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SHORT COMMUNICATION

# GLUCOSYLATION OF QUERCETIN BY A MAIZE POLLEN ENZYME*

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**Abstract**—A glucosyltransferase present in maize pollen catalyses the formation of isoquercitrin from quercetin and uridine 5′-diphosphoglucose. The reaction also required reduced sylfhydryl groups and was stimulated by addition of magnesium ions. Two pH optima were observed for the catalytic activity, a minor one at 6·2 and a major one at 8 2. The enzyme has a temperature optimum of 37° and can be inhibited by mercuric salts. Whole pollen readily served as the source of the enzyme in the reaction mixture.

## INTRODUCTION

THE GLUCOSYLATION of flavonoids is a common reaction in the metabolism of phenolic compounds in plants.[1] However much of the evidence for these reactions has been obtained by feeding the aglycone to the intact plant. Relatively little information is available concerning plant organelles or cell free extracts. Glucosyltransferase activity in cell free extracts from plants has been reported by Barber,[2] and Barber and Chang.[3] Of particular interest is the glucosylation of quercetin to form isoquercitrin (quercetin-3-glucoside) catalyzed by a cell free extract of mung beans (*Phaseolus aureus*) by the following reaction:[2]

$$\text{Quercetin} + \text{UDPglucose} \longrightarrow \text{UDP} + \text{Isoquercitrin (I)}.$$

In this system uridine 5′-diphosphoglucose (UDPglucose) or thymidine 5′-diphosphoglucose were utilized equally well as glucose donors.

Investigations concerned with the distribution of anthocyanin and its precursors in maize (*Zea mays*) revealed the presence of quercetin and isoquercitrin in seed and pollen. Subsequently the enzyme (UDPglucose:quercetin glucosyltransferase) that catalyses reaction I was discovered in the pollen. First efforts to rupture the pollen and extract the enzyme met with failure; therefore a preliminary investigation was made, to determine the requirements for the glucosylation reaction by using whole pollen as the source of the catalytic activity. The results of these studies are presented and discussed in this paper.

## RESULTS

### *Identification of Isoquercitrin*

The product of the reaction was identified as isoquercitrin by chromatography (Table 1) and also by spectrophotometric studies.

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[1] W. Z. HASSID, *Science* **165,** 137 (1969).
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TABLE 1. $R_f$ VALUES FOR ISOQUERCITRIN

| Compound | $R_f$ Solvent* A | B | C |
|---|---|---|---|
| Reaction product | 0·45 | 0·59 | 0·11 |
| Isoquercitrin | 0·47 | 0 60 | 0 12 |
| Quercetin | 0 05 | 0 74 | 0 61 |

* See Experimental for solvent systems and media.

In spectral studies enzymatically produced and standard isoquercitrin were found to have absorption maxima in ethyl acetate at 257 and 358 nm and quercetin had maxima at 253 and 368 nm.

Acid hydrolysis of the enzymatically produced and standard isoquercitrin yielded quercetin and glucose which were easily identified by conventional chromatographic techniques.[4]

*Properties of the Glucosyltransferase System*

Demonstration of catalytic activity in the pollen required the addition of UDPglucose and reduced sulfhydryl groups to the system. Addition of $Mg^{2+}$ gave a 20 per cent increase in enzyme activity. The optimum incubation time for the enzyme was 150 min. Examination of the reaction suspension under a microscope showed that the pollen grains were breaking up during the incubation. Several attempts were made to determine Michaelis–Menten constants for UDPglucose, and for quercetin using pollen as the source of enzyme without consistent results.

The glucosyltransferase had a clearly defined requirement for UDPglucose as the glucose donor for the reaction. Other nucleotide sugars involving the separate bases: thymine, adenine, cytosine and guanine were also assayed for glucose donor activity with a trace of activity from thymidine 5′-diphosphoglucose. Addition of adenosine 5′-triphosphate to the system as a protective agent for the nucleotide sugars had no effect.

Glucosyltransferase activity was assayed over a pH range from 5·8 to 9·0 using citrate–phosphate buffer from 5·8 to 7·0 and tris (hydroxymethyl) aminomethane buffer from 7·4 to 9·0. Activity optima were observed for the enzyme at pH 6·2 and 8·2 with almost double the activity at pH 8·2 as at 6·2. Deletion of the buffer from the system resulted in near total loss of the catalytic activity.

Enzymatic activity was markedly inhibited by addition of *p*-chloromercuribenzoate (0·5 mM) or mercuric chloride (0·25 mM) to the reaction mixture. Glucosyltransferase activity was optimum at 37° and could be destroyed by suspension of the pollen in buffer and heating for 10 min at 100°.

