Flavonol 3-O-Methyltransferase in Plant Tissues

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Flavonol 3-O-methyltransferase activity has been detected in Calamondin orange peel and root tissue, tobacco cell culture and young shoots of *Chrysosplenium americanum*. The enzyme was purified from the latter tissue by precipitation with ammonium sulphate, ion-exchange chromatography and finally by chromatofocusing on Polybuffer exchanger at pI 4.8. The focused enzyme exhibited strict stereospecificity towards quercetin, MW of 65000 daltons and pH optimum of 7.5–8.5. The apparent K_m values for quercetin and S-adenosyl-L-methionine were 1.4 and 70 μM, respectively.

O-Methylated flavonoids are known for their wide spread occurrence in plants [1], with the 3-OMe-flavones accounting for almost 55% of the substituted flavonols [2]. The 3-OH group of flavonols exhibits the highest nuclear electron density as compared with other hydroxy groups on the flavonoid ring system [3]. This characteristic feature was found to compare well with the high methyl acceptor ability of a number of 3-hydroxyflavones by cell-free extracts [4].

Except for O-methylation of ring B of flavonoids which has been thoroughly investigated [5–9], there is a lack of knowledge on the enzymic O-methylation of ring A as well as the heterocyclic ring. Recent work in this laboratory [4, 10–12] has demonstrated the *in vitro* O-methylation of a number of flavonoid compounds including the flavonols, galangin (3,5,7-trihydroxyflavone) and quercetin (3,5,7,3′,4′-pentahydroxyflavone). We wish to report here on the 3-O-methylation of flavonols by enzyme preparations from three different plant sources and some properties of the enzyme involved. These were Calamondin orange peel and root tissue (I), tobacco pith cell culture (II) and *Chrysosplenium americanum* young shoots (III).

The plant material (I-III) was frozen in liquid nitrogen, mixed with Polyclar AT and homogenised with 0.1 M phosphate buffer, pH 7.6 containing 5 mM EDTA, 10 mM diethylammonium diethyldithiocarbamate and 14 mM 2-mercaptoethanol, then centrifuged at 20000×*g* for 15 min. After treatment with Dowex 1X2, the supernatnat was fractionated with solid ammonium sulphate and the protein which precipitated between 35–70% satn. was separated by centrifugation, desalted on Sephadex G-25 and was used as the enzyme source of plant material I. In the case of II, the desalted pellet was further purified on a DEAE-cellulose column using a linear gradient of 0-250 mM KCl and fractions were tested for OMT activity. In the case of III, the protein extract was treated as in II, except for one-step elution from the ion-exchange column using 125 mM KCl; and the protein fractions which exhibited OMT activity were precipitated with ammonium sulphate and desalted. The desalted protein was further purified by chromatofocusing on Polybuffer exchanger (Pharmacia). The bound protein was eluted with Polybuffer-94 which generated a linear gradient of pH 6–4 and fractions were collected for 3-OMT assay. The standard enzyme assay was the same as that described previously [4] using S-adenosyl-L-[$^{14}CH_3$]methionine as the methyl group donor. The reaction products were extracted with a mixture of ethyl acetate-benzene (1:1, v/v) and chromatographed on cellulose or Polyamid-6 TLC plates then autoradiographed.

Calamondin orange enzyme preparation catalysed the 3-O-methylation of both galangin and quercetin; though the latter was twice as efficient methyl acceptor as the former [4]. Tobacco cell culture OMT, on the other hand, utilised galangin more efficiently than quercetin. Almost 95% of the reaction product was found in 3-OMe-galangin (Fig. 1) which co-chromatographed with an authentic sample. Whereas the partially purified OMT preparations of *C. americanum* (ammonium sulphate pellet and DEAE-cellulose fractions) catalysed the successice O-methylation of quercetin to its 3-OMe- and 3,7-diOMe-derivatives (Fig. 2A, B), the chromatofocused enzyme O-methylated quercetin exclusively at

Abbreviations: OMT, O-methyltransferase

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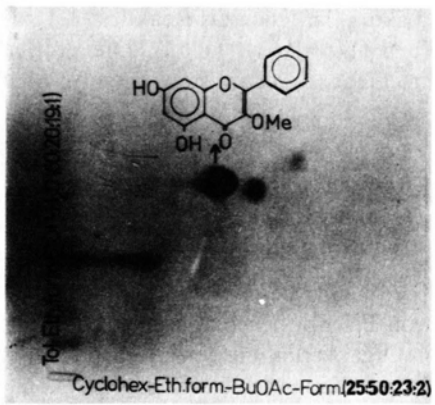

Fig. 1. Photograph of an autoradiograph of the O-methylated products of galangin catalysed by a partially purified enzyme preparation of tabacco cell culture. The major product co-chromatographed with 3-OMe-galangin.

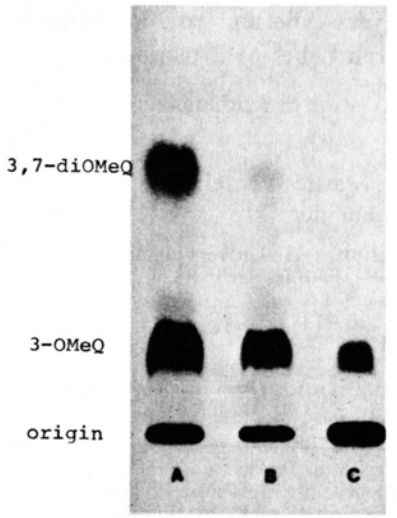

Fig. 2. Photograph of an autoradiograph of the O-methylated products of quercetin catalysed by different enzyme preparations from *C. americanum*: A, desalted ammonium sulphate pellet; B, after chromatography on DEAE-cellulose; C, after chromatofocusing on Polybuffer exchanger (Q = quercetin).

the 3-position (Fig. 2C). The fact that it did not accept any of the flavonols tested (galangin, kaempferol, myricetin, quercetagetin or gossypetin) indicates strict stereo specificity and strongly suggests its designation as quercetin 3-O-methyltransferase. This enzyme did not react with any of the phenylpropanoids tested (caffeic, ferulic or 5-OH-ferulic) nor with the flavones apigenin or luteolin.

The 3-OMT of *C. americanum* which focused at pI 4.8 had an apparent molecular weight of 65000 daltons, a pH optimum of 7.5–8.5 and required Mg^{2+} for activity. The apparent K_m values for quercetin and S-adenosyl-L-methionine wer 1.4 and 70 µM, respectively. The purification data and kinetic properties of this novel enzyme will be reported elsewhere.

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