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Transfer Reaction of β-D-Glycofuranose by β-Glycosidase

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Transfer reaction of β-D-glucofuranose from phenyl β-D-glucofuranoside to methanol by almond emulsin was recognized. Extent of glucofuranosyl transfer from different aryl β-D-glucofuranoside to alcohol was determined. Influence of pH on the glucofuranosyl transfer was examined. The course of the transfer reaction was also reported.

Enzymic transfer of β-D-galactofuranose and β-D-glucofuranosiduronic acid was also investigated.

In view of the above facts, it was concluded that β-D-glycofuranosyl residue was transferred by β-glycosidase from phenol to alcohol and conversion of furanose ring to pyranose ring was not occured in the course of enzymic transfer reaction.

In the preceding papers, it was clarified that rabbit liver β-glucuronidase,[2] almond emulsin β-glucosidase[3] and almond emulsin β-galactosidase[4] hydrolyze β-D-glucofuranosiduronic acid, β-D-glucofuranoside and β-D-galactofuranoside respectively.

The enzymic hydrolysis of aryl β-D-glucopyranosiduronic acid,[5,6] aryl β-D-glucopyranoside[7] and aryl β-D-galactopyranoside[8] in the presence of a small amount of various kinds of alcohol is accompanied by the transfer of a part of pyranose residue to the alcohol, resulting in the formation of corresponding alkyl β-D-glucopyranosiduronic acid, alkyl β-D-glucopyranoside and alkyl β-D-galactopyranoside respectively.

As for transfer reaction of furanose, Miwa and Ishizawa[9] reported that β-D-fructofuranose residue of saccharose was transferred to methanol by β-fructofuranosidase and methyl β-D-fructofuranoside was formed.

The present investigation has been undertaken in order to clarify whether or not glycofuranosyl transfer reaction from phenol to alcohol may also be found in β-glycosidase, such as β-glucosidase, β-galactosidase and β-glucuronidase.

Experimental

Gas Chromatography (GLC)——Gas chromatography was performed on a Hitachi K-53 gas chromatograph equipped with a hydrogen flame ionization detector. The Golay column Z (SE 30) and the stainless steel tube packed with 3% SE 52 on 60—80 mesh Chromosorb W were used. The trimethylsilylation reaction was carried out as described by Sweeley, *et al.*[10] and the trifluoroacetylation was carried out as described by vilkas, *et al.*[11]

Paper Chromatography (PPC)——Ascending development was employed with filter paper, Toyo Roshi No. 50. Solvent systems employed were (A) BuOH–AcOH–H_2O (4:1:5), (B) AcOEt–AcOH–H_2O (5:2:2). The products were detected by spraying the $NaIO_4$–benzidine reagent on the chromatogram.

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Thin-Layer Chromatography (TLC)——Plates were made with Wako gel B-5 and developed at room temperature by ascending method. Solvent systems employed were (A) $CHCl_3$–MeOH–AcOEt (20:8:2), (B) BuOH–AcOH–H_2O (4:1:1). Products were detected by spraying 50% H_2SO_4 and heating.

Enzyme——Almond emulsin and *E. coli* β-glucuronidase were obtained from Sigma Chemical Co. The rabbit liver β-glucuronidase was partially purified according to a modification of the method of Fishman and Talalay.[12]

