Breakdown of heptuloses in *Escherichia coli*

In view of the increasing interest in the role of sedoheptulose phosphate in the metabolism of glucose and pentose in animals[6], plants[1], and micro-organisms[5], it appears worthwhile to report some experiments on the degradation of various heptuloses by *E. coli*, which have been carried out in the frame of a broader study to be reported elsewhere.

The organism was cultivated on a mineral medium[3] containing 0.2% glucose, D-xylose, D-ribose or L-arabinose, as well as on 0.5% yeast extract with and without 0.05% glucose. Cells were harvested from 16–24 hour-old cultures, vigorously aerated at 37° C. Oxidation was measured at 30° by the conventional Warburg techniques. The cells (25 mg of wet cells), suspended in 0.05 *M* phosphate buffer, pH 6.8, did not oxidize sedoheptulose, glucoheptulose, mannoheptulose or sedoheptulosan monohydrate, nor did oxidation of sedoheptulose occur in the presence of glucose or ribose.

In a further series of experiments, the transfer of the terminal phosphate group from adenosine triphosphate (ATP) to heptuloses in cell-free extracts prepared by grinding the bacteria with alumina powder[8] was studied manometrically[4].

Only the extract prepared from cells grown on yeast extract-glucose-medium, phosphorylated sedoheptulose to a considerable extent (Fig. 1). Extracts of cells grown on yeast extract alone phosphorylated sedoheptulose slowly and only when the concentration of the enzyme was increased five-fold (equivalent to 150 mg of wet cells). No other heptuloses were affected by the extracts. (Mannoheptulose was tested only with extracts of yeast-glucose grown cells.)

Sedoheptulose is not only phosphorylated by the above extract, but is also oxidized (Fig. 1). The inability of the corresponding intact cells to oxidize the heptose could, therefore, be attributed to a permeability barrier. That the phosphorylation product is probably further metabolized by extracts of *E. coli* is indicated by the degradation of sedoheptulose-7-phosphate, which takes place in these extracts, as has previously been demonstrated[2].

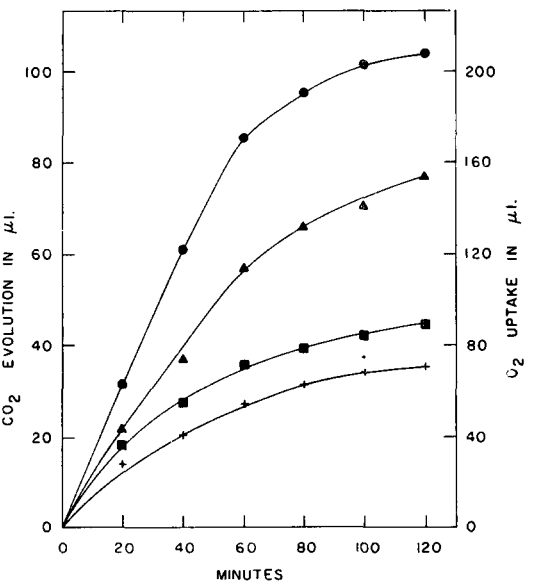

Fig. 1. Oxidation and phosphorylation of sedoheptulose by extracts of *E. coli* grown on yeast-extract glucose medium. Oxidation (●——●): the flasks contained in the main compartment 0.9 ml extract (equivalent to 180 mg wet cells); 0.1 ml (0.1 mg) of triphosphopyridine nucleotide and 0.1 ml of $MgCl_2 \cdot 6H_2O$ (0.09 *M*). One side arm carried 0.3 mg of phenazine methosulphate in 0.2 ml of tris buffer pH 7.0; in the second arm: 0.1 ml of ATP (sodium salt) (9 μM) and 0.6 ml (9 μM) substrate. The center well contained 0.2 ml 20% KOH. Final volume, 2.0 ml; gas phase, air; temperature, 30° C. Endogenous values subtracted. Phosphorylation: (▲——▲) sedoheptulose, (■——■) endogenous; (+——+) eluted chromatographed filter paper treated as that used in the preparation of sedoheptulose. Each flask contained in the main well, 0.15 ml of bacterial extract (equivalent to 30 mg wet cells); 0.4 ml of 0.08 *M* $NaHCO_3$; 0.1 ml of 0.4 *M* KF; 0.1 ml of $MgCl_2 \cdot 6H_2O$ and 0.2 ml of substrate (3 μM). The side arm received 9 μM ATP in 0.4 ml of 0.02 *M* $NaHCO_3$. Final volume, 2.0 ml; gas phase, 75% N_2 5% CO_2; temperature, 30° C.

