SC 11081

Effect of temperature on the enzymatic synthesis of unsaturated fatty acids in *Torulopsis utilis*

Growing or resting cells of *Torulopsis utilis* oxidatively desaturate oleic acid to linoleic acid[1]. This transformation has now been demonstrated with oleyl-CoA in a cell-free system. The enzyme preparations are especially active when *T. utilis* is grown microaerobically at suboptimal temperatures (19°). With stearyl-CoA as the substrate, oleate formation requires only a particulate fraction, whereas both particles and a supernatant fraction are necessary for the introduction of the second double bond to form linoleate.

Torulopsis utilis (ATCC 8205) was grown without agitation either at 19° or at 30° for 5–7 days in 2-l Erlenmeyer flasks filled to the neck with the medium described by KLEIN[2]. The harvested cells were crushed twice in a Hughes press and diluted with 2 vol. of 0.13 M phosphate buffer (pH 7.2). The supernatant obtained after 3 centrifugations at 17 000 × *g* for 15 min was used as the source of enzyme.

After incubation the reaction mixtures (Table I) were saponified, the extracted

TABLE I

The complete system contained 0.25 μmole TPNH, 50 μmoles phosphate buffer (pH 7.2), 16 mμmoles [1-^{14}C]oleyl-CoA (25 000 disintegrations/min) or 16 mμmoles [1-^{14}C]stearyl-CoA (100 000 disintegrations/min), 100 000 × *g* particles (2.0 mg protein) and 100 000 × *g* supernatant (10 mg protein) in a final volume of 0.6 ml. Incubations were carried out in air at 30° for 90 min with shaking. The subscripts indicate the temperature at which the yeast was grown.

Acyl-CoA added	*Enzyme*	*Unsaturated fatty acids formed*	
		Oleate (%)	*Linoleate (%)*
Stearyl-CoA	$Particles_{30}$	34	2
	$Particles_{30}$ + $supernatant_{30}$	41	8
	$Particles_{19}$	77	6
	$Particles_{19}$ + $supernatant_{19}$	52	27
Oleyl-CoA	$Particles_{30}$	—	2
	$Particles_{30}$ + $supernatant_{30}$	—	9
	$Particles_{19}$	—	8
	$Particles_{19}$ + $supernatant_{19}$*	—	34
	$Supernatant_{19}$	—	6
	$Particles_{19}$ + $supernatant_{30}$	—	27
	$Particles_{30}$ + $supernatant_{19}$	—	9

* Yields of up to 60% of linoleate are obtained with enzyme from Torulopsis grown at 15° in a medium consisting of 2% glucose, 0.6% ammonium chloride, 0.3% yeast extract and 0.5% bacto-peptone in 0.1 M monobasic potassium phosphate.

fatty acids were separated from other lipids on silicic acid columns and the methyl esters subsequently isolated by gas chromatography[1]. In one experiment linoleate was isolated on silica-gel thin-layer plates impregnated with silver nitrate[3] and brominated after addition of carrier linoleate. The tetrabromide derivative was crystallized 5 times to constant specific activity[4].

Under the standard reaction conditions described in Table I, cell-free extracts from 30° cultures converted about 50% of stearyl-CoA to oleate, but less than 10% of added oleyl-CoA to linoleate. In contrast, extracts from 19° cells desaturated more than 80% of stearyl-CoA to unsaturated acids and 40% of oleyl-CoA to linoleate. Both desaturations required TPNH. After incubation with stearyl-CoA over 70% of the ^{14}C was present in neutral lipids and phospholipids and less than 3% in free fatty acids.

Cell-free extracts of Torulopsis were fractionated by centrifugation at 100 000 $\times$ g for 90 min. The resulting particles and supernatant were tested individually and in combination after the particles had been washed once in 0.1 M phosphate buffer (Table I). When stearyl-CoA was the substrate, the particles alone catalyzed conversion to oleate with high efficiency. However, further desaturation to linoleate did not occur to a significant extent unless the supernatant was also added. With oleyl-CoA as substrate, linoleate formation also depended on both particles and supernatant. The supernatant alone was virtually inactive.

