21. On Glucotransferase.

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In the course of his researches on the biochemistry of Salicaceae, J. Rabaté¹⁾ noticed that by the action of a particular enzyme contained in leaves of *Salix purpurea* or *Gaultheria procumbens* salicin is hydrolyzed to saligenin and glucose but a part of the cleaved glucose is simultaneously transferred to the primary alcohol group of saligenin forming another β -glucoside.*

He also indicated that in the hydrolysis by this enzyme of phenol- β -d-glucoside or picein (*p*-hydroxyacetophenone- β -d-glucoside) the presence of a small amount of methanol gives rise to the formation of methyl- β -d-glucoside by the transfer of split glucose to methanol. As it was found that the added glucose could not be utilized for the synthesis of methylglucoside, he considered that only glucose in nascent state is responsible for this reaction.

Assuming that this reaction is comparable with the formation of ethylchlorophyllide by chlorophyllase, enzymatic transaminations and phosphorylation processes in fermentation and glycolysis, we have studied the enzymatic transfer of glucose and confirmed the finding of Rabaté.²⁾

Recently B. Axelrod reported the enzymatic transfer of phosphate from arylphosphate to various hydroxyl compounds without mediation of nucleotide and named the enzyme "phosphotransferase".³⁾ So in the enzymatic transfer of glucose it seems appropriate to denote the enzyme as "glucotransferase", where aryl- β -glucoside represents glucose donor and alcohol its acceptor.

As Rabaté already stated, this enzyme reaction is not a simple reversal of the hydrolysis, since no synthesis of β -glucoside is achieved when the enzyme acts upon the mixture of dilute aqueous

1) Bull. Soc. Chim. biol. 19 (1935), 572; 20 (1938), 449.

* This was named β -salicylglucoside by E. Bourquelot et H. Hérissé, (J. Pharm. et Chim. (7) 8 (1913), 49.

2) T. Miwa, K. Mafune and S. Furutani, Medicine and Biology, 1 (1942), 80 (in Japanese).

3) J. Biol. Chem. 172 (1948) 1; 176 (1948), 295.

solution of glucose and alcohol.

For the elucidation of the nature of glucotransferase it is essential to determine whether this enzyme catalyzes only the glucose transfer or both transfer and hydrolysis.

Of enzyme preparations so far examined, we have not yet been able to obtain glucotransferase preparation entirely devoid of hydrolyzing activity. It may be possible that the hydrolytic action is an inherent character of this enzyme. However, as will be described in the following paragraph, evidences were given to show that the enzymatic hydrolysis of aryl- β -glucosides, at least in part, due to the action of an usual β -glucosidase present in glucotransferase preparation.

Enzyme preparation.

Rabate used the powder of dried leaves as an enzyme preparation. As the enzyme could not be obtained free from cell residues, he considered that it is firmly bound to protoplasm. However, we could extract the enzyme without difficulty by adjusting the pH of the solvent to weak alkaline.

Powder of dried green leaves collected in July to August was shaken several hours at room temperature with 7 to 10 times its weight of 0.3 per cent diammonium phosphate which had been made to pH 9 with dilute ammonia. It was strained with cloth and the dark brown extract was deproteinized at pH 4 by the addition of dilute acetic acid.

The filtrate was dialyzed against running water and clarified by centrifugation. The enzyme solutions used in the following experiments have been prepared from *Sambucus Sieboldiana* var. *typica*, *Populus nigra* and *Equisetum arvense*.

Substrate and determination of the enzyme action.

p-Nitrophenol- or phenol- β -D-glucoside was used as substrate. By the separate determination of aglucon and glucose liberated, the course of the enzyme action was pursued.

Glucose transfer from *p*-nitrophenol- β -D-glucoside to alcohol.

By the action of the enzyme a part of glucose cleaved from *p*-nitrophenol- β -D-glucoside is transferred to alcohol so that in presence of alcohol the amount of free glucose is always smaller than that of liberated *p*-nitrophenol. In absence of alcohol *p*-nitrophenol and glucose are produced in equimolecular ratio.

TABLE I.

Enzymatic transfer of glucose from *p*-nitrophenol- β -D-glucoside to alcohol.

Reaction mixture :

0.048 M *p*-nitrophenol- β -d-glucoside 2.0 ml
 0.2 M acetate buffer, pH 4.8 2.0 ml
 Enzyme solution (from *Sambucus Sieboldiana*) 4.0 ml
 10 M alcohol or water 2.0 ml
 Reaction temperature 30°.

