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Vibrational and electronic spectroscopy of the retro-carotenoid rhodoxanthin in avian plumage, solid-state films, and solution

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ABSTRACT

Rhodoxanthin is one of few retro-carotenoids in nature. These chromophores are defined by a pattern of single and double bond alternation that is reversed relative to most carotenoids. Rhodoxanthin is found in the plumage of several families of birds, including fruit doves (*Ptilinopus*, Columbidae) and the red cotingas (*Phoenicircus*, Cotingidae). The coloration associated with the rhodoxanthin-containing plumage of these fruit dove and cotinga species ranges from brilliant red to magenta or purple. In the present study, rhodoxanthin is characterized *in situ* by UV–Vis reflectance and resonance Raman spectroscopy to gain insights into the mechanisms of color-tuning. The spectra are compared with those of the isolated pigment in solution and in thin solid films. Key vibrational signatures are identified for three isomers of rhodoxanthin, primarily in the fingerprint region. Electronic structure (DFT) calculations are employed to describe the normal modes of vibration, and determine characteristic modes of retro-carotenoids. These results are discussed in the context of various mechanisms that change the electronic absorption, including structural distortion of the chromophore or enhanced delocalization of π-electrons in the ground-state. From the spectroscopic evidence, we suggest that the shift in absorption is likely a consequence of perturbations that primarily affect the excited state of the chromophore.

Introduction

Some of the most vibrant colors of feathers are derived from carotenoids. Colors such as yellow, red, or purple in feathers can be partly attributed to unique carotenoids that are formed metabolically from a small number of dietary carotenoids. The absorption characteristics of these molecules depend in large part upon the length of double-bond conjugation, which can vary substantially. In one remarkable example, 16 different carotenoids were recently identified in the plumage of cotingas, and their double-bond conjugation lengths were found to span all values from 9 to 15, inclusive [1].

Apart from the expected effect that different carotenoids have on coloration, there are also intriguing examples of feathers with the same composition of chromophore(s), yet varied coloration. These cases have long been of interest [2–5] and there are several possible explanations for the variation. First, structural coloration can influence or even dominate the overall color of a feather, independent of the pigments [6–8]. Second, other non-carotenoid pigments, particularly melanin, contribute to the coloration of some bird feathers [9,10]. Third, the electronic absorption of carotenoids can be tuned by polarizing influences (e.g. hydrogen bonding, or nearby charges) in the keratin environment. These types of specific interactions between the carotenoids and surrounding protein are considered likely causes for substantial color-tuning in various carotenoproteins, e.g. within crustaceans [11,12].

An additional mechanism of color-shifts is based upon electronic interaction between neighboring carotenoid molecules. The mechanism has been considered a possibility for protein-bound astaxanthin in crustaceans [13,14] as well as ketocarotenoids within avian plumage [5]. Electronic interactions between chromophores are of exceptional interest, because several photophysical processes that are not possible for a monomer become available for two or more coupled chromophores. In particular, the process of singlet exciton fission, whereby a singlet exciton forms two triplet excitons on neighboring chromophores, becomes possible for carotenoids [15–20]. Despite the evidence for singlet fission in biological systems with carotenoids, no functional role for the mechanism has been determined. We recently proposed that intermolecular singlet fission may provide a photoprotective advantage for carotenoids, via the partitioning of absorbed photonic energy over two chromophores, rather than a single chromophore [18]. Evidence supporting this idea has yet to be found; nevertheless, our interest in the topic has led us to

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investigate natural systems where electronic interaction between carotenoids could play a role in their excited-state dynamics.

The five birds that are the focus of the present study (Fig. 1) all have the carotenoid rhodoxanthin as the principle chromophore in portions of their plumage. The pattern of single/double bond alternation of rhodoxanthin, a retro-carotenoid, is reversed from that found for the vast majority of carotenoids, such as zeaxanthin or β-carotene (Fig. 2). Despite the shared chromophore, the coloration of the feathers probed here varies from crimson red to burgundy, pink, or purple. We considered exciton coupling to be among the possible reasons for the variation. Four of the birds are fruit doves in the genus *Ptilinopus* within the Columbidae family: Wompoo Pigeon (*Ptilinopus magnificus*), Beautiful Fruit Dove (*Ptilinopus pulchellus*), Yellow-bibbed Fruit Dove (*Ptilinopus solomonensis*), and Jambu Fruit Dove (*Ptilinopus jambu*). The presence of rhodoxanthin in this genus has been known for decades [2]. One cotinga species, *Phoenicircus carnifex* (Cotingidae), was also included in our study because the bright red plumage of this bird is also known to contain rhodoxanthin [1,21]. Our principle aim was to gain an understanding of rhodoxanthin *in situ*, both from vibrational and electronic spectroscopy. The combined approach of *in situ* resonance Raman and electronic spectroscopy has been a powerful one for identifying various causes of coloration in carotenoid/protein systems, including structural distortion, π-electron delocalization, and exciton coupling [4,5,11,22,23].

At the outset, the lack of resonance Raman spectra of retro-carotenoids in the literature hampered our exploration of the chromophores in the feathers. One reason for the dearth of spectra is that retro-carotenoids are far less common in nature than carotenoids having the normal pattern of single/double bond alternation [24]. Rhodoxanthin occurs in various plants [25–34], lichens [35], and animals including birds [1–3,21,36–39], and fish [40]. One can infer that vibrational resonance Raman spectra of rhodoxanthin may have been acquired previously [41–43]. However, the prior reports did not make a clear connection between spectra (or peak positions) and the carotenoid. Infrared spectra or band positions of retro-carotenoids have been reported as part of synthetic efforts [44–47]. However, the analyses of IR spectra of retro-carotenoids are sparse and tend to focus on a small portion of the spectrum [48–50]. To our knowledge, there is only a single example in the literature where a full IR spectrum of rhodoxanthin is illustrated [51].

