TRANSFORMATION OF GLUCOSE TO 3-KETOGLUCOSE WITH THE CELLS OF AGROBACTERIUM TUMEFACIENS

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The biochemical conversion of disaccharides to their corresponding glycoside-3-uloses has been reported by three research groups (Bernaerts et al., 1958, 1960a, 1960b, 1961, 1963a, 1963b; Feingold et al., 1961; and Fukui et al., 1963a, 1963b, 1963c), and the chemical structures of 3-ketosucrose, 3-ketotrehalose, 3-ketomaltose and 3-ketolactose were confirmed to be α-D-ribo-hexopyranosyl-3-ulose-β-D-fructofuranoside, α-D-ribo-hexos-3-ulose-(1-1)-α-D-glucopyranoside, D-ribo-hexos-3-ulose (1-4)-α-D-glucopyranoside and D-xylo-hexos-3-ulose-D-glucopyranoside, respectively (Fukui et al., 1963a, 1963b). As one of their physiological activities it was found that every glycoside-3-ulose shows a striking inhibitory effect on the sucrose transport system (Fukui et al., 1964a) of Agrobacterium tumefaciens.

Of the monosaccharide-3-uloses, a new sugar, D-ribo-hexos-3-ulose (trivial name 3-ketoglucose, Fukui et al., 1963d) was first isolated as crystalline form from the hydrolyzate of 3-ketosucrose, and its physiological function as competitive inhibitor on the sucrose uptake reaction was also demonstrated. (Fukui et al., 1964a).

The transformation of glucose to 3-ketoglucose, however, has never been reported, so that in this paper the author deals with the accumulation of 3-ulose in glucose medium and discusses the carbohydrate metabolism by the cells of the plant tumour-inducing microorganism, Agrobacterium tumefaciens.

Materials and Methods

Uniformly labeled D-glucose-C^{14} (7.1 mc/mmole) was obtained from Daiichi Pure Chemical Co., Ltd., Tokyo. Whereas unlabeled sugars were commercial preparations, 3-ketoglucose was prepared from the enzymatic hydrolyzate of crystalline 3-ketosucrose according to the method presented previously (Fukui et al., 1963d). A strain of Agrobacterium tumefaciens IAM-1525 was used in this study; it was obtained from the Japanese Federation of Culture Collection of Microorganisms (J.F.C.C.). The culture medium of McIntire et al. (1942) was applied with the omission of zinc ions and the phosphate concentration was tripled over the values given by these authors. Sucrose (2 %) was used as the carbon source. Under these conditions the pH of the culture medium did not fall below 6.8 during the growth period. The cells of A. tumefaciens were grown on a reciprocal shaker at 28° for approximately 28 hours until the cell count reached 2-3 X 10^9 per ml. Cells were removed by centrifugation and washed with 1.0 X 10^{-2}M phosphate buffer, pH 7.0.

3-Ketoglucose reacted readily in the Nelson(1944) modification of the micro Somogyi method at 30° where 95% of maximum color was attained in 2 hours. D-glucose was determined by using "Glucostat" which does not show any activity on 3-ketoglucose. Descending paper chromatography was carried out on sheets of Toyo-filter paper No. 51. The following solvent systems were effective to separate 3-ketoglucose from glucose (Fukui et al., 1963d): (A) methylethylketone, acetone, H_2O (3:1:0.6); (B) acetone, acetic acid, H_2O (4:1.2:1). Paper electrophoresis was performed in 0.05 M sodium tetraborate at pH 9.2 in a cooled pressure plate apparatus at 30 volts/cm for 2 hours. Alkaline silver nitrate and periodate benzidine were used as a general sugar reagent and urea-phosphate (Wise et al., 1955) for ketosugar. The urea-phosphate gave a blue color with 2-ulose and its derivatives, namely fructose, sorbose, sucrose and raffinose, and a clearly distinguishable deep red color with 3-ulose. For the separation of 3-

ketoglucose from the reaction mixture containing glucose, the adsorption technique on activated carbon Darco 60 (Fukui et al., 1963d) was also employed, followed by the elution with 50% aqueous ethanol. For the detection and determination of spots resulting from paper chromatography and electrophoresis the radioactivities on the paper were also measured by means of automatic gas flow counter, Aloka, Tokyo Musen Co., Ltd., Tokyo.

Results and Discussion

Formation of 3-ketoglucose from glucose

During glucose-(U)-C^{14} metabolism with resting cells of A. tumefaciens which were grown on sucrose medium, the accumulation of an unknown compound could be demonstrated as an intermediate as seen in Fig.-1, and no other substances were formed in the medium throughout this reaction.

