Enzyme and Microbial Technology 81 (2015) 63–71

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Stepwise conversion of flavonoids by engineered dioxygenases and dehydrogenase: Characterization of novel biotransformation products

Heike Overwin[a], Myriam González[b], Valentina Méndez[b], Franco Cárdenas[b], Michael Seeger[b], Bernd Hofer[a,*]

[a] Department of Chemical Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany
[b] Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química & Center for Nanotechnology and Systems Biology & Centro de Biotecnología, Universidad Técnica Federico Santa María, Valparaíso, Chile

ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form 28 July 2015
Accepted 5 August 2015
Available online 7 August 2015

Keywords:
Flavonoid
Chalcone
Hydroxylation
Diol
Dioxygenase
Dehydrogenase

ABSTRACT

Flavonoids are a large group of plant secondary metabolites that exert various biological and pharmacological effects. In this context, the generation of derivatives is of considerable interest. The introduction of hydroxy groups is of particular relevance, as they are known to be involved in many of the biological interactions and furthermore enable additional modifications, such as glycosylations. Bacterial aryl-hydroxylating dioxygenases (ARHDOs) have proven to be very useful for the conversion of aromatic structures into versatile building blocks for different kinds of derivatizations. Such enzymes have been used with varying success for the oxidation of flavonoids. In order to find better ARHDOs for the hydroxylation of such substrates, we carried out biotransformation trials with a collection of hybrid ARHDOs of different origin, using resting cells of recombinant strains. This identified enzymes able to transform all of the flavonoids examined, typically in yields above 50%. It also showed that moderately reactive substituents of flavonoids, such as hydroxy or amino groups, can lead to spontaneous follow-up reactions with the dienediol structures generated by dioxygenation. A report of flavanone epoxidation, a reaction never before observed to be catalyzed by an ARHDO, is challenged by our results. All ARHDOs examined converted this substrate into a dehydrogenase-transformable dihydrodiol. All dihydrodiols obtained by dioxygenation of the examined flavonoids were successfully re-aromatized into catechols by a bacterial dehydrogenase. These metabolites were usually stable. However, the catechols formed from flavanone and 2'-hydroxy-chalcone, respectively, were interconvertible under mild conditions. Altogether, we isolated and characterized 13 compounds that have not previously been described. The biotransformations reported here give access to novel flavonoid derivatives that may be applied for biological screens as well as for further modification, such as glycodiversification.

1. Introduction

Flavonoids and the structurally as well as metabolically related chalcones (Fig. 1) are a diverse group of bioactive compounds of natural origin [1,2]. They are ubiquitous secondary metabolites of plants and have been shown to exert various biological and pharmacological effects, including influence on certain forms of cancer, cardiovascular disease, diabetes mellitus, allergic diseases, bacterial infections and cholesterol metabolism, stimulation of the immune system, as well as anti-inflammatory and anti-viral activities [1,3–5].

Given their various biological effects, it is of considerable interest to further diversify these compounds by chemical, biological or chemo-enzymatic means. Particularly relevant naturally occurring reaction types for compound modifications, so-called tailoring reactions, are hydroxylations, methylations and glycosylations [6]. Hydroxylations are of particular importance, on the one hand, as hydroxy groups are crucially involved in many beneficial effects of flavonoids, on the other hand, as these reactions generate novel reactive sites for further modification, such as glycosylation.

Abbreviations: ARHDO, aryl-hydroxylating dioxygenase; DHD, dihydrodiol; GC–MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatography; LC–HRMS, liquid chromatography–high resolution mass spectrometry; NMR, nuclear magnetic resonance.

* Corresponding author at: Helmholtz Centre for Infection Research, Department of Chemical Biology, Inhoffenstraße 7, D-38124 Braunschweig, Germany. Fax: +49 531 61813499.
E-mail address: bernd.hofer@helmholtz-hzi.de (B. Hofer).

