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Biosynthesis of curcuminoids and gingerols in turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*): Identification of curcuminoid synthase and hydroxycinnamoyl-CoA thioesterases

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Abstract

Members of the Zingiberaceae such as turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale* Rosc.) accumulate at high levels in their rhizomes important pharmacologically active metabolites that appear to be derived from the phenylpropanoid pathway. In ginger, these compounds are the gingerols; in turmeric these are the curcuminoids. Despite their importance, little is known about the biosynthesis of these compounds. This investigation describes the identification of enzymes in the biosynthetic pathway leading to the production of these bioactive natural products. Assays for enzymes in the phenylpropanoid pathway identified the corresponding enzyme activities in protein crude extracts from leaf, shoot and rhizome tissues from ginger and turmeric. These enzymes included phenylalanine ammonia lyase, polyketide synthases, *p*-coumaroyl shikimate transferase, *p*-coumaroyl quinate transferase, caffeic acid *O*-methyltransferase, and caffeoyl-CoA *O*-methyltransferase, which were evaluated because of their potential roles in controlling production of certain classes of gingerols and curcuminoids. All crude extracts possessed activity for all of these enzymes, with the exception of polyketide synthases. The results of polyketide synthase assays showed detectable curcuminoid synthase activity in the extracts from turmeric with the highest activity found in extracts from leaves. However, no gingerol synthase activity could be identified. This result was explained by the identification of thioesterase activities that cleaved phenylpropanoid pathway CoA esters, and which were found to be present at high levels in all tissues, especially in ginger tissues. These activities may shunt phenylpropanoid pathway intermediates away from the production of curcuminoids and gingerols, thereby potentially playing a regulatory role in the biosynthesis of these compounds.

Keywords: Polyketide synthase; Phenylpropanoid pathway; Rhizome; Acyltransferase; Phenylalanine ammonia lyase; O-methyltransferase; Medicinal plant

1. Introduction

Powdered turmeric (*Curcuma longa* L.) rhizome is widely used as a food additive, especially in curries, and has been very popular in traditional Asian medicine for the treatment of a number of conditions, including hepatic disorders and rheumatism (Miquel et al., 2002). In addition, anti-inflammatory, antiulcerogenic and antitumor activities, among others, have been described for turmeric (Claeson et al., 1994; Joe et al., 2004). The most important constituent of turmeric, curcumin (**1**), has been shown to possess many of these properties. Ginger (*Zingiber officinale* Rosc.), also a member of the Zingiberaceae and an important component of traditional Asian herbal medicine, is used for management of such symptoms as the common cold, digestive disorders, rheumatism, neuralgia, colic and motion-sickness, as well as being an important spice to flavor foods and beverages. [6]-Gingerol (**2**), the major gingerol in ginger rhizomes, has been found to possess many

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interesting pharmacological and physiological activities, such as anti-inflammatory, analgesic and cardotonic effects (Mascolo et al., 1989; Mustafa et al., 1993).

Because of their importance to human health and nutrition, some initial investigations into the biosynthesis of the curcuminoids and gingerols were performed over 25 years ago (Denniff and Whiting, 1976; Macleod and Whiting, 1979; Denniff et al., 1980). These initial radiotracer feeding studies suggested that these compounds are derived from intermediates in the phenylpropanoid pathway that are condensed with other molecules, derived in turn from the acetate and short and medium-chain fatty acid pathways. Based on these results, Schröder (1997) proposed that enzymes similar to polyketide synthases are most likely responsible for formation of the basic backbone structure of these compounds and would utilize coenzyme A derivatives of the intermediates that were suggested from the studies carried out during the 1970s. Based on these results, two biosynthetic pathways can readily be envisaged for the production of each of these two groups of compounds. The curcuminoids could be formed from condensation of two molecules of *p*-coumaroyl-CoA with one molecule of malonyl-CoA via the action of a polyketide synthase (or similar) enzyme, perhaps involving an additional diketide intermediate, as has been suggested recently by Bernd Schneider's group (Brand et al., 2006). The resulting bisdemethoxycurcumin (**3**) would then be transformed through demethoxycurcumin (**4**) into curcumin (**1**) via two sequential rounds of hydroxylation followed by *O*-methylation (see bottom pathway in Fig. 1). Alternatively, it is likely that the curcuminoid synthase enzyme may utilize the CoA esters of both *p*-coumaric acid (**5**) and ferulic acid (**6**) as substrates. In this case, the central pathway in Fig. 1 could be operative, and the hydroxylation and *O*-methylation reactions that lead to formation of the methoxyl functional groups in curcumin (**1**) would be the same reactions as those found in the general phenylpropanoid pathway. Likewise, the gingerols could be produced via related pathways, as outlined in Fig. 1, with the addition of reduction steps necessary to eliminate the double bond present in the *p*-coumaric acid-derived intermediate(s). This reduction could occur prior to condensation or after condensation of the phenylpropanoid derived moiety with the CoA ester of the corresponding short chain aliphatic alcohol. Enzymes in all of these proposed pathways would include one or more polyketide synthases, cytochrome p450 hydroxylases, and *S*-adenosyl-L-methionine-dependent *O*-methyltransferases. The present investigation

