Pathway of Triglyceride Biosynthesis During Seed Ripening

J. M. S. MATHUR, Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Abstract

The investigations on the mode of triglyceride biosynthesis during ripening of *Solanum indicum, Salvadora persica, Mimusops hexandra, Aegle marmelos* and *Mesua ferrea* seeds, using chromatographic techniques have shown that these fats follow essentially a stepwise mechanism involving the formation of mono- and diglycerides as intermediate products. This is in agreement with the general in vitro enzymatic esterification by bacteriolipase, castor bean lipase and pancreatic lipase. The experimental basis of the Quantum mechanism of lipogenesis, postulated earlier for *Cocos nucifera* and recently for many phanerograms, has been shown to be inadequate and hence the validity of Quantum hypothesis appears to be doubtful.

Introduction

Hilditch (1) has demonstrated that the in vivo triglyceride synthesis is a two step reaction wherein free fatty acids, the primary products of synthesis, undergo lipasic esterification during ripening through the formation of mono and diglycerides, as indicated by initial high acid values gradually decreasing during ripening to almost nil in fully mature seeds. The slow reaction was attributed to insufficiency of either glycerol or enzyme. Terroine (2) explained it on the basis of excess water becoming the limiting factor in total synthesis of triglycerides, as in vitro (3–6).

Kartha (7) postulated the Quantum biosynthesis of fat in ripening coconut (*Cocos nucifera*) involving direct esterification by lipases without intermediate formation of mono- and diglycerides. In an earlier communication Mathur (8) reported the Quantum lipolysis of fat without release of mono- and diglycerides during germination of peanut (*Arachis hypogea*) and cotton (*Gossypium virnar*) seeds, as previously shown by Kartha and Mathur (9) for *Brassica juncea, B. campestris, Sesamum orientale* and *S. indicum*. Kartha and Nainawati (18) reported Quantum biosynthesis for several seed fats of phanerograms. However, recently using chromatographic techniques Mathur (19) has shown that fat biosynthesis during ripening of seven seed fats, viz., *Hibiscus cannabinus, Shorea robusta, Tectona grandis, Achras zapota, Albizzia lebbek, Cinnamomum camphora* and *Salvadora oleoides,* follows an essentially stepwise mechanism through intermediate formation of mono- and diglycerides.

Since in vitro enzymatic esterification by bacteriolipase (11), castor bean lipase (12) and pancreatic lipase (13) proceeds via mono- and diglycerides, the in vivo results, if substantially different, would be quite important. Moreover, using dissimilar procedures Kartha and Nainawati (18) and Mathur (19) have reported contradictory results for biosynthesis of *Achras zapota* seed fat. Therefore it was considered worthwhile to study the mode of lipogenesis in a number of seeds during maturing by more sophisticated and reliable procedures before the concept of Quantum biosynthesis of fat is generalized and its significance with reference to glyceride structure studied.

In mono- and diglycerides the hydroxyl groups contribute polar characteristics to the molecules providing a basis for adsorption and separation by chromatography, which offers a high degree of precision and accuracy and has, therefore, been adopted as an analytical tool for the present studies on the pathway of triglyceride biogenesis in a number of ripening oleaginous seeds.

Experimental Procedures

The fat from sun-dried seeds of *Solanum indicum, Salvadora persica, Mimusops hexandra, Aegle marmelos* and *Mesua ferrea,* collected at 3–6 different stages of ripening, was extracted by the cold percolation method (14) and was used for further studies after refining with 1% potassium hydroxide containing 5% ethanol, as usual.

Acetyl values were determined by the method of Kartha and Mathur (10) as simplified by Mathur (15).

For qualitative work the micro thin layer chromatographic procedure of Osman et al. (16) was adopted, with slight modifications. The components separated into three well defined zones (rows of spots or bands) in the following order of increasing height travelled :monoglycerides, diglycerides and triglycerides.

After drying the chromatoplates, the zones were detected by the following methods: (a) spraying with 2–7′ dichlorofluorescein (1% solution in 70% ethanol) and viewing under UV light, (b) spraying with chromic-sulphuric mixture (saturated solution of $K_2Cr_2O_7$ in 80% H_2SO_4 by weight) and charring on a hot plate at 200 C, (c) briefly exposing to iodine vapors.

Micro-chromatoplates showed the presence of mono- and diglycerides in all samples and quantitative separations were carried out by chromatography on a silica gel column by the method of Quinlin and Weiser (17).