## DISCUSSION

Quercetin has been identified previously in pollen[5] and its 3-glucoside in the husks of maize.[6] Evidence presented here indicate that isoquercitrin and the glucosyltransferase involved in isoquercitrin biosynthesis are also present in maize pollen.

[4] YU S. OVADOV, E. V. EVTUSHENKO, V. E. VASKOVSKY, R. G OVODOVA and T. F. SOLOVEVA, *J. Chromatog.* **26,** 111 (1967).
[5] C. T. REDEMANN, S. H. WITTWER, C. D. BALL and H. M. SELL, *Arch. Biochem. Biophys.* **25,** 27 (1950).
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The product of the enzymatic reaction was clearly identified as isoquercitrin in chromatographic and spectrophotometric studies. Acid hydrolysis of the reaction product further supported its identification as isoquercitrin, since the products of hydrolysis were quercetin and glucose.

Rupture of the pollen grains during the incubation period suggests the release of the enzyme into the medium where the reaction then occurs. This would explain the extended length of the incubation period since the rate of the reaction would depend on the rate at which the enzyme was released into the medium. This would also explain the difficulties encountered in obtaining Michaelis constants for the enzyme since these values are dependent on the rate of the reaction during the initial part of the incubation period when the enzyme is saturated with substrate.

The apparent specificity of the enzyme for UDPglucose as the glucose donor is in contrast to the enzyme from mung beans which utilized either the uridine or thymidine nucleotide sugar.[2] In contrast to the requirement for added glucose donor the relatively small increase in isoquercitrin production by added divalent metal ion suggests the presence of divalent metal ions in the pollen. The added reduced sulfhydryl groups in the system probably serve to protect the enzyme against the action of oxidative enzymes in the pollen. The results obtained in the inhibitor studies, for the temperature optimum and pH optima for the maize pollen glucosyltransferase are consistent with the properties of similar enzymes from other plant sources. Preliminary evidence in substrate specificity studies indicates the enzyme will also catalyse formation of the glucoside of kampferol.

The satisfactory use of whole pollen in a reaction system as a source of enzyme should prove a valuable tool in the study of metabolic processes in maize pollen. Further, the haploid genetic nature of pollen should make it possible to readily investigate gene–enzyme relationships in biosynthetic processes in pollen. Such studies are in progress in this laboratory. Although production of a soluble form of the enzyme by conventional enzyme isolation techniques has proved difficult, preliminary results indicate that this can be accomplished.

## EXPERIMENTAL

*Pollen.* Pollen was collected at anthesis from plants in the field, sieved with a No. 70 mesh screen, freeze-dried and stored under vacuum at −10° until used.

*Chemicals.* The chemicals used in this study were obtained commercially with the exception of isoquercitrin which was prepared according to methods previously described.[7]

*Assay of enzymatic activity.* Samples were incubated at 37° for 150 min. The standard reaction mixture contained 1·65 μmoles of UDPglucose, 100 μmoles of tris (hydroxymethyl) aminomethane buffer (pH 8·2 + 0·01 M mercaptoethanol), 10 μmoles of $MgCl_2$, 25 mg of pollen and $H_2O$ to 2·6 ml. After incubation isoquercitrin was extracted with ethyl acetate, the solvent removed, the product suspended in ethanol and chromatographed in solvent C on Magnesol (magnesium acid silicate. 36 gm of magnesium acid silicate and 4 g of $CaSO_4$ with 120 ml of $H_2O$ gives 5 plates[8]). The plates were air dried before using. The isoquercitrin spot was scraped from the plate, suspended in $H_2O$, acidified and extracted with ethyl acetate. Absorbance of the ethyl acetate at 360 nm was then measured.

*Chromatographic studies.* Chromatographic separations were carried out on Whatman 3 MM paper in the following solvents: A = isopropanol–$H_2O$ (11:39); B = *n*-butanol–acetic acid–$H_2O$ (12:3:5). TLC was carried out in solvent C: toluene–ethyl formate–formic acid (5:4:1). Chromatography of sugars was carried out using $NaH_2PO_4$ impregnated silica gel plates in *n*-butanol–methanol–$H_2O$ (5:3:1).[4]

*Acid hydrolysis of isoquercitrin.* Standard and enzymatically produced isoquercitrin were hydrolysed in 1% $H_2SO_4$ at 100° for 2 hr under $N_2$. Quercetin was extracted into ethyl acetate, the solvent removed and the

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[8] R. L. Larson, *J. Chromatog.* **43**, 287 (1969).

residue suspended in ethanol for chromatography. The aqueous phase containing the sugar was neutralized with $BaCO_3$, filtered and freeze dried. The sugar was chromatographed with standard sugars by silica gel TLC.

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