Result and Discussion

Transfer Reaction of β-D-Glucofuranose

The incubation mixture contained 6 mg of phenyl β-D-glucofuranoside, 2.5 ml of methanol, 30 mg of almond emulsin and 22.5 ml of 0.1 M acetate buffer, pH 5.0. After incubation for 1 hr at 40°, 100 ml of ethanol was added to the reaction mixture and filtered. The filterate was concentrated under reduced pressure at 40°, and made up to 3 ml with water. The solution was deionized by passing through 6 ml of IR-120 (H^+) and 9 ml of IRA-410 (OH^-) successively. The deionized solution was evaporated under reduced pressure at 40°, and the residue was analyzed by PPC, TLC and GLC. Table I shows PPC and TLC of hexoses and hexosides. Paper chromatogram and thin-layer chromatogram of reaction mixture indicated a spot corresponding to methyl D-glucofuranoside, but identification of β- configuration of the product could not be made by PPC and TLC as indicated in Table I. Fig. 1 shows the gas chromatogram of glucose and four isomers of methyl D-glucoside. Glucose and four isomers of methyl D-glucoside could be completely resolved by using Golay column Z. In the reaction mixture, only the peak of methyl β-D-glucofuranoside, but not those of the other isomer of methyl D-glucoside, was observed. In the presence of ethanol as acceptor, ethyl β-D-glucofuranoside was identified. From these experiments, it seems most reasonable to conclude that conversion of furanose ring to pyranose ring does not occur in the course of the enzymic transfer of glycosyl residue from phenol to alcohol. Moreover, as with the transfer of β-D-glucopyranose,[7] the β- configuration of D-glucofuranose remained unchanged.

TABLE I. PPC and TLC of Hexoses and Hexosides

Compound	PPC *Rf* values with solvents A	PPC *Rf* values with solvents B	TLC *Rf* values with solvents A	TLC *Rf* values with solvents B
Glucose	0.17	0.19	0.13	0.46
Methyl α-D-glucofuranoside[13]	0.41	0.45	0.44	0.68
Methyl β-D-glucofuranoside[13]	0.42	0.45	0.46	0.68
Methyl α-D-glucopyranoside[14]	0.31	0.38	0.31	0.64
Methyl β-D-glucopyranoside[14]	0.30	0.39	0.33	0.63
Ethyl β-D-glucofuranoside[15]	0.59	0.58	0.56	0.80
Ethyl β-D-glucopyranoside[16]	0.44	0.50	0.43	0.70
Galactose	0.17	0.17	0.06	0.49
Methyl α-D-galactofuranoside[17]	0.38	0.40	0.39	0.62
Methyl β-D-galactofuranoside[17]	0.44	0.43	0.42	0.71
Methyl α-D-galactopyranoside[18]	0.26	0.33	0.28	0.55
Methyl β-D-galactopyranoside[19]	0.25	0.31	0.22	0.51
Methyl β-D-glucofuranosiduronolactone[20]			0.68	

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Two milligrams of authentic specimen of methyl β-D-glucofuranoside was incubated with 10 mg of almond emulsin in 45 ml of 0.01M acetate buffer, pH 5.0, at 40°. In this experiment, 0.01M acetate buffer was employed, and deionization process was omitted, since recovery of authentic methyl β-D-glucofuranoside was low by this deionization process. After incubation, 60 ml of ethanol was added to the 15 ml of reaction mixture, and the solution was filtered. The filterate was evaporated under reduced pressure, and the unhydrolyzed substrate in the residue was determined by GLC. Amount of methyl β-D-glucofuranoside hydrolyzed for incubation of 1 hour, 3 hours and 5 hours was 62.0%, 68.5% and 70.8% respectively. Ethyl β-D-glucofuranoside was treated with almond emulsin as described above. The amount of ethyl β-D-glucofuranoside hydrolyzed for 1, 3 and 5 hours incubation was 54.4%, 66.7% and 70.0% respectively.

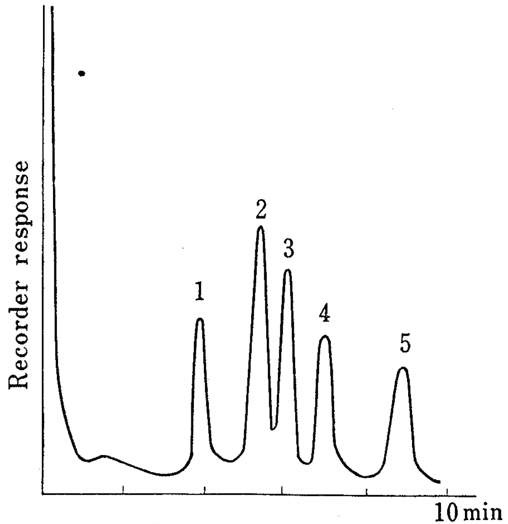

Fig. 1. Gas Chromatogram of the Trifluoroacetates of Glucose and Methyl D-Glucosides

