Talanta 117 (2013) 133–138

Contents lists available at ScienceDirect

Talanta

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

Profiling differences in chemical composition of brain structures using Raman spectroscopy

Marko Daković [a,*], Aleksandra S. Stojiljković [a], Danica Bajuk-Bogdanović [a], Ana Starčević [b], Laslo Puškaš [b], Branislav Filipović [b], Snežana Uskoković-Marković [c], Ivanka Holclajtner-Antunović [a]

[a] Faculty of Physical Chemistry, Studentski trg 12-16, University of Belgrade, Belgrade, Serbia
[b] Institute of Anatomy Niko Miljanić, School of Medicine, Dr Subotića 4, University of Belgrade, Belgrade, Serbia
[c] Faculty of Pharmacy, Vojvode Stepe 450, University of Belgrade, Belgrade, Serbia

ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form
30 August 2013
Accepted 30 August 2013
Available online 6 September 2013

Keywords:
Raman spectroscopy
Brain tissue
Chemical composition
Independent component analysis

ABSTRACT

Raman spectroscopy enables non-invasive investigation of chemical composition of biological tissues. Due to similar chemical composition, the analysis of Raman spectra of brain structures and assignment of their spectral features to chemical constituents presents a particular challenge. In this study we demonstrate that standard and independent component analysis of Raman spectra is capable of assessment of differences in chemical composition between functionally related gray and white matter structures. Our results show the ability of Raman spectroscopy to successfully depict variation in chemical composition between structurally similar and/or functionally connected brain structures. The observed differences were attributed to variations in content of proteins and lipids in these structures. Independent component analysis enabled separation of contributions of major constituents in spectra and revealed spectral signatures of low-concentration metabolites. This provided finding of discrepancies between structures of striatum as well as between white matter structures. Raman spectroscopy can provide information about variations in contents of major chemical constituents in brain structures, while the application of independent component analysis performed on obtained spectra can help in revealing minute differences between closely related brain structures.

1. Introduction

Raman spectroscopy is a widely used method applied for qualitative, quantitative and structural analysis in chemical, biochemical and pharmaceutical research [1,2]. The ability for non-destructive or minimally destructive assessment of chemical composition and molecular interactions makes Raman spectroscopy suitable for *in vitro* as well as *in vivo* analysis of biological samples [3,4]. The application of this technique in assessment of compositional differences between normal and tissue affected by pathology provides information complementary to histological analysis [5,6]. So far, Raman spectroscopy has been applied in studies of normal brain tissue [7–9], detection and differentiation of various pathologies, including brain tumors [10–12], skin diseases [13,14], carcinoma of the neck [15], prostate [16] etc. However, the obtained spectra are very complex due to overlapping contributions of different molecular species which makes their analysis difficult.

The analysis and differentiation of Raman spectra of brain tissues presents a particular challenge due to their very similar chemical composition. Mizuno et al. established that spectra of gray and white matter essentially do not differ from those obtained from the rat brain [7]. They reported that dominant lines in Raman spectra arise from lipids and proteins, where contribution of the former is more pronounced in white matter. Sajid et al. [9] analyzed the intensity ratio of stretching vibrations in CH_2 and CH_3 groups (I_{CH2}/I_{CH3}) and found that the value of this parameter is lower in gray matter, indicating higher protein content [9]. Santos et al. used a single optic probe to acquire high wavenumber Raman spectra (2700–3100 cm^{-1}) of several structures present in coronal sections of the porcine brain [17]. They reported almost complete distinguishing of structures based on hierarchical cluster analysis of obtained spectra. Wolthuis et al. demonstrated that Raman spectroscopy can be a powerful tool for accurate determination of water concentration in the brain [18]. A number of studies dealt with spectra based distinguishing of normal brain tissue and tumors arising from brain parenchymal cells. However, to our

* Corresponding author. Tel.: +381112630796; Mob.: +381611147410.
E-mail address: marko@ffh.bg.ac.rs (M. Daković).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.08.058

knowledge there have been no studies dealing with Raman spectroscopy of structures which belong to human white and gray matter and reveal the spectral and compositional differences between them.

In this study, we demonstrate the ability of Raman spectroscopy to distinguish human gray matter structures: caudate nucleus (CN), putamen (PT) and cerebellar gray matter (CLG) as well as white matter structures: corticospinal tract (CST), pons (PN) and *septum pellucidum* (SP). We show that spectra provide the ability to reveal differences in the chemical composition of these structures using the classical approach classical approach (analysis of spectral line intensities) and the method of independent component analysis (ICA).

2. Materials and methods

2.1. Sample preparation

Specimens of deep gray matter (caudate nucleus and basal nuclei), cerebral gray matter and white matter (pons, nerve tract) and *septum pellucidum* were obtained from the Collection of brain tissues of the Institute of Anatomy of the School of Medicine, Belgrade. All samples in the collection were taken from a single cadaver (Program of Body Donation for Medical Research, Institute of Anatomy, Belgrade) within 6 h after death, kept in liquid nitrogen for two hours and stored at −80 °C. Half an hour before cutting, the specimens were transferred to a refrigerator at −20 °C. Ten consecutive 25 μm thick sections were cut (Leica Cryocut 1800) from specimens and transferred to microscopic plates. Prior to acquisition of Raman spectra, which was performed within 3 h after slicing, preparations were kept at −20 °C.

