The synthesis of glycogen in yeast

Enzymes catalysing the synthesis of glycogen from UDPG have been found in animal tissues[1-3]. A similar enzyme has now been purified about 500-fold from baker's-yeast extracts. The purification procedure involves the following steps: (a) grinding of the lyophilized yeast and subsequent extraction with 0.05 *M* Tris-maleate buffer, pH 7.5, followed by dialysis; at this stage, part of the enzyme is sedimentable at 20,000 × *g*; (b) treatment with 1% digitonin, which brings most of the enzyme into solution; (c) immediate precipitation with satd. $(NH_4)_2SO_4$, and dialysis; (d) adsorption on retrograded amylose[4], and (e) elution from amylose with a conc. (100 mg/ml) glycogen solution, buffered at pH 7.5. Practically all the trehalose-synthesizing enzyme[5] remains in the supernatant, in the amylose-adsorption step.

No phosphorylase or amylase could be detected in the purified preparation under the usual assay conditions for these enzymes. A slight amylase activity was, however, observable in the presence of Ca^{++}.

Glycogen is required for activity, as shown in Table I. Since the enzyme used in this experiment was adsorbed on retrograded amylose, it would seem that the latter does not function as primer.

When [^{14}C]UDPG was used as substrate, the amount of radioactivity incorporated into glycogen was equivalent to the UDP formed, as can be seen in Table II.

TABLE I

GLYCOGEN REQUIREMENT FOR ACTIVITY OF THE ENZYME

The complete system contained 0.35 μmole UDPG, 1 μmole G-6-P, 2.4 mg glycogen, 10 μmoles Tris-maleate buffer, pH 7.5, 1 μmole EDTA and 0.01 ml of amylose suspension containing adsorbed enzyme (step d), in a total volume of 0.1 ml. After incubating for 30 min at 37° the UDP formed was determined as described by LELOIR AND GOLDEMBERG[1].

Incubation mixture	*UDP formed (mμmoles)*
Complete system	61
Complete system *minus* glycogen	6
Complete system *minus* glycogen and G-6-P	1

TABLE II

STOICHIOMETRY OF THE REACTION

The incubation mixture contained 0.5 μmole [^{14}C]UDPG (uniformly labeled in the glucose moiety, specific activity 17,200 counts/min/μmole), 1 μmole G-6-P, 10 μmoles Tris-maleate buffer, pH 7.5, 1 μmole EDTA and 0.02 ml of purified enzyme (step e) containing 2 mg of glycogen, in a total volume of 0.1 ml. After 90 min incubation at 30° UDP was determined according to LELOIR AND GOLDEMBERG[1]. Glycogen was isolated and counted as described by LELOIR *et al*[2].

Incubation mixture	*UDP formed (μmoles)*	*Glucose incorporated into glycogen*	
		(counts/min)	*(μmoles)*
Complete system	0.20	3140	0.18
Complete system *minus* G-6-P	0.14	2300	0.13
Complete system, UDPG added at the end of incubation	—	20	—

Abbreviations: UDPG, uridine diphosphate glucose; UDP, uridine diphosphate; G-6-P, glucose 6-phosphate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate.

After treatment of the labeled glycogen with β amylase, the radioactivity was recovered in the liberated maltose.

While G-6-P increased the activity of yeast glycogen synthetase, the stimulation obtained was usually less than 2-fold (see Table II) as compared with 15- to 20-fold in the case of the animal enzymes[1,2].

A full report of these findings will be published elsewhere.

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The identification of lysolecithin in lipid extracts of brain

The presence of lysolecithin in lipid extracts of a number of mammalian tissues has recently been reported[1–5]. Its presence in extracts of brain has not, as far as we are aware, been described, although PIGHINI[6] in 1932 had obtained an alcohol-soluble, ether-insoluble substance from human brain which, both from its solubility properties and from certain of its biological actions, appeared to resemble a lysophosphatide. It was decided therefore to re-investigate PIGHINI's work, using more modern chromatographic methods for the separation of lysolecithin. Initial experiments showed that lipid extracts of normal human brain, prepared by PIGHINI's method, contained a haemolytic substance having the same R_F value as lysolecithin. We therefore next decided to extract the brain lipids by the method of FOLCH *et al.*[7] and to characterise this haemolytic substance more precisely.

Human brains were obtained post-mortem as soon as possible after death from cases in which there had been no clinical evidence of nervous disorder. Meninges were removed and the surface of the brain washed free from blood in gently running tap-water. Cerebral cortical grey matter was then dissected off and extracted. In addition, a number of scattered plaques were dissected from the unfixed brain of a case of multiple sclerosis.