The supernatant fraction of Torulopsis required for the oleate–linoleate conversion appeared to contain at least two factors, one heat labile and one heat stable. These factors could be fully replaced by a comparable fraction from *Saccharomyces cerevisiae* (ATCC 12341), an organism which does not form polyunsaturated fatty acids whether grown at 30° or 10°. Rat-liver extract was also active in replacing the Torulopsis supernatant. FAD and Fe^{2+}, which can substitute for the supernatant fraction in the desaturating system of *Mycobacterium phlei*, had no effect on either of the two desaturation reactions in Torulopsis.

In order to establish whether the more effective synthesis of linoleate at suboptimal temperatures is attributable to an increased activity of the particles or of the supernatant, or possibly of both fractions, particles from 30° and 19° yeast were incubated with the non-corresponding supernatant fractions. The results given in the table clearly show that the increase in enzyme activity is due solely to the particulate fraction. This stimulatory effect of suboptimal growth temperatures cannot be explained by an unusual temperature sensitivity of the desaturating system, since enzyme activity is reduced at lower assay temperatures.

Torulopsis cells grown at suboptimal temperatures contain a much greater proportion of the more highly unsaturated fatty acids, a phenomenon which has already been observed for mesophilic and psychrophilic yeasts by KATES AND BAXTER[6]. The present results showing that the lower growth temperatures stimulate the activity of the desaturating enzymes provide an explanation for this shift of the fatty acid spectrum to higher levels of unsaturation.

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Purification of bovine plasminogen

Partial purifications of bovine plasminogen (EC 3.4.4.14) have been described[1–4] but few characterizing data given. When it was reported[5,6] that, among other ω-amino acids, ε-ACA and lysine markedly increased the solubility of sparingly soluble human plasminogen preparations at neutral pH, these amino acids were tried in the purification of human plasminogen[7,8]. Recently WALLÉN[9,10] published an improved method for the purification of human plasminogen in which the rather specific interaction between plasminogen and ε-ACA was utilized. This method was used for bovine material with suitable modifications. The plasminogen preparation obtained was almost homogeneous in starch-gel electrophoresis at different pH values. It contains aspartic acid as N-terminal amino acid.

The starting material used was fresh bovine citrated plasma (1 volume 0.14 M trisodium citrate and 9 volumes blood). The purity and yields throughout the different steps were followed by analyses of the freeze-dried fractions. The proteolytic activity obtained after activation with urokinase (human urokinase[11] containing about 500 Ploug and Kjeldgaard units/mg) was usually determined by a caseinolytic method[12], but the purer fractions were also assayed for fibrinolytic activity by a clot lysis technique using reagents free from plasminogen[13]. Homogeneity was tested by starch-gel electrophoresis[14]. The N-terminal analyses were carried out by the Edman technique as applied by ERIKSSON AND SJÖQUIST[15].

The procedure elaborated for the purification of bovine plasminogen is as follows: From 30 l of bovine citrated blood, 16 l of plasma are obtained and Fraction I (mainly fibrinogen) precipitated according to Method 6 of COHN *et al.*[16]. After centrifugation the supernatant is adjusted to pH 5.3 with acetic acid, whereupon most of the plasminogen precipitates. This precipitate (Fraction IP), which usually contains about 100 g of protein (dry weight), is used directly for further fractionation. It is suspended in 3.3 l distilled water at 0° by means of a vibrating mixer (Vibro-Mischer, Chemap A.G., Männedorf, Switzerland) and the slurry is acidified to pH 4.5–4.6 with acetic acid. To the opaque solution obtained, 74 ml of 4 M sodium acetate are added, giving an ionic strength of about 0.08 and a pH of 5.4 $\pm$ 0.1. The plasminogen activity again precipitates. It is then dissolved and precipitated once more as described above. The precipitate thus obtained is suspended in 1.2 l distilled water at 0° by using the Vibro-Mischer and the pH is adjusted to 4.5–4.6 with acetic acid

Abbreviation: ε-ACA, ε-aminocaproic acid.