2.2. Raman instrumentation and spectra acquisition

Micro-Raman spectra of specimens were recorded in situ on a DXR Raman Microscope (Termo Scientific). The 532 nm line of a diode-pumped solid state high brightness laser was used as the exciting radiation and the power of illumination at the sample surface was 10 mW. Collection of the scattered light was made through an Olympus microscope with infinity corrected confocal optics, 25 μm pinhole aperture, standard working distance objective 50×, grating of 1800 lines/mm and resolution of 2 cm^{-1}. Acquisition time was 15 s with 10 scans. The laser spot diameter on the sample was 1 μm. Thermo Scientific OMNIC software was used for spectra collection and manipulation. For each of the specimens 10 Raman spectra were collected in the fingerprint region 600–1800 cm^{-1}. All spectra of each tissue were recorded from different spots within the same small area of a corresponding tissue specimen, so that tissue inhomogeneity effects could be minimized. None or slight degradation of samples was observed during experiments.

2.3. Analysis of Raman spectra

K-means cluster analysis (KMCA) (SPPS v 13) was performed on raw spectra obtained from a particular tissue in order to diminish the influence of surface heterogeneities which can be the source of errors in separation of components by ICA. The final number of clusters was set to be 2 and 100 iterations were performed. A cluster which contained a higher number of spectra was considered representative and served as input in the preprocessing step.

Raman spectra were preprocessed using Matlab® 2010a based package "Raman processing" [19]. Steps included application of a median filter in order to reduce noise in spectra, followed by subtraction of fluorescence and normalization of the spectrum by subtraction of minimal and then dividing by maximal intensity value. After these procedures intensities in spectra were in the range 0–1. Difference spectra were calculated for all tissue combinations in order to trace differences in content of major constituents (lipids and proteins).

2.4. Independent component analysis of Raman spectra

Independent component analysis is a statistical technique that extracts the source signals from a data matrix of multisensor recordings without *a priori* knowledge of constituents in analyzed samples [20]. The obtained data (x) can be represented as a linear combination of mutually independent components (s):

$$x_i = a_{i1}s_1 + a_{i2}s_2 + \cdots a_{in}s_n \text{ for all } i \quad (1)$$

where a_{in} represents abundance of the n-th component in a complex signal. It is more convenient to use vector matrix notation instead of sums used in the previous equation. Complex signal and independent components can be represented in the form of row vectors $\boldsymbol{x}=[x_1\ x_2 \ldots x_j]$ and $\boldsymbol{s}=[s_1\ s_2 \ldots s_n]$, while coefficients a_{ni} form mixing matrix **A**:

$$\mathbf{x} = \mathbf{A}\mathbf{s} \quad (2)$$

The problem of finding independent components is confined to obtaining the unmixing matrix **W** which satisfies equation

$$\mathbf{s} = \mathbf{W}\mathbf{x} \quad (3)$$

There are several different approaches for estimation of independent components. The FastICA algorithm uses the fixed-point iteration scheme for finding the maximum of non-Gaussianity of components, and the maxICA is based on maximizing of output entropy etc. The details of various techniques for ICA implementation can be found elsewhere [21,22].

Independent component analysis has been applied in analysis of various fields including medical image processing, analysis of recordings from electroencephalography (EEG), magnetoencephalography (MEG) [23,24], color decomposition of histologically stained samples etc. Spectroscopic data had been analyzed using this technique: it was successful in separation of signals of pure chemical compounds from their mixtures, obtaining spectra of major fractions in urine, etc. However, assignment of independent components to a particular chemical compound in spectra of human tissues cannot be achieved, which is a consequence of complex composition. Nevertheless, inspection of spectral features present in them may reveal signatures of constituents which were virtually absent or masked by other lines in original spectra.

Independent component analysis was performed in two steps using the FastICA toolbox [25] in Matlab 2010a and preprocessed spectra as input. First, principal component analysis (PCA) was performed in order to establish the major sources of variability in spectra and to achieve data reduction. Components whose sum of variances contributed with more than 99.0% to spectra were retained and whitened. In the second step the FastICA algorithm was performed using 10,000 iterations and fine tuning and the number of independent components was set to be equal to the number of retained principal components.

3. Results and discussion

3.1. Raman spectra of brain tissues

The average Raman spectra of gray (caudate nucleus, basal ganglia, cerebellar gray matter) and white matter structures (cerebral white matter, pons) as well as the *septum pellucidum* are given in Fig. 1. In all spectra the bands arising from contributions of lipids (1064, 1089, 1268, 1296, 1309, 1439 and 1659 cm^{-1}) [26],