peak: 1: glucose, 2: methyl β-D-glucofuranoside, 3: methyl α-D-glucofuranoside, 4: methyl α-D-glucopyranoside, 5: methyl β-D-glucopyranoside
column: Golay column Z (45 m × 0.5mm *i.d.*)
temperature: column 125°
carrier gas: N_2 0.5kg/cm^2

In addition to the Fischer's report[21] that emulsin did not form reducing sugar from methyl β-D-glucofuranoside, although the experimental detail was not described, haworth, *et al.*[22] reported that the emulsin did not hydrolyze ethyl β-D-glucofuranoside using probably polarimetric method. However, in this study hydrolysis of methyl and ethyl β-D-glucofuranoside by almond emulsin was clearly observed by means of gas chromatography. In the preceding paper,[3] hydrolysis of aryl β-D-glucofuranoside by almond emulsin was described. Therefore, it may be concluded that alkyl β-D-glucofuranoside as well as aryl β-D-glucofuranoside was hydrolyzed by almond emulsin. The extent of transfer reaction of β-D-glucofuranose is listed in Table II. After incubation, 2 ml of reaction mixture was removed and the liberated aglycon was measured by the method of preceding paper,[3] 23 ml of reaction mixture was evaporated under reduced pressure, and dryed in dessicator. A newly formed β-D-glucofuranoside was determined by GLC. As listed in Table II, various aryl β-D-glucofuranoside function as glucofuranosyl donor in transfer reaction. Fig. 2 shows influence of pH on the β-D-glucofuranosyl transfer from phenyl β-D-glucofuranoside to methanol. The maximum ratio of β-D-glucofuranosyl transfer to released aglycon was at pH 5.0. From Fig. 3 it may be seen that the velocity rates for both hydrolysis and transfer was a function of time, and the ratio of β-D-glucofuraonsyl transfer to released aglycon decreased slowly with time passing. It seems likely that a newly formed methyl β-D-glucofuranoside was hydrolyzed slowly with time.

Transfer Reaction of β-D-Galactofuranose

Experimental procedure of transfer reaction of β-D-galactofuranose was the same as was used for β-D-glucofuranosyl transfer, except that 60 mg of phenyl β-D-galactofuranoside and 50 mg of almond emulsin were used. Methyl β-D-galactofuranoside formed by transfer of β-D-

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Table II. Enzymic Transfer of β-D-Glucofuranose and β-D-Galactofuranose

Donor substrate	Donor substrate concentration (M)	Acceptor	Acceptor concentration (M)	Aglycon liberated (μM)	Glycofuranoside formed (μM)	Transfer (%)
Phenyl β-D-glucofuranoside[3]	0.001	MeOH	2.5	5.6	3.4	60.7
Guaiacyl β-D-glucofuranoside[3]	0.001	MeOH	2.5	7.1	4.5	63.3
m-Tolyl β-D-glucofuranoside[3]	0.001	MeOH	2.5	22.0	7.6	34.5
Phenyl β-D-glucofuranoside	0.001	EtOH	2.5	4.9	2.9	59.3
Phenyl β-D-galactofuranoide[23]	0.01	MeOH	2.5	1.6	0.57	35.6
Guaiacyl β-D-galactofuranoside[4]	0.01	MeOH	2.5	1.5	0.55	36.7
m-Tolyl β-D-galactofuranoside[4]	0.01	MeOH	2.5	2.2	0.68	30.9
Guaiacyl β-D-galactofuranoside	0.01	EtOH	2.5	2.3	0.57	24.9