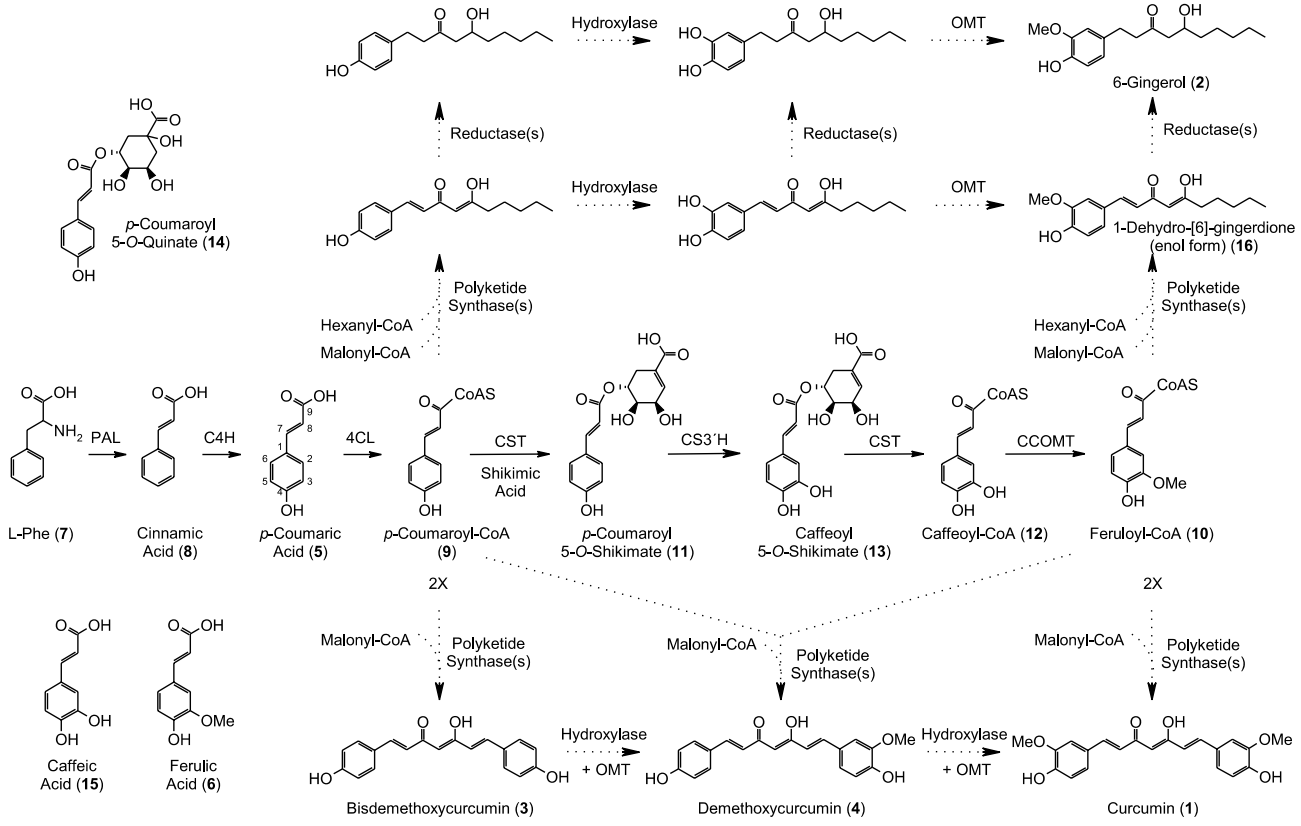

Fig. 1. Proposed biosynthetic pathway to curcuminoids and gingerols in turmeric and ginger. Enzymes are as follows: PAL = phenylalanine ammonia lyase; C4H = cinnamate 4-hydroxylase; 4CL = 4-coumarate:CoA ligase; CST = *p*-coumaroyl shikimate transferase; CS3′H = *p*-coumaroyl 5-*O*-shikimate 3′-hydroxylase; OMT = *O*-methyltransferase; CCOMT = caffeoyl-CoA *O*-methyltransferase. All conversions have been demonstrated in other species, except for those catalyzed by the polyketide synthases, the reductase, and the hydroxylases and OMTs that would convert bisdemethoxycurcumin (**3**) via demethoxycurcumin (**4**) to curcumin (**1**) (indicated by dashed arrows).