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Production of deuterated zeaxanthin by *Flavobacterium multivorum* and its detection by resonance Raman and mass spectrometric methods

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Abstract

Flavobacterium multivorum, a zeaxanthin-producing organism, was grown aerobically in a medium prepared with deuterated water. Atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and resonance Raman spectroscopy (RRS) analysis revealed ~75% replacement of hydrogen by deuterium atoms as indicated by the molecular mass cluster at around m/z 600. Deuterated zeaxanthin upon excitation with a 488 nm laser exhibited characteristic resonance Raman vibrational modes at 1161 and 1504 cm^{-1} as compared to 1007, 1159 and 1525 cm^{-1} for undeuterated zeaxanthin. HPLC/APCI-MS and HPLC/RRS were specific and sensitive with limits of detection of 2.5 pg and 50 ng, respectively.

Introduction

Dietary xanthophyll carotenoids, such as lutein [(3R,3′R,6′R)-β,ϵ-carotene-3,3′-diol] and zeaxanthin [(3R,3′R)-β,β-carotene-3,3′diol], act as preventive antioxidants against age-related macular degeneration (AMD), the leading cause of blindness in the developed world (Snodderly 1995, Moeller *et al.* 2000). The human retina accumulates lutein and zeaxanthin in very high concentrations and there are various non-dietary metabolites of lutein and zeaxanthin present in substantial quantities including *meso*-zeaxanthin [(3R,3′S)-β,β-carotene-3,3′diol] and 3′-oxolutein [(3-hydroxy-3′-oxo-β,ϵ-carotene)] (Bernstein *et al.* 2001). Although Khachik *et al.* (2002) proposed a mechanism for the inter-conversion of these xanthophyll metabolites, the enzymatic or photochemical basis for these interconversions is unknown. Metabolite labeling of dietary carotenoids with heavy isotopes in animal models is an important method to understand the mechanism of proposed interconversions.

HPLC with UV–visible absorption detectors are used routinely for analysis of carotenoids from microbial sources but it is impossible to differentiate between deuterated and undeuterated product using HPLC and UV–visible absorption detection alone. HPLC can be coupled with an in-line atmospheric pressure chemical ionization mass spectrometer (APCI-MS) to distinguish between labeled and unlabeled carotenoids. Resonance Raman spectroscopy (RRS) is an alternative method that could potentially distinguish between labeled and unlabeled carotenoids because heavy isotope substitution will alter the vibration modes of molecules, and in line HPLC-Raman detectors for other compounds have been reported by other groups (Marquardt *et al.* 1999, Wang *et al.* 2000). In this work, we have used