Archives of Biochemistry and Biophysics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Review

Optical detection methods for carotenoids in human skin

Igor V. Ermakov *, Werner Gellermann

Department of Physics and Astronomy, University of Utah, Salt Lake City, UT 84112, USA

ARTICLE INFO

Article history:
Received 26 November 2014
and in revised form 16 January 2015
Available online xxxx

Keywords:
Carotenoids
Antioxidants
Human skin
Raman spectroscopy
Reflection spectroscopy

ABSTRACT

Resonance Raman spectroscopy and reflection spectroscopy are non-invasive optical quantitative methods for the measurement of carotenoid antioxidant levels in human skin *in vivo*. Since all tissue carotenoids are derived from the diet, optical monitoring in skin may serve as an objective indicator for fruit and vegetable intake, and more broadly also as an effective biomarker for integrative health. The two optical methods have already found enthusiastic application in the Nutritional Supplement Industry where they are used with large populations to measure skin carotenoid uptake upon consumption of carotenoid-containing dietary supplements. Applications in medical fields such as nutrition science and epidemiology have been awaiting rigorous correlation studies between the optical carotenoid detection methods and the established gold standard detection method of high-performance liquid chromatography, which requires excised tissue samples. In this article we review the principles of the methods along with the current status of validations so the reader can assess the merits of the optical methods in their respective fields of interest.

Introduction

Carotenoids play an important protective role in healthy human tissues through their optical filtering and/or antioxidant functions [1]. They cannot be synthesized by the human body; rather they need to be taken up from the diet, where they exist in high concentrations in a wide range of fruits and vegetables [2]. The most common carotenoids in the diet are alpha-carotene, beta-carotene, beta-cryptoxanthin, lycopene, lutein, zeaxanthin, phytoene and phytofluene [3]. Usually all these carotenoids are absorbed from the diet by the intestine and transported by lipoproteins through the bloodstream to various target tissues [4]. Carotenoid levels in the blood stream are correlated with dietary intake, typically increasing significantly in response to fruit and vegetable consumption in intervention settings [5]. In the retina, where the carotenoid uptake is mediated by binding proteins, only lutein and zeaxanthin are selectively taken up from the blood stream. They are deposited in high concentrations into the macular region of the retina, where they help prevent or delay the onset of age-related macular degeneration [6]. In skin, all carotenoids are taken up that are present in the blood stream. Here, the carotenoids may help prevent premature skin aging [7] and certain skin cancers [8]. Skin is a relatively stable storage medium for carotenoids and non-invasive optical measurements in this tissue may serve as a novel non-invasive biomarker for fruit and vegetable intake. Since adequate fruit and vegetable consumption is closely linked to reductions in chronic diseases such as various cancers [9], cardiovascular disease [10], age-related degenerative diseases [11] and obesity [12], rapid painless optical screening methods for large populations could have tremendous utility. It would be possible to identify populations at particular risk for inadequate intake of fruits and vegetables with these screening methods, and to evaluate the success of interventions aimed at increasing fruit and vegetable intake.

In this manuscript, we review the principles of the two spectroscopy methods, validation studies carried out to date, and their feasibility for applications in field and clinical settings.

Resonance Raman spectroscopy, RRS

RRS is a laser spectroscopy method that detects the characteristic vibrational/rotational energy levels of a molecule. Carotenoid molecules are particularly well suited for RRS-based detection since their chain-like carbon backbone is conjugated. It consists of a sequence of alternating carbon double – and single bonds (C=C and C–C bonds, respectively), with the outer electron of the molecule free to move along the chain. This leads to strong absorption bands in the blue region of the optical spectrum as shown in Fig. 1(a) and the possibility to obtain resonantly enhanced Raman scattering responses when exciting the carotenoids with suitable

* Corresponding author at: Department of Physics and Astronomy, 115 S 1400 E RM 201, Salt Lake City, UT 84112, USA. Fax: +1 801 581 4801.
E-mail address: ermakov@physics.utah.edu (I.V. Ermakov).

http://dx.doi.org/10.1016/j.abb.2015.01.020
0003-9861/

blue laser lines or other spectrally narrowed light sources. The RRS responses occur as spectrally sharp spectral lines (Stokes lines) at discrete frequencies that are downshifted from the excitation laser frequency by the respective vibrational frequencies of the carotenoid backbone constituents, i.e. at shifts that are due, respectively, to the C=C and C–C stretch frequencies, and the rocking frequency of the CH_3 molecules attached on the side of the carbon backbone [13]. As an example, we show in Fig. 1(b) a typical RRS spectrum of carotenoids for a solution in methanol, featuring three strong and clearly resolved carotenoid Stokes lines at ~1525 cm^{-1} (C=C stretch), 1159 cm^{-1} (C–C stretch), and 1008 cm^{-1} (C–CH_3 rocking motions). A relatively weak line exists also at ~960 cm^{-1}. It is due to out-of-plane wagging motions of the chain hydrogens (C–H groups), coupled with C=C torsional modes [14,15], but its intensity is too weak for quantitation purposes.

In homogeneous, optically thin solvent systems, the Raman scattering intensity of any of the Stokes lines, I_s, is linearly correlated with the carotenoid concentration, N, according to the relation

$$I_s = N \cdot \sigma \cdot I_L \quad (1)$$

where σ and I_L represent the Raman scattering cross section and laser intensity, respectively. The RRS line intensity can therefore be used as quantitative optical measure for the carotenoid concentration.

