	+ +
	Ol				+ + + +	+ +	+
	Lin					+ + + +	+
Acetabularia mediterranea	Ac	15	10	5	70	Trace	Trace
	Ol				84	16	Trace
	Lin					91	5§

* Microdroplets of precursors (Ac: 1-[^{14}C]acetate, Ol: 1-[^{14}C]oleate, Lin: 1-[^{14}C]linoleate, Lin*u: U-[^{14}C]linoleate) as ammonium salts, were placed on the surface of the intact tissues and the labelled fatty acids studied 20 hr after.

† *Ginkgo biloba* gave 6% of 16:3 with acetate.

‡ *Marchantia polymorpha* gave, in addition, 3% of 16:2, 9% of 16:3 and 1% of 18:3 γ with acetate.

§ *Acetabularia mediterranea* gave, in addition, 2% each of 18:3 γ and 18:4 acids with linoleate.

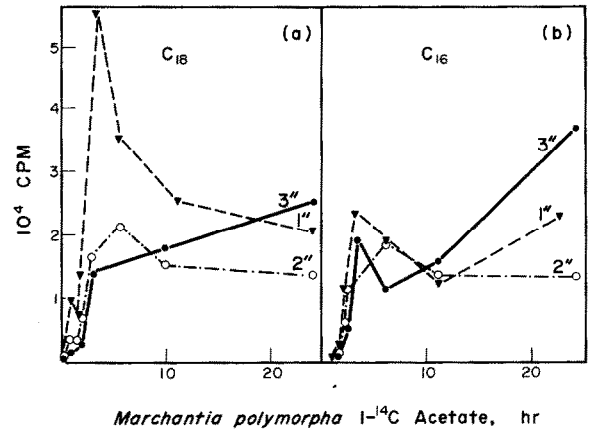

Fig. 1. Kinetics of incorporation of 1-[^{14}C]acetate in the C_{16} and C_{18} unsaturated fatty acids of the growing thallus of *Marchantia polymorpha*. The curves: ——, 1″; – · – · –, 2″; and ——, 3″, are those for the mono-, di- and tri-unsaturated acids in each series, $C_{16}C_{18}$.

vert oleic acid into linoleic acid and linoleic acid into linolenic acid fairly readily (Table 1). However, roots which contain only a small quantity of linolenic acid (e.g. those of *Vicia faba*) only convert oleic acid into linoleic acid and cannot carry out the second desaturation step. The same applies to maturing seeds. Those which synthesize important amounts of α-linolenic acid [e.g. *Linum usitatissimum* (flax) and *Brassica napus* (rape)] readily convert both oleic acid into linoleic acid and linoleic acid into linolenic acid. In sunflower seeds (*Helianthus annuus*), on the other hand, which contain only low quantities of α-linolenic acid, only the first desaturation step occurs (Table 1).

The two steps of desaturation have also been demonstrated in several lower plants. In the growing thallus of *Marchantia polymorpha*, the kinetics of incorporation of 1-[^{14}C]acetate into fatty acids (Fig. 1) shows that this tissue synthesizes not only α-linolenic acid but also large amounts of hexadecatrienoic acid ($C_{16:3}$) (which is concentrated, together with α-linolenic acid, in the galactolipids of the tissue: (Mache and Tremolières, unpublished data). The kinetics of incorporation suggest that the pathway for the $C_{16:3}$ acid in *Marchantia polymorpha* is analogous to that for α-linolenic acid.

The two desaturating steps were also demonstrated in the two algae examined. *Acetabularia mediterranea* synthesizes polyunsaturated fatty acids very slowly, but nevertheless can convert 1-[^{14}C]linoleate into α-linolenic acid, γ-linolenic acid ($C_{18:3}\Delta^{7,9,12}$) and octadecatetraenoic acid ($C_{18:4}\Delta^{7,9,12,15}$) thus showing that extra desaturating enzymes are present.

In conclusion, this paper shows that the biosynthesis of α-linolenic acid by successive desaturations of oleic acid to linoleic acid and of linoleic acid to linolenic acid is encountered very widely in plant tissues which contain and synthesize linolenic acid. Hence this pathway must be considered as the major pathway of α-linolenic acid biosynthesis in plants.

EXPERIMENTAL

Material. Lupinus luteus and *Vicia faba* seedlings were grown according to Oursel *et al.* [9]. *Linum usitatissimum*, *Brassica napus* and *Helianthus annuus* seeds and flowers were provided by C.N.R.A. 78000 Versailles (France). *Pisum sativum* (var. Alaska) seedlings were grown according to Tremolières [5]. *Spinacia oleracea* plants were purchased at the local market. Algae were kindly provided by Professor Puiseux-Dao, Laboratoire de Biologie Cellulaire Végétale, Université de Paris VII. All other materials were provided by the Museum d'Histoire Naturelle, Paris.

Incubation conditions. Na 1-[^{14}C]acetate (45 mCi/mM), NH_4 1-[^{14}C]oleate (51·7 mCi/mM) and NH_4 1-[^{14}C]linoleate (50 mCi/mM) were obtained from C.E.A. (Saclay France) and were placed as microdroplets on the surface of the tissues examined or added in the culture medium of algae.

Analytical methods. Lipids were extracted by the method of Bligh and Dyer [10]. Fatty acid methyl esters were prepared according to Metcalfe *et al.* [11] and analysed by gas–liquid radio-chromatography [5].

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