Incubation mixture (25 ml of total volume) contained donor substrate in the amount required to give the indicated final concentration, a volume of alcohols in a final concentration of 2.5M, 30 mg of almond emulsin except transfer reaction of β-D-galactofuranose in which 50 mg of enzyme preparation was employed; the remaining volume consisted of 0.01 M acetate buffer, pH 5.0. Incubation time was 1 hour at 40.°

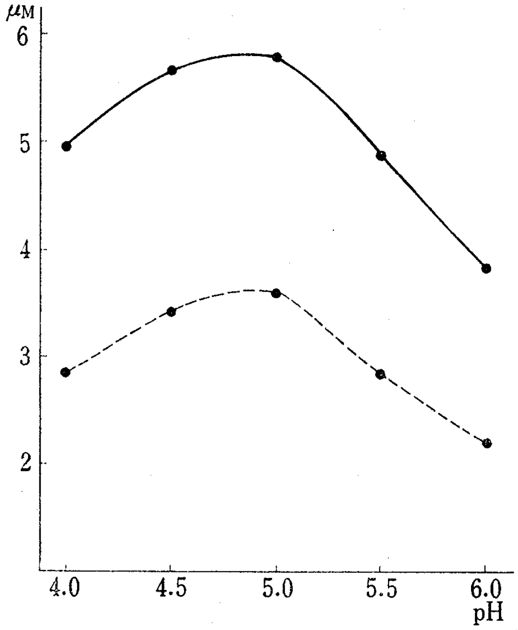

Fig. 2. Effects of pH on the β-D-Glucofuranosyl Transfer from Phenyl β-D-Glucofuranoside to Methanol

Incubation mixture contained 6 mg of phenyl β-D-glucofuranoside, 2.5 ml of methanol, 30 mg of almond emulsin and 22.5 ml of 0.01 M acetate buffer. Incubation time was 1 hour at 40°.

—●—: phenol liberated

---●---: methyl β-D-glucofuranoside formed

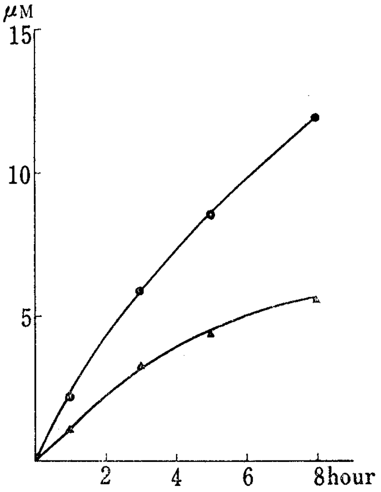

Fig. 3. Time Course of β-D-Glucofuranosyl Transfer from Phenyl β-D-Glucofuranoside to Methanol

Incubation mixture was as stated in the legend to Fig. 2. In this experiment, acetate buffer, pH 5.0 was employed.

—●—: phenol liberated

—▲—: metyl β-D-glucofuranoside formed

galactofuranose residue from phenyl β-D-galactofuranoside to methanol was identified by PPC, TLC and GLC. *Rf* values of galactose and methyl D-galactosides were listed in Table I. Fig. 4 shows gas chromatogram for galactose and four isomers of methyl D-galctoside. In the reaction mixture, only the peak for methyl β-D-galactofuranoside was observed by means of gas chromatography, but any for the other isomers was not. As with the transfer reaction of β-D-glucofuranose, conversion of the β-D-galactofuranose ring was not occurred in the course of transfer reaction. The extent of transfer reaction of β-D-galactofuranose is listed in Table II.