http://dx.doi.org/10.1016/j.enzmictec.2015.08.001
0141-0229/

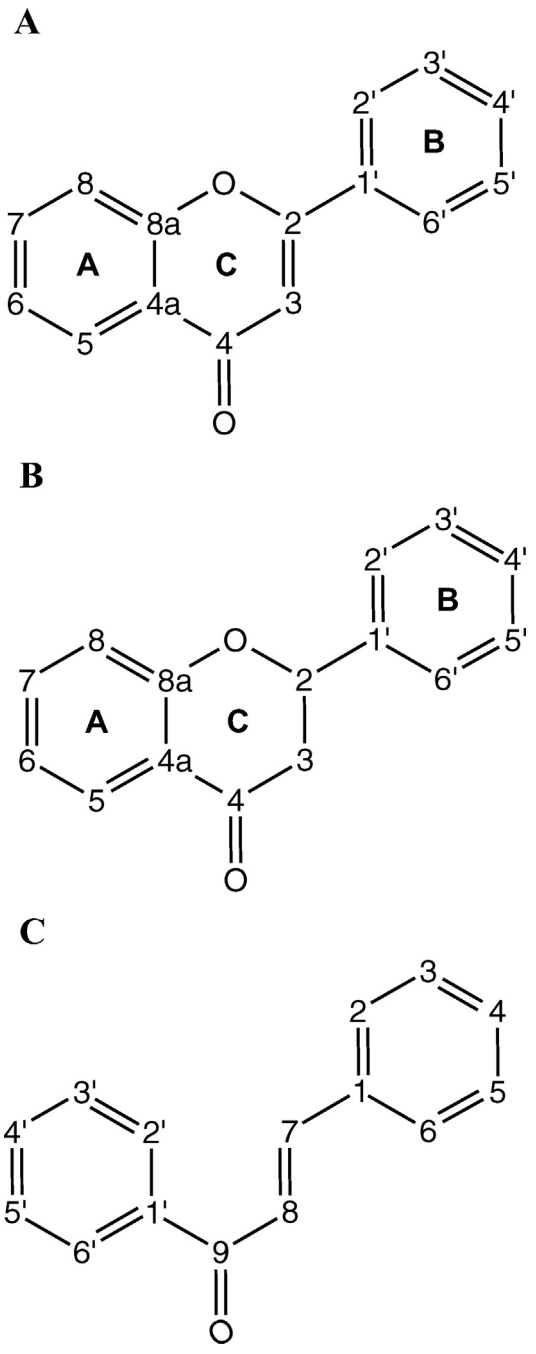

Fig. 1. Atom numbering of flavones (A), flavanones (B) and chalcones (C). Parts A and B also show the lettering of flavonoid rings.

Microbial hydroxylations of flavonoids are mostly carried out by fungi and typically target carbons of the A ring or the *para* position (4′) of the B ring (Fig. 1). The majority of these reactions are monohydroxylations. Dihydroxylations are usually directed towards a side chain, but not to the flavonoid scaffold [7]. It has already been shown that bacterial ARHDOs are able to attack flavonoids [8–17]. This group of enzymes is of particular interest for three reasons. Firstly, they hydroxylate other carbons than mentioned above. Secondly, they generate products which can be further converted into phenolic or catecholic moieties by chemical or enzymatic means, respectively. Thirdly, the dioxygenation reaction converts the targeted ring into a cyclic diene substructure that opens up the way for a diverse range of further chemical modifications of the parent compound [18]. In the case of flavonoids, the direct dioxygenation products, so-called dihydrodiols or dienediols, have rarely been isolated and characterized. Publications dealing with the dioxygenation of flavonoids rather have described the formation of phenols and catechols, which are follow-up products of the dioxygenations.

In the present work, we characterized both, the direct dioxygenation products and their catecholic daughter products, obtained through dehydrogenation of the former. For all of the examined flavonoids a successful dioxygenation was observed, which for most of these compounds has previously never been described. As the hydroxylation of flavonoids and related compounds, catalyzed by ARHDOs, is not always successful and often inefficient, we first screened our selection of compounds against a collection of mostly artificial ARHDOs that have previously been generated in our lab and have been shown to possess different and fairly wide substrate ranges [19–21]. All substrates were biotransformed by at least three of these enzymes. This resulted, in addition to expected metabolites, in a number of unexpected products and reactions. Thirteen of the obtained compounds have never been described before.

2. Materials and methods

2.1. Chemicals

Compounds used as substrates were of the highest purity available. 5-Hydroxy, 6-amino- and 7-aminoflavone were obtained from Sigma–Aldrich (Munich, Germany), 3- and 7-hydroxyflavone, flavanone, 2′-hydroxyflavanone and *trans*-2′-hydroxychalcone were obtained from Tokyo Chemical Industry (Eschborn, Germany).

2.2. Plasmids

All of the plasmids are based on the phage T7 expression vector pT7-6. pAIA111 [22] carries *bphA1A2A3A4* (collectively referred to as *bphA*) of *Burkholderia xenovorans* LB400 (gene accession no. M86348.1). The following plasmids contain a modified *bphA1A2A3A4* gene cluster from *B. xenovorans* LB400 in which part of *bphA1* has been replaced by the respective segment from an ARHDO gene from *Pseudomonas* sp. strain B2A (accession no. AJ544518.1, resulting in plasmid pAIA1102), *Pseudomonas* sp. strain B4 (accession no. AJ544519.1, plasmid pAIA1104), *Ralstonia* sp. strain B15 (accession no. AJ544523.1, plasmid pAIA1115), *Rhodococcus opacus* BIE-20 (accession no. AJ544524.1, plasmid pAIA1121) or from metagenomic DNA (accession no. FR877589.1, plasmid pAIA1B15 and accession no. FR877629.1, plasmid pAIA1C18). The construction of pAIA1104 has previously been described [21]. The other plasmids were constructed similarly by deletion of major parts of the *bphBC* genes from the respective previously described precursor plasmids pAIA6102, pAIA6115 and pAIA6121 [19] or pAIA6B15 and pAIA6C18 [20]. Deletions were done by digestion with *Ppu*MI (pAIA6102 and pAIA6B15) or *Bsp*EI (pAIA6115, pAIA6121 and pAIA6C18) and recircularization of the largest fragment, following standard methods [23]. Plasmid pAIA20, efficiently expressing the *bphB* gene of *B. xenovorans* LB400 (accession no. X66122.1), was constructed by deletion of major parts of the *bphC* gene from pDD372 [24], which contains *bphBC*, by digestion with *Eag*I and recircularization, following standard methods [23].

2.3. Bacterial strains and culture conditions

The *Escherichia coli* strains used in this study were all derivatives of the host BL21(DE3)[pLysS] [25]. They harboured one of the plasmids described above. The strains were grown in Luria–Bertani medium [23] at 37 °C, unless otherwise indicated. Chloramphenicol