Alleged Role of Fructofuranose in the Synthesis of Levan

THE view was long widely entertained that cells synthesize macromolecules of polysaccharides and proteins by a reversion of the process of hydrolysis. It has been suggested accordingly that the synthesis of the polyfructoside levan specifically from aldosido< >fructofuranosides (sucrose, raffinose) involves two distinct steps: first, hydrolysis of the substrate; secondly, polymerization of fructofuranose by a condensation involving removal of water[1]. Bacteria which form levan from sucrose do so also from raffinose[2]. This polymerative type of sucrose degradation is concurrent with an ordinary hydrolytic inversion[3,4]. The same bacteria ferment levan[3]. Investigators might be tempted by these correlations to consider the enzyme system, levansucrase, to be but a mixture of invertase and polyfructosidase. It is shown below, however, that this view cannot be valid.

(*a*) Owen[5] added yeast invertase solution to cultures of bacteria growing in a sucrose medium. The addition did not augment but inhibited formation of levan by the cells. The findings suggested that invertase is not an essential component of the levan-forming enzyme, but since it referred to living and proliferating cells a final conclusion in this respect could not be drawn[6]. The recent preparation of cell-free levansucrase from *Aerobacter*[3] has made it possible to carry out Owen's experiment in conditions free from the criticisms to which his earlier attempt is open. It has been found that addition to this levansucrase of yeast invertase in amount sufficient to double the rate of sucrose decomposition does not affect levan production in solutions containing a high initial concentration of sucrose (more than 5 per cent). In solutions containing levan-formation-limiting concentrations of sucrose (less than 2 per cent), addition of yeast invertase to the levansucrase caused inhibition, rather than augmentation, of levan production.

On the view that fructofuranose is the substrate actually polymerized by levansucrase, it is still possible to explain the failure of invertase to accelerate levan production from a non-limiting concentration of sucrose by assuming that in this process hydrolysis, though essential, is not a rate-limiting step. This complicating possibility is eliminated where the rate of levan production is known to be dependent on the sucrose concentration. It is similarly eliminated in reactions carried out on raffinose. Levan is produced much more slowly from raffinose than from an equivalent concentration of sucrose[7]. If the reaction proceeds via fructofuranose, the rate-limiting step on raffinose can only be hydrolysis, subsequent steps by the terms of the theory being identical for both sucrose and raffinose. Yet addition to levansucrase of enough yeast invertase to render the rate of raffinose hydrolysis by the enzyme mixture equal to the rate of conversion of sucrose by levansucrase alone failed to augment, and in the long run inhibited, the rate of production of levan from raffinose. The conclusion is therefore confirmed that invertase is irrelevant to levan production from sucrose and raffinose by levansucrase.

(*b*) There is further direct evidence that fructofuranose is not a substrate which can be polymerized by levansucrase. Isbell and Pigman[8] have concluded on the basis of measurements of optical rotation that fructose in aqueous solution is an equilibrium mixture of fructofuranoses and fructopyranoses. The recent demonstration that glucose-1-phosphate (Cori ester) and fructose form a dynamic equilibrium with sucrose and phosphoric acid in the presence of a specific enzyme[9] corroborates this view. Addition of fructose to sucrose does not inhibit levan production from the latter, yet fructose itself, although it presumably contains ready fructofuranose, is not converted into levan by levansucrase[7]. Similarly, levansucrase fails to form levan from reaction mixtures in which fructofuranose is sustainedly liberated in *statu nascendi*, for example, in reaction mixtures of methyl gamma fructoside + yeast invertase, and of inulin inulase.

(*c*) Extracts of an *Aerobacter*, although they produce levan from sucrose and hydro'yse the latter as well, do not hydrolyse levan. Thus they contain levansucrase and invertase but no polyfructosidase (levanase)[7]. On the other hand, takadiastase and an extract of a *Torula* yeast have been found to hydrolyse levan and inulin as well as sucrose, yet produced no levan from the latter. Thus they contain invertase as well as polyfructosidase yet are without levansucrase.

The conclusion is therefore indicated that fructofuranose is not an intermediary of levan synthesis from aldosido< >fructofuranosides. The view of macromolecular biosynthesis as a reversion of hydrolase action apparently fails to describe levan production, even as it fails to depict the biological production of glycogen and starch.

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A Blind Woodlouse

WHEN examining some specimens of *Armadillidium vulgare* (Latr.), kindly sent to me by Dr. H. W. Howard of Cambridge from the University Farm, I noticed that a female specimen of the variety "Black type B" of Howard[1] was entirely void of eyes or any trace of them. In this example there is no trace of any visual elements or even pigment. The chitin on the cephalon, where the eyes should be, is very slightly convex, shelving down laterally and inwardly.

During the past thirty years, I have examined many thousands of specimens referable to this species, but I have never met with one in which the eyes were absent, or in any other species of terrestrial isopod excepting in truly cavernicolous ones.

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