Purification of Recombinant Flavanone 3β-Hydroxylase Using Bio-Scale CHT5-I Hydroxyapatite Column

Introduction

Flavanone 3β-hydroxylase (FHT), a typical 2-oxoglutarate-dependent dioxygenase, catalyzes the 3β-hydroxylation of 2*S*-flavanones to 2*R*,3*R*-dihydroflavonols which are intermediates in the biosynthesis of flavanoids, catechins, proanthocyanidins, and anthocyanidins in plants. To understand the complex reaction mechanism, the active site of the hydroxylase, and especially its interaction with the substrates and cofactors of the enzyme reaction, knowledge of the crystal structure of the protein is required.

The *E. coli*-expressed enzyme from *Petunia hybrida* was purified to apparent homogeneity by a three-step chromatographic procedure which included rapid, high resolution chromatography on a 5 ml Bio-Scale CHT5-I ceramic hydroxyapatite column (Bio-Rad, catalog # 751-0023).

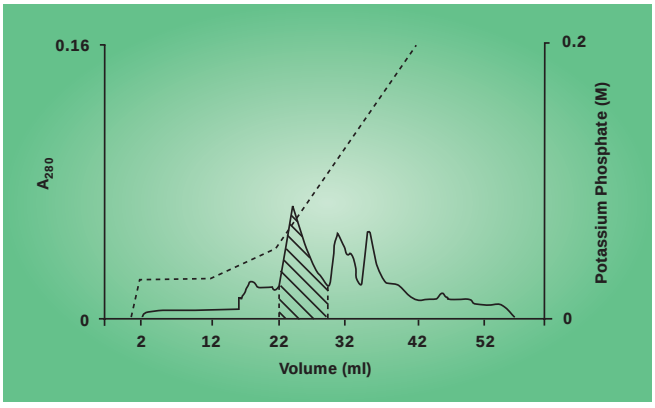

Fig. 1. Purification of recombinant flavanone 3β-hydroxylase from Petunia hybrida *on a Bio-Scale CHT5-I column. Protein load: 3 mg; Buffer A: 50 mM imidazole-HCl, 5 mM potassium phosphate, 2 mM DTT, pH 6.8; Buffer B: 200 mM potassium phosphate, 2 mM DTT, pH 6.8; Gradient: 12.5%–25% B in 10 ml, 25%–100% B in 40 ml; Flow rate: 1 ml/min; Detection: 280 nm.*

Results

Recombinant flavanone 3β-hydroxylase was expressed in *E. coli*. The cells were lysed and the resulting supernatant from a 45% saturated ammonium sulfate precipitation step was fractionated on a butyl HIC column. The FHT-containing fraction was concentrated and further fractionated using a gel filtration column. The FHT-containing fractions from the gel filtration column were pooled and loaded directly onto a 5 ml Bio-Scale CHT5-I column. Bound proteins were eluted by a two-step linear gradient from 25–50 mM in 10 ml and 50–200 mM potassium phosphate in 40 ml (Figure 1). Pure enzyme was obtained in the fractions containing 50–80 mM potassium phosphate. The purity of the enzyme was investigated by polyacrylamide gel electrophoresis (Figure 2).

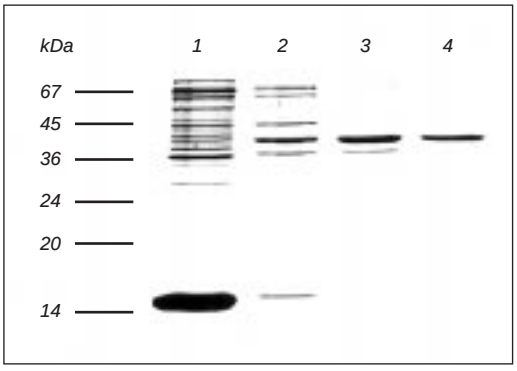

Fig. 2. SDS-PAGE analysis for Petunia hybrida *flavanone 3β-hydroxylase from* E. coli*. Fractions were run on a 12.5% Mini-PROTEAN® II gel and stained with Coomassie® blue stain. Lane 1: Soluble fraction of the* E. coli *cells expressing FHT (crude extract). Lane 2: Combined FHT-active fractions after hydrophobic interaction chromatography on Fractogel EMD Butyl (S). Lane 3: Combined FHT-active fractions after size exclusion chromatography on a Fractogel EMD BioSEC 650 (S). Lane 4: Purified FHT after chromatography on Bio-Scale CHT 5-I ceramic hydroxyapatite column.*

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Purification Table for Recombinant *Petunia hybrida* FHT from *E. coli* Cells (7 g)

PURIFICATION STEP	TOTAL PROTEIN (mg)	SPECIFIC ACTIVITY (U/mg)	PURIFICATION (FOLD)	TOTAL RECOVERY (%)
Crude Extract	236	0.05	1	100
Bio-Scale CHT5-I Column	0.535	2	40	9

Data is not provided for the butyl HIC and gel filtration steps

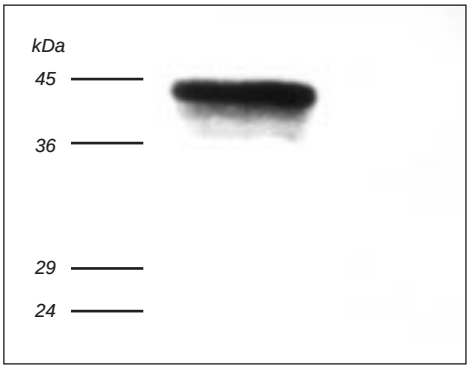

Fig. 3. Immunoblot of FHT after Bio-Scale CHT5-I hydroxyapatite column probed with specific polycolonal antiserum from rabbit.

The purification procedure was monitored by immuno dot blot analysis for FHT protein (Figure 3) and enzyme activity assays of the individual fractions after each of the chromatographic steps. The table contains the essential purification parameters.

Data courtesy of Dr Richard Lukacin, Institute for Pharmaceutical Biology, University of Marburg, Germany

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