

## 1 Nomenclature

### EC number

2.4.1.249

### Systematic name

UDP-glucose:delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside 3'-O-glucosyltransferase

### Recommended name

delphinidin 3',5'-O-glucosyltransferase

### Synonyms

UA3'5'GT <1> [1]

UA3'GT <1> [1]

UA5'GT <1> [1]

UDP-glucose:anthocyanin 3',5'-O-glucosyltransferase <1> [1]

## 2 Source Organism

<1> *Clitoria ternatea* (UNIPROT accession number: A4F1Q6) [1]

## 3 Reaction and Specificity

### Catalyzed reaction

UDP-glucose + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside = UDP + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3'-O- $\beta$ -D-glucoside (<1> UDP-glucose:anthocyanin 3,5-O-glucosyltransferase has both, UA3GT and UA5GT activities, highly regioselective to the 3 and 5 OH groups [1])

UDP-glucose + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3'-O- $\beta$ -D-glucoside = UDP + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3',5'-di-O- $\beta$ -D-glucoside (<1> UDP-glucose:anthocyanin 3,5-O-glucosyltransferase has both, UA3GT and UA5GT activities, highly regioselective to the 3 and 5 OH groups [1])

### Natural substrates and products

**S** UDP-D-glucose + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside <1> (<1> 3-O-glucosyltransferase activity, first reaction of UA35GT in the biosynthesis of ternatins, transferring two glucosyl groups in a stepwise manner [1]) (Reversibility: ?) [1]

- P** UDP + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3'-O- $\beta$ -glucoside
- S** UDP-D-glucose + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -glucoside-3'-O- $\beta$ -D-glucoside <1> (<1> 5-O-glucosyltransferase activity, second reaction of UA35GT in the biosynthesis of ternatins, transferring two glucosyl groups in a stepwise manner [1]) (Reversibility: ?) [1]
- P** UDP + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -glucoside-3',5'-di-O- $\beta$ -D-glucoside

### Substrates and products

- S** UDP-D-glucose + cyanidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside <1> (Reversibility: ?) [1]
- P** UDP + cyanidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3'-O- $\beta$ -D-glucoside (<1> 24.3% relative activity compared to delphinidin 3-O-(6-O-malonyl)- $\beta$ -D-glucoside as substrate [1])
- S** UDP-D-glucose + delphinidin 3,3',5'-di-O- $\beta$ -D-glucoside <1> (<1> 3.3% relative activity compared to delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside as substrate [1]) (Reversibility: ?) [1]
- P** UDP + delphinidin 3,3',5'-tri-O- $\beta$ -D-glucoside
- S** UDP-D-glucose + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside <1> (<1> 3-O-glucosyltransferase activity, first reaction of UA35GT in the biosynthesis of ternatins, transferring two glucosyl groups in a stepwise manner [1]; <1> 3-O-glucosyltransferase activity, catalyzed by UA35GT [1]) (Reversibility: ?) [1]
- P** UDP + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3'-O- $\beta$ -glucoside
- S** UDP-D-glucose + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -glucoside-3'-O- $\beta$ -D-glucoside <1> (<1> 5-O-glucosyltransferase activity, second reaction of UA35GT in the biosynthesis of ternatins, transferring two glucosyl groups in a stepwise manner [1]; <1> 5-O-glucosyltransferase activity, catalyzed by UA35GT [1]) (Reversibility: ?) [1]
- P** UDP + delphinidin 3-O-(6''-O-malonyl)- $\beta$ -glucoside-3',5'-di-O- $\beta$ -D-glucoside
- S** UDP-D-glucose + delphinidin 3-O- $\beta$ -D-glucoside <1> (<1> 3.9% relative activity compared to delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside as substrate [1]) (Reversibility: ?) [1]
- P** UDP + 3,3'-di-O- $\beta$ -D-glucoside
- S** UDP-D-glucose + petunidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside <1> (Reversibility: ?) [1]
- P** UDP + petunidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside 5'-O- $\beta$ -D-glucoside (<1> 123% relative activity compared to delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside as substrate [1])
- S** Additional information <1> (<1> no activity detected with cyanidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3-O- $\beta$ -D-glucoside, pelargonidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside, delphinidin 3,5-di-O- $\beta$ -D-glucoside. No activity with delphinidin 3-O-(6''-O-malonyl)- $\beta$ -glucoside-3-O- $\beta$ -D-glucoside using UDP-D-galactose as sugar donor [1]) (Reversibility: ?) [1]
- P** ?