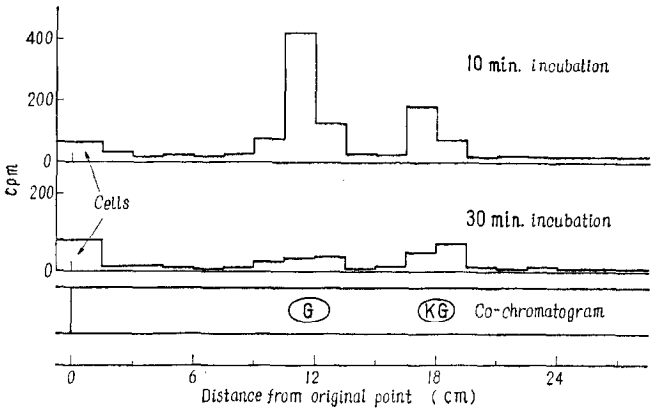

Fig.-1. Paper Chromatography of Reaction Mixture under the Solvent System of B

Components of reaction mixture (2.0 ml) were as follows; cell suspension 0.2 ml; M/10 Tris-buffer, pH 8.2, 1.0 ml; glucose-(U)-C^{14} 0.3 ml (3 µmoles, specific radioactivity 2.5 X 10^5 cpm per µmole); and dist.-water 1.3 ml. Reaction was carried out with shaking in Warburg vessels at 30°.
G; glucose, KG; 3-ketoglucose

By applying the same method (Fukui et al., 1963d) which was used for the confirmation of 3-ketoglucose isolated from the hydrolyzate of

3-ketosucrose, the intermediate could be identified as 3-ketoglucose from the results shown below.

The migration rate of the material on the paper chromatography showed complete agreement with that of authentic samples of 3-ketoglucose under the solvent systems A and B. The spot gave a reddish color with the spray reagent of urea-phosphate. This color test strongly suggests that the unknown material possesses a keto group at C-3 position.

The radioactive intermediate was eluted from the paper with distilled water, followed by reduction with $NaBH_4$. After the reduction, the mixture was treated with resins to remove inorganic substances, then the products were concentrated to syrup in vacuo.

The migration rates of the products on paper chromatography and paper electrophoresis were estimated from their radioactivities. As a result, two polyols were formed from an intermediate by the reduction of $NaBH_4$ and were identified as sorbitol and allitol.

Rates of Formation and Utilization of 3-Ketoglucose during Glucose Metabolism

The rates of formation of 3-ketoglucose from glucose with resting cells of A. tumefaciens are given in Fig.-2A and 2B. They show that at initial 30 minutes incubation a rapid accumulation of the 3-ulose takes place at a rate of approximately 2.0 μmoles/ g cells/ min at conditions of pH 8.2, Tris-buffer; 1.0 X 10^{-1}M concentration of substrate; and 30°. And it was also found that the uptake rate of glucose was 13.6 μmoles/ g cells/ min under the same conditions as above. After 2 hours incubation the accumulation reached the maximum where the concentration of the 3-ulose was 1.0 to 1.2 X 10^{-3}M in a reaction medium. On the other hand, the uptake rate of 3-ketoglucose could be estimated to be 10.9 μmoles/ g cells/ min at a 3-ulose concentration of 1.0 X 10^{-3}M which is the maximum level during the glucose metabolism. As can be seen in Fig.-2B, even after 60 or more minutes incubation the rate of 3-ketoglucose formation is found to be very rapid because of a dramatic increase of specific

radioactivities in the formed 3-ulose from labeled glucose which was added after 60 minutes incubation.

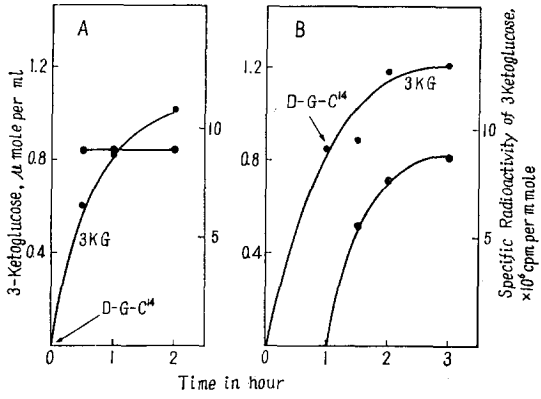

Fig.-2. Formation curve of 3-Ketoglucose from Glucose with the Resting Cells of A. tumefaciens
A) glucose-(U)-C^{14} was added at 0 time ; B) glucose-(U)-C^{14} was added at 60 minutes incubation
Components of reaction mixtures were as follows: cell suspension 1.0 ml, 50 mg; M/10 Tris-buffer, pH 8.2, 1.0 ml; 5 X 10^{-1} M glucose, 1.0 ml; and distilled water plus glucose-(U)-C^{14} (7.1 mc/ m mole) up to 5.0 ml. Specific radioactivity of glucose in the mixture is 9.0 X 10^{6} cpm per mole. Reaction was carried out in Monod's tube with shaking.
•---•---• ; 3-ketoglucose
•---•---⊗ ; specific radioactivity of 3-ketoglucose

This finding means that the turnover of 3-ketoglucose in the reaction mixture is very fast during glucose metabolism of the cells.

Considering the metabolic rates toward glucose and 3-ketoglucose, speculation on the glucose catabolic step in A. tumefaciens would be thus: the conversion of glucose to 3-ketoglucose is the first reaction, followed by the efflux of the product from cell space to medium, then the 3-ulose gets again into the cells and is metabolized.

The phenomenon of quantitative excretion of 3-ketosucrose from the cells of the same strain of A. tumefaciens during the oxidative sucrose metabolism (Fukui et al., 1964b) strongly supports this speculation.

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