Discussion

The results of the present studies are given in Table I, and show that in all species examined the amounts of mono- and diglycerides decrease with a corresponding increase in triglycerides as seed ripening proceeds. Triglycerides predominate with only traces of mono- and diglycerides being present in fully ripe seeds. This indicates that during triglyceride biosynthesis in these seeds, fatty acids esterify with glycerol in steps involving formation of intermediate products. This is not in agreement with Kartha's (7) studies in *Cocos nucifera,* and studies of Kartha and Nainawati (18) on several seed fats of phanerograms.

Kartha's (7) method employed castor oil as the control sample and no attempts were made to study the behavior of mono- and diglycerides under the reaction conditions used. The unstandardized method might give incomplete acetylation. When mono- and diglycerides isolated by column chromatography from seeds at several stages of fat development (Table I) were subjected to acetylation under the conditions specified by the method of Kartha and Mathur (10), an increase in weight of only a few milligrams occurred. This is contrary to the expected high theo-

TABLE I
Results of Studies on Biosynthesis of Some Seed Fats

Sample No.	Seed	Stage	Fat Synthesized, %	Acetyl value	Monoglycerides		Diglycerides		Triglycerides		M.M.W. of F.F.A.
					Wt. %	Acetyl value	Wt. %	Acetyl value	Wt. %	Acetyl value	
1	*Solanum indicum*										
		I	6.2	13.6	81.5	17.3	16.2	6.9	2.3	9.2	272
		II	16.9	21.0	57.7	8.9	33.9	11.2	8.4	16.9	.....
		III	38.1	17.4	17.8	14.3	35.2	12.0	47.0	13.3	.....
		IV	63.3	11.9	6.4	9.9	24.5	0.9	69.1	14.0	284
		V	80.0	10.5	3.0	7.0	10.4	6.4	86.6	13.2	.....
		VI	100	10.1	0.1	.....	0.0	.....	99.9	8.8	.....
2	*Salvadora persica*										
		I	11.7	4.9	74.3	1.4	17.6	4.4	8.1	11.2	.....
		II	42.8	6.2	38.6	3.6	28.7	9.6	32.7	6.0	270
		III	81.6	3.3	11.0	4.0	22.6	2.1	66.4	9.2	282
		IV	100	4.2	0.4	.....	0.6	.....	99.0	4.8	.....
3	*Mimusops hexandra*										
		I	3.3	11.5	89.0	13.2	9.2	17.5	1.8	7.3	.....
		II	19.4	6.9	60.2	5.8	21.7	8.6	18.1	11.1	276
		III	50.1	3.8	28.7	9.0	26.3	13.8	45.0	8.0	.....
		IV	79.0	4.7	3.6	8.6	6.1	6.6	90.3	17.3	.....
		V	100	6.0	0.0	.....	0.8	.....	99.2	9.2	282
4	*Aegle marmelos*										
		I	15.6	16.9	71.8	13.2	22.0	4.8	6.2	2.8	284
		II	29.5	18.2	31.3	5.9	39.4	11.9	29.3	9.0	.....
		III	57.7	8.3	12.9	7.3	20.5	5.5	66.6	14.2	.....
		IV	90.1	11.4	5.8	13.6	18.2	16.2	76.0	5.9	272
		V	100	9.9	0.0	.....	0.2	.....	99.8	11.3	.....
5	*Mesua ferrea*										
		I	18.6	3.8	67.7	12.0	23.0	6.1	9.3	8.1	280
		II	73.9	10.5	16.3	17.2	12.7	9.2	71.0	7.0	280
		III	100	4.2	0.2	.....	0.6	.....	99.2	5.8	.....

retical values. The presence of higher free fatty acids at all stages of growth is revealed by the mean molecular weights (Table I). Kartha's (7) assumption of the continual conversion of all higher fatty acids immediately after synthesis to neutral glycerides and that this conversion occurs at all stages of ripening is based on false analytical data.

The absence of higher fatty acids in *Cocos nucifera* could be their delayed synthesis rather than rapid utilization for fat formation. No reason has been assigned as to why there could not be a delay in glycerol synthesis and its insufficiency when the mode leading to its synthesis differs from that of fatty acid synthesis.

Kartha's (7) explanation for the difference in mechanism in vitro and in vivo based on the fact that the enzyme complex changes during isolation, does not appear to be logical without evidence as to how there could be such major changes in enzymes during their simple isolation so as to cause a reverse mode of action. This investigation suggests that all the fats studied were biosynthesized in steps, involving the formation of intermediate mono- and diglycerides, as has been shown for general in vitro enzymatic esterification. The experimental basis of Kartha's (7) Quantum fat biosynthesis is shown inadequate and hence the validity of Quantum hypothesis of lipogenesis appears doubtful.

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