In this connection it may be interesting to record that a red yeast, *Rhodotorula mucilaginosa*, has been isolated in this laboratory from an exposed solution of mannoheptulose and has been found to utilize this sugar as well as glucose. After this investigation had been completed, SATO *et al.*[10] reported the isolation of a *Bacillus* strain which can utilize sedoheptulose adaptively, and MOORE *et al.*[9] demonstrated the utilization of sedoheptulose and the oxidation of heptoses by various bacteria.

Sedoheptulose was prepared by hydrolysis of sedoheptulosan monohydrate in 0.2 *N* HCl for half an hour at 100° C. It was separated by descending chromatography (Whatman No. 1), using water-saturated phenol as a solvent. The band corresponding to R_F value 0.44 was cut out, washed with ether, and eluted with water by the capillary descent procedure. Mannoheptulose was prepared, in collaboration with Dr. E. SIMON (Weizmann Institute of Science) from avocado pears (Collinson variety) by the method of LAFORGE[7].

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On the prosthetic group of succinic dehydrogenase*

The problem of the prosthetic group of succinic dehydrogenase has been the subject of much conjecture, but a definitive experimental approach to its study had to await the isolation of the pure enzyme. Since the suggestion of AXELROD *et al.*[1], based on nutritional experiments that flavin may be the prosthetic group of the enzyme, the presence of a flavin moiety in the dehydrogenase has been widely speculated upon. Other investigators favored the view that the primary dehydrogenase is a hemoprotein, possibly identical with cytochrome B. The availability of essentially homogeneous preparations of the enzyme[2] has opened the way to a critical re-examination of the problem.

The authors have reported that the prosthetic group of the dehydrogenase contains iron but not in the form of hemin[2,3]. The total iron content equals the inorganic iron which is liberated as Fe^{++} by boiling or acidification. Ultracentrifugally homogeneous preparations of the dehydrogenase, prepared from *fresh* mitochondrial acetone powders contain 1 g atom of Fe^{++} per 60,000 to 65,000 g protein; similar preparations isolated from stored acetone powders (several months at -10°) contain 1 g atom Fe^{++} per 110,000 to 130,000 g. From the preliminary value of the molecular weight (about 140,000, based on sedimentation rate and diffusion[3]), it may be concluded that the two types of preparations contain 2 and 1 g atom of Fe^{++} per mole, respectively. Since by physico-chemical criteria the two preparations appear identical and since the specific activity varies with the iron content[2], it seems that both iron atoms are needed for full activity and that one of the two becomes readily labilized and lost on storage or purification. In contrast, the iron in the one-iron type of preparation is so strongly held that neither dialysis against buffer or Fe^{++}-chelators nor passage through cation exchangers liberates it, although it is set free on denaturation. Iron complexors (*o*-phenanthroline, 8-oxyquinoline, thiocyanate, α,α'-bipyridyl, versene, iron-specific versene) do not inhibit the enzyme but the apo-protein of crystalline β_1-globulin from plasma strongly inhibits the dehydrogenase; this can be prevented and partly reversed by ferrous iron. While *o*-phenanthroline does not inhibit the enzyme, it forms a red complex with it and the resulting spectrum is similar to but not identical with that of ferrous *o*-phenanthrolinate. The *o*-phenanthroline compound of succinic dehydrogenase has full activity; it is bleached by succinate in the same way as the free enzyme, and it does not dissociate on dialysis.

The absorption spectrum of succinic dehydrogenase (Fig. 1) is atypical for a flavoprotein: there is no definite maximum in the region of 460 or 375 mμ; there is a gradually increasing absorption below 400 mμ as well as a measurable absorption in the entire visible range. The enzyme is partly bleached by hydrosulfite and the resulting difference spectrum (Fig. 1, insert) exhibits a maximum at 460 mμ. Succinate bleaches the enzyme less completely than hydrosulfite and this reduction is inhibited by malonate. The preparation shown in Fig. 1 is the one-iron type; the two-iron enzyme shows almost 50% more color at and below 450 mμ, whereas above 520 mμ, where the iron moieties of other ferroflavoproteins[4] are thought to absorb light, the two-iron enzymes shows twice as much color.

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