Acceptor	Reaction time hrs.	<i>p</i> -Nitrophenol- β -d-glucoside hydrolyzed		Glucose liberated millimoles	Glucose transferred to alcohol millimoles	Glucose transferred, % of <i>p</i> -nitrophenol hydrolyzed
		millimoles	%			
methanol	19.5	3.8	40	2.6	1.2	32
	49.5	7.7	80	4.5	3.2	41
	67.5	8.2	85	4.7	3.5	42
	146.0	9.6	100	5.5	4.1	43
	360.0	9.6	100	5.7	3.9	42
	1200.0	9.6	100	6.4	3.2	33
Ethanol	19.5	3.8	40	2.8	1.0	32
	49.5	7.7	80	4.2	3.5	46
	67.5	8.2	80	4.7	3.5	42
	360.0	9.6	100	6.0	3.0	37
	1200.0	9.6	100	7.8	1.8	19
None	19.5	2.8	30	2.9	0	
	49.5	6.7	70	6.9	0	
	67.5	7.7	80	7.8	0	
	146.0	9.6	100	9.6	0	

It will be seen that of the glucose split from *p*-nitrophenol- β -d-glucoside about 30 to 45 per cent have been transferred to alcohol. It is also worthy of note that the amount of alkylglucoside once formed diminished again with prolonged reaction time. This might be due to contamination of the enzyme preparation with usual β -glucosidase which performs hydrolysis but not glucose transfer.

By the action of a enzyme from *Salix Sachalinensis* upon the solution of 3 gm phenol- β -d-glucoside and 33 gm methanol in 50 ml of water, 0.35 gm pure methyl- β -d glucoside of m. p. 119° and $[\alpha]_D^{15} = -32.6^\circ$ (in H₂O) was obtained.

Glucotransferase not a synthesizing enzyme.

Formation of alkyl- β -d-glucoside by this enzyme can not be ascribed to the synthesizing action in the usual sense, since added glucose is not utilized for the synthesis of alkyl-glucoside.

In the reaction mixture containing ethanol, enzyme and glucose in place of arylglucoside, the change in the content of free glucose was pursued but no decrease could be ascertained. (TABLE II).

TABLE II.

Reaction mixture :

0.048 M d-Glucose	2.0 ml
0.2 M Acetate buffer, pH 4.8	2.0 ml
Enzyme solution	4.0 ml
10 M Ethanol	2.0 ml
Reaction temperature 30°	

Enzyme from	Reaction time, hrs.	Free glucose, millimoles
<i>Sambucus Sieboldiana</i> var. <i>typica</i>	0	9.0
	12	10.0
	24	9.7
* <i>Equisetum arvense</i>	0	9.6
	93	10.2
	120	9.8
* <i>Plantago major</i> var. <i>asiatica</i>	0	9.6
	24	9.6
	74	9.6
	312	9.8

Hydrolyzing and transfer action of the enzyme preparation.

As stated above, the results in Table I favor the view that our enzyme preparations contain usual β -glucosidase together with glucotransferase. If this is the case, the activity ratio of hydrolysis to glucose transfer might be altered by suitable fractional inactivation of the enzyme preparation

Following experiment showed that by heat inactivation a significant change in activity ratio had taken place. Glucotransferase appeared to be more susceptible to heat than β -glucosidase. (TABLE III).

TABLE III.

Heat inactivation.

0.048 M Phenol- β -d-glucodide	5.0 ml
0.5 M Acetate buffer, pH 4.8	2.0 ml
80% Methanol	3.0 ml
Enzym solution (from poplar)	10.0 ml
Reaction time : 6 days, Reaction temperature 30°	

Heating	A Phenol- β -glucoside hydrolyzed millimoles	B Glucose transferred to methanol millimoles	$\frac{B}{A} \times 100$
None	9.2	4.8	52
45°, 15 min	8.8	3.1	35
55°, 15 min	6.7	2.6	37
65°, 15 min	3.6	0.82	23

* Activity of these enzyme solutions for glucotransferase had been tested in separate experiments.

These results may be taken as an additional evidence in support of the view that the enzyme solution contains β -glucosidase along with glucotransferase.

Occurrence of glucotransferase.

In addition to the plants employed in this research as enzyme source, following plants were found to contain glucotransferase. Presumably the occurrence of this enzyme is very wide.

Leaves.

Salix amygdalina var. *nipponica*.

S. babylonica

S. gracilistyla

Gaultheria Miqueliana

Betula latifolia

Prunus yedoensis

Polygonum Reynoutria

Smilax China

Flower.

Robinia pseudacacia

Fruit.

Gaultheria Miqueliana

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