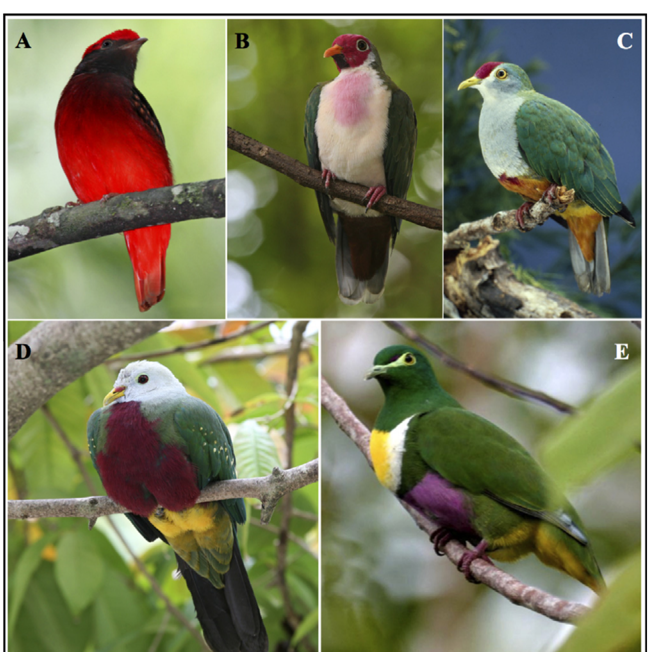

Fig. 1. Photos of bird species with rhodoxanthin in portions of their plumage: (A) Black-necked Cotinga, *Phoenicircus nigricollis* (close relative of *Ph. carnifex*), (B) Jambu fruit dove, *Ptilinopus jambu*, (C) Beautiful fruit dove, *Ptilinopus pulchellus* (D) Wompoo pigeon, *Ptilinopus magnificus* (E) Yellow-bibbed fruit-dove, *Ptilinopus solomonensis*. Photo credits: (A) Wim de Groot; (B and C) T. Friedel/VIREO; (D) W. Peckover/VIREO; (E) Mehd Halaouate.

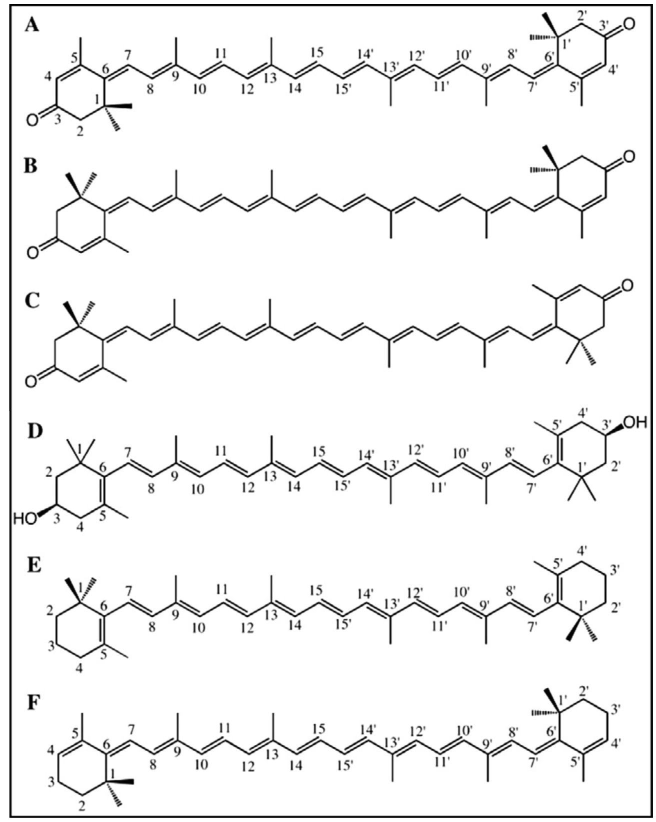

Fig. 2. Chemical structures of (A) (6E,6′E)-rhodoxanthin, (B) (6Z,6′E)-rhodoxanthin, (C) (6Z,6′Z)-rhodoxanthin, (D) (3R,3′R)-zeaxanthin, (E) *β*-carotene, and (F) (6E,6′E)-isocarotene.

Given the lack of any comprehensive report or analysis of vibrational spectra of retro-carotenoids, we have explored the resonance Raman spectroscopy of rhodoxanthin within feathers (*in situ*), and as an isolated chromophore, in solution and in thin solid films. The spectra of three isomers of rhodoxanthin are compared with (3R,3′R)-zeaxanthin, which has a well-understood vibrational spectrum [52]. Assignments of the bands of the retro-carotenoid were aided by the results from density functional theory (DFT[1]) calculations. The vibrational spectra of the carotenoids in the feathers, as well as their absorption spectra, are evaluated with the aim of assessing various mechanisms for the different colors of these feathers.

Methods and materials

Pigment extraction and analysis

The feathers of this study were obtained from the University of Kansas Natural History Museum (Lawrence, KS, USA) and the Yale Peabody Museum of Natural History (Yale University, New Haven, CT, USA). The five feather types are described as follows: (1) red

[1] *Abbreviations used:* DFT, density functional theory; MTBE, methyl-*tert*-butyl ether.