**Inhibitors**

- N-ethylmaleimide <1> (<1> 10 mM, 16% activity retained [1]) [1]  
 diethylidicarbonate <1> (<1> 10 mM, 16% activity retained [1]) [1]  
 dithioerythritol <1> (<1> 10 mM, 83% activity retained [1]) [1]  
 iodoacetate <1> (<1> 10 mM, 84% activity retained [1]) [1]  
*p*-chloromercuribenzoic acid <1> (<1> 10 mM, completely inhibited [1]) [1]

**Metals, ions**

- CaCl<sub>2</sub> <1> (<1> 10 mM, 82% activity retained [1]) [1]  
 EDTA <1> (<1> 10 mM, 75% activity retained [1]) [1]  
 FeCl<sub>2</sub> <1> (<1> 10 mM, 73% activity retained [1]) [1]  
 MgCl<sub>2</sub> <1> (<1> 10 mM, 88% activity retained [1]) [1]  
 ZnCl<sub>2</sub> <1> (<1> 10 mM, 62% activity retained [1]) [1]  
 Additional information <1> (<1> completely inhibited by 10 mM CuCl<sub>2</sub> [1]) [1]

**Turnover number (s<sup>-1</sup>)**

- 4.4 <1> (delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside, <1> UDP-glucose as sugar donor [1]) [1]  
 4.9 <1> (UDP-D-glucose, <1> delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3-O- $\beta$ -glucoside as substrate [1]) [1]  
 5.1 <1> (UDP-D-glucose, <1> delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside as substrate [1]) [1]  
 5.4 <1> (delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3'-O- $\beta$ -glucoside, <1> UDP-glucose as sugar donor [1]) [1]

**Specific activity (U/mg)**

- 0.000276 <1> (<1> UA5GT activity, substrate: delphinidin 3,3-di-O- $\beta$ -D-glucoside [1]) [1]  
 0.000324 <1> (<1> UA3GT activity, substrate: delphinidin 3-O- $\beta$ -D-glucoside [1]) [1]  
 0.002 <1> (<1> UA3GT activity, substrate: cyanidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside [1]) [1]  
 0.01 <1> (<1> UA5GT activity, substrate: petunidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside [1]) [1]  
 0.98 <1> (<1> UA3GT activity, substrate: delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside [1]) [1]  
 2.29 <1> (<1> UA5GT activity, substrate: delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3-O- $\beta$ -glucoside [1]) [1]

**K<sub>m</sub>-Value (mM)**

- 0.0389 <1> (delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside) [1]  
 0.138 <1> (delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3'-O- $\beta$ -glucoside) [1]  
 1.49 <1> (UDP-D-glucose, <1> apparent, delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside as saturated substrate [1]) [1]  
 6.18 <1> (UDP-D-glucose, <1> apparent, delphinidin 3-O-(6''-O-malonyl)- $\beta$ -D-glucoside-3-O- $\beta$ -glucoside as saturated substrate [1]) [1]

**pH-Optimum**

7.5-8 <1> (<1> pH 7.5 and pH 8.0 in potassium phosphate buffer and Tris-HCl buffer, respectively [1]) [1]

**4 Enzyme Structure****Molecular weight**

48000 <1> (<1> gel filtration [1]) [1]

50800 <1> (<1> SDS-PAGE [1]) [1]

**Subunits**

monomer <1> (<1> 1 \* 58000, SDS-PAGE [1]) [1]

**5 Isolation/Preparation/Mutation/Application****Source/tissue**

petal <1> [1]

**Purification**

<1> (from petals, which accumulate polyacylated anthocyanins and ternatins, using ammonium sulfate precipitation, DEAE Toyopearl, Reactive yellow 86, Cellufine HAP, and Mono Q chromatography, UA5'GT: 1007fold purified, UA3'GT: 346 fold purified) [1]

**References**

- [1] Kogawa, K.; Kato, N.; Kazuma, K.; Noda, N.; Suzuki, M.: Purification and characterization of UDP-glucose: anthocyanin 3',5'-O-glucosyltransferase from *Clitoria ternatea*. *Planta*, **226**, 1501-1509 (2007)