Transfer Reaction of β-D-Glucofuranosiduronic Acid

The incubation mixture contained 15 mg of 2-naphthyl β-D-glucofuranosiduronic acid, 5 ml of methanol, 1 ml of *E. coli* β-glucuronidase solution (20.000 Fishman unit) and 44 ml of 0.1 M acetate buffer, pH 5.0. After incubation for 3 hours at 38°, 200 ml of ethanol was added to the reaction mixture and filtered. The filterate was evaporated under reduced pressure at 40°, and made up to 3 ml with water. When the solution was passed through 12 ml of IR-120, by this deionization process a newly formed methyl β-D-glucofuranosiduronic acid was converted to methyl β-D-glucofuranosiduronolactone. An attempt to obtaine authentic specimen of free methyl β-D-glucofuranosiduronic acid from its barium salt was unsuccessfull. Methyl β-D-glucofuranosiduronolactone was obtained, although barium methyl β-D-glucofuranosiduronate was very carefully treated with oxalic acid or sulfuric acid. Therefore, one might identify a transferred product, methyl β-D-glucofuranosiduronic acid as methyl β-D-glucofuranosiduronolactone. The effluent passed through IR-120 was evaporated under reduced pressure, the residue was purified by alumina column chromatography, using EtOH–MeOH as developer. Methyl β-D-glucofuranosiduronolactone was identified by TLC and GLC. *Rf* value of methyl β-D-glucofuranosiduronolactone was indicated in Table I. Fig. 5 shows gas chromatogram of transfer reaction mixture. In the preceding paper,[24] it was shown that the extent of transfer of β-D-glucopyranosiduronic acid by *E. coli* β-glucuronidase was effective, but the extent of the transfer by rabbit liver β-glucuronidase was very small. When rabbit liver β-glucuronidase preparation was used in this transfer reaction, methyl β-D-glucofuranosiduronolactone could not be identified.

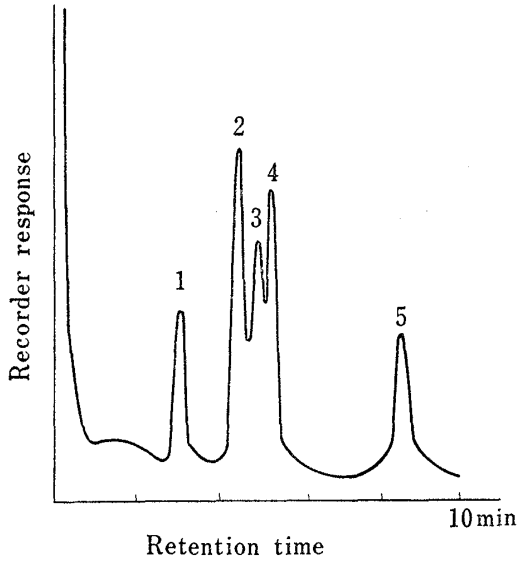

Fig. 4. Gas Chromatogram of Trifluoroacetates of Galactose and Methyl D-Galactosides

peak: 1: galactose, 2: methyl β-D-galactofuranoside, 3: methyl α-D-galactofuranoside, 4: methyl α-D-galactopyranoside, 5: methyl β-D-galactopyranoside
column: Golay column Z (45 m × 0.5 mm *i.d.*)
temperature: column 140°
carrier gas: N_2 0.5 kg/cm²

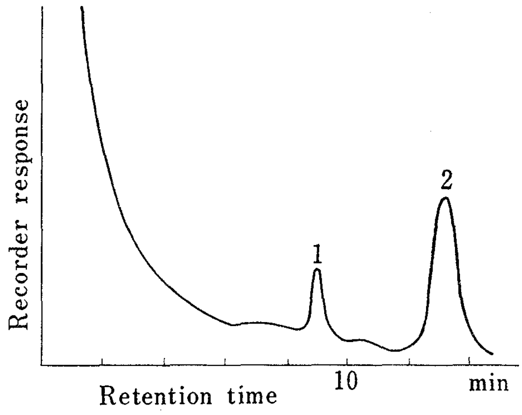

Fig. 5. Gas Chromatogram of the Trimethylsilyl Esters of the Reaction Mixtures

peak: 1: methyl β-D-glucofuranosiduronolactone, 2: glucuronolactone
column: 3% SE 52 on 60—80 mesh Chromosorb w (stainless steel tube 1 m × 3mm *i.d.*)
temperature: column 170°
carrier gas: N_2 0.6 kg/cm²

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