The unique Raman scattering properties of carotenoids led us to explore RRS for the non-invasive quantitative optical measurements of carotenoids and their spatial distributions in living human tissue environments. Challenges could be anticipated in this complex tissue matrix from other tissue chromophores existing in the measured tissue volume simultaneously with the carotenoids of interest, and from laser intensity restrictions due to safety constraints. However quantitative RRS detection could be readily demonstrated in the human retina (macula) [16–18], in human skin [13,19–22] and in oral mucosal tissue [13].

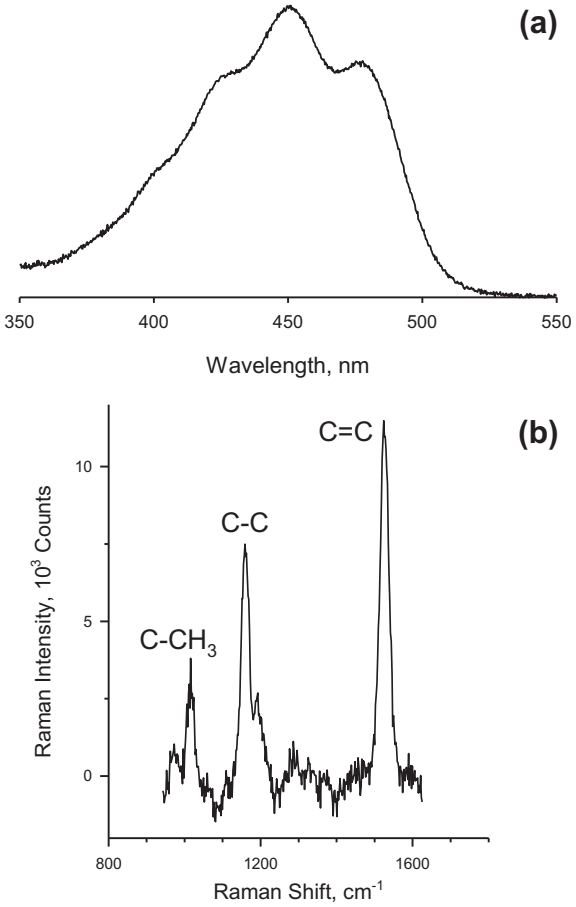

Fig. 1. (a) Absorption spectrum of β-carotene in methanol and (b) corresponding Resonance Raman spectrum, RRS, obtained under laser excitation with 488 nm. The vibronic substructure in the absorption behavior and the Raman pattern of three high frequency lines in the 1000–1550 cm^{-1} range are spectral carotenoid characteristics useable for their noninvasive detection in living human tissues.

A prototype RRS instrument used for skin carotenoid measurements is shown in Fig. 2(a). It consists of a 488 nm solid-state laser, a spectrograph/CCD array combination for detection of the backscattered light, and a computer for exposure control, data acquisition, processing, and display. Excitation and backscattered light is routed to and from the skin tissue site of interest with the help of fiber optics and a light delivery and collection module that is placed in direct contact with the skin tissue site of interest. Light backscattered from the skin is filtered to reject the laser excitation light, spectrally dispersed by a compact, small focal length spectrograph, and recorded with an interfaced CCD array. Using custom developed software algorithms, the recorded spectra are analyzed for the intensity of the strongest RRS line at the carbon double bond (C=C) frequency at 1525 cm^{-1}, which under 488 nm excitation occurs at 527 nm. RRS spectra with high signal to noise ratios (approximately 50:1 in best cases) can be obtained with ~9 mW exposure for 30 s at a laser excitation disk at the skin surface of ~2.5 mm diameter [13,23].

Fig. 2(b) shows the computer display after a single *in vivo* measurement. The measured raw spectrum, shown in the left panel, features a strong spectrally broad "autofluorescence" background along with the three weak tissue Raman carotenoid lines, which are superimposed on this background. Even though the autofluorescence intensity is about 100 times stronger compared to the carotenoid RRS lines, it is possible to quantify the latter with high accuracy by employing a detector with high dynamic range. After fitting of the fluorescence background with a higher order polynomial and subsequent subtraction from the raw spectrum, an isolated skin carotenoid RRS spectrum is obtained (right panel) that is virtually undistinguishable from the RRS spectrum of a pure β-carotene solution (Ref. Fig. 1(b)).

The skin carotenoid RRS response originates from contributions of all carotenoid species present in the tissue volume that absorb in the blue wavelength region [23]. Since all individual C=C Raman line positions are identical at the instrument's spectral resolution, the intensity of the C=C RRS line at 1525 cm^{-1} can be used as a measure for the combined concentrations of all carotenoids existing in the measured skin tissue volume (with the exception of the "color-less" phytoene and phytofluene, which absorb in the UV range).

The RRS intensity varies with excitation light intensity and of course depends also on the sensitivity of the instrumentation (light throughput, optical alignment, specifications of CCD detector array, etc.). It is therefore susceptible to intensity fluctuations and requires an external calibration standard for routine sensitivity checks and cross-calibration purposes between instruments. A suitable external standard is sodium nitrate, featuring a Raman line which is in close proximity to the carotenoid RRS lines of interest. The RRS intensities of the standard are measured prior to a skin carotenoid measurement session and used to correct any deviations of the instrument response from its original sensitivity potentially occurring. A comprehensive description of the RRS methods for quantitative skin carotenoid measurements is published elsewhere [13,22,23].

Selective RRS detection of carotenes and lycopene in human skin

Skin carotenoid RRS measurements under blue excitation, as described above, provide a measure for the total concentration of all carotenoid species absorbing in that wavelength region since