Formation of N-phenyl-D-fructosylamine by enzymic transfructosylation from sucrose to aniline*

In enzymic transglycosylation reactions the acceptors for transferred sugar residues are not necessarily restricted to hydroxy compounds like simple alcohols and sugars but other compounds such as inorganic phosphate and carboxylic acids have been shown to be involved[1]. On the other hand the occurrence of N- and S-glycosides in nature suggests, as we have pointed out[2], that amines and thiol compounds may also serve as glycosyl acceptors. These considerations prompted us to look for enzymic transglycosylation reactions in which amines are utilized as acceptors. This paper describes the formation of N-phenyl D-fructosylamine by the enzymic transfructosylation between sucrose and aniline.

In the case of the enzymic formation of N-glycosides of amines special attention must be paid to the fact that spontaneous condensation of free sugars with amines is liable to take place even under physiological conditions[3]. Therefore, in the present experiments the non-enzymic formation of N-glycosides was carefully controlled.

A mixture of sucrose and aniline dissolved in phosphate buffer, pH 7.0, was incubated with a solution of takadiastase. The detection of the reaction products was made by paper chromatography as shown in Fig. 1.

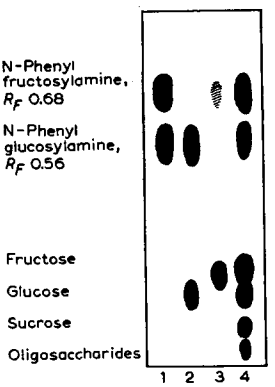

Fig. 1. Paper chromatogram showing enzymic formation of N-phenylfructosylamine. Reaction mixture contained 1 ml 1 *M* sugar, 0.1 ml aniline, 1.9 ml 0.2 *M* phosphate, pH 7.0 and 2 ml of dialyzed solution of 30% takadiastase. Incubated at 30° for 5 h. Ascending paper chromatography on Toyo No. 50 filter paper; solvent, *n*-butanol–ethanol–water (100 : 15 : 30), spraying reagent, benzidine–trichloroacetic acid for aldose, resorcinol–HCl for ketose, Ehrlich's reagent for aniline. 1. Synthetic N-phenyl fructosylamine and N-phenyl glucosylamine as standard. 2. Aniline, glucose and enzyme. 3. Aniline, fructose and enzyme. 4. Aniline, sucrose and enzyme.

On the chromatogram spots were recognized corresponding to sucrose, glucose, fructose and oligosaccharides arising from sucrose by transfructosylation. Two spots with higher R_F also appeared. The color reaction revealed that the one with R_F 0.68 was composed of fructose and aniline and the other with R_F 0.56 of glucose and aniline. These two spots were identified as those of N-phenylfructosylamine and N-phenylglucosylamine by reference to the R_F values and color reactions of the compounds synthesized according to HONEYMAN and coworkers[4,5]. Controls in which sucrose was substituted for either glucose or fructose showed that under these experimental conditions spontaneous formation of N-phenylglucosylamine was considerable whereas that of N-phenylfructosylamine was almost negligible. The enzymic formation of N-phenylfructosylamine was also observed when tris(hydroxylmethyl)aminomethane of the same pH was used in place of phosphate buffer.

For the isolation of N-phenyl fructosylamine, 95 ml 0.264 *M* sucrose, 5 ml

* Presented at the 10th Symposium on Enzyme Chemistry of Japan, Sapporo, July 13, 1958.

aniline, 50 ml 0.2 *M* phosphate, pH 7.5 and 100 ml dialyzed 30 % takadiastase were incubated at 30°. After 6 h the mixture was heated at 100° for 3 min and filtered. The filtrate was concentrated *in vacuo* to a thin syrup and added to 4 vol. hot ethanol. The ethanolic filtrate was concentrated *in vacuo* until crystals began to appear and placed in a refrigerator to complete crystallization. From the control incubation mixture, in which sucrose was replaced by an equimolar mixture of glucose and fructose, no such crystals were obtained. The product was recrystallized twice from ethanol yielding 249 mg. The substance had m.p. 151° (decomp., uncorr.), unchanged by admixture with synthetic N-phenylfructosylamine, m.p. 151°. $[\alpha]_D^{12}$ —186° → —175° in pyridine (*c*, 0.5). (Found: C, 56.36; H, 6.72; N, 5.54. $C_{12}H_{17}ON_5$ requires C, 56.46; H, 6.71; N, 5.49.) The synthetic sample had $[\alpha]_D^{12}$ —213° → —196° under these conditions. BARRY AND HONEYMAN[5] gave for their synthetic N-phenyl-D-fructosylamine m.p.151°; $[\alpha]_D^{19}$ —209° → —164.1° pyridine (*c*, 1). Hydrolysis of the isolated compound with 0.5 *N* HCl at 15° yielded fructose and aniline in equimolar proportion. The infrared spectra of the enzymic product and the synthetic compound were identical.

Apart from a slight disagreement of the specific rotation, the results obtained would indicate that the substance produced by the action of takadiastase upon a mixture of sucrose and aniline was identical with N-phenyl-D-fructosylamine reported by HONEYMAN *et al.*[5].

A fructopyranoside structure has been assigned to the N-phenyl-D-fructosylamine of BARRY AND HONEYMAN[5], as was also the case with *p*-ethoxyphenylfructosylamine[6] and *p*-methoxyphenylfructosylamine[7]. The identity of our enzymic product with the synthetic N-phenylfructosylamine suggests that a change in ring structure of the fructosyl group took place, either enzymically or spontaneously.

As regards the anomer structure of aryl-D-fructosylamines the β-form has been considered probable because of their upward mutarotation and the formation of β-1,3,4,5-tetraacetyl-D-fructose by acetylation followed by hydrolysis[5]. The upward mutarotation as observed with our enzymic product suggests also its β-fructoside form.

With the yeast invertase preparation the formation of N-phenyl fructosylamine was also observed in a similar way.

To Sankyo Co. Ltd. the authors wish to acknowledge with gratitude the generous gift of takadiastase preparation.

The present work was supported in part by a grant from the Ministry of Education.

Botanical Institute, Faculty of Science,
Tokyo Kyoiku University, Otsuka, Tokyo (Japan)

TOMOO MIWA
MASARU TAKESHITA
SAHEYE NAKAMURA

[1] G. J. DUTTON, *Biochem. J.*, 64 (1956) 693.
[2] T. MIWA, K. TAKANO, A. YASUMURA, H. SUZUKI AND K. ISHIZAWA, *Symposium on Enzyme Chemistry, Japan.*, 11 (1956) 225.
[3] G. P. ELLIS AND J. HONEYMAN, *Advances in Carbohydrate Chem.*, 10 (1955) 95.
[4] J. HONEYMAN AND A. R. TATCHELL, *J. Chem. Soc.*, (1950) 967.
[5] C. P. BARRY AND J. HONEYMAN, *J. Chem. Soc.*, (1952) 4147.
[6] R. KUHN AND L. BIRKOFER, *Ber.*, 71 (1938) 621.
[7] B. HELFERICH AND W. PORTZ, *Ber.*, 86 (1953) 604.

Received May 22nd, 1959