Biophysical Chemistry 182 (2013) 58–63

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Raman micro-spectroscopy: A powerful tool for the monitoring of dynamic supramolecular changes in living cells

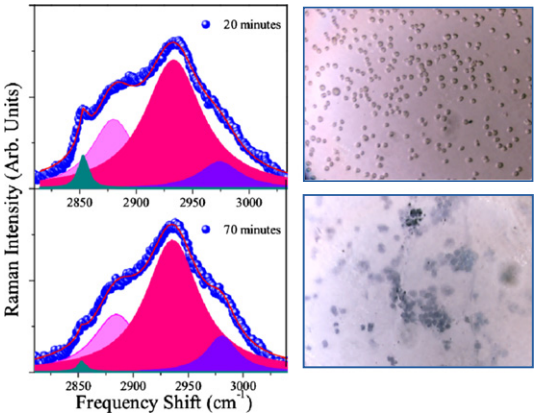
Silvia Caponi [a,b,*], Lavinia Liguori [c], Alessandra Giugliarelli [d], Maurizio Mattarelli [e], Assunta Morresi [d], Paola Sassi [d], Lorena Urbanelli [f], Carlo Musio [a]

[a] Istituto di Biofisica, Consiglio Nazionale delle Ricerche, c/o Fondazione Bruno Kessler, Via alla Cascata 56/C, 38123 Trento, Italy
[b] Dipartimento di Fisica, Università di Trento, Via Sommarive 14, 38050 Povo, Trento, Italy
[c] Equipe SyNaBi, Laboratoire TIMC UMR CNRS 5525, Université J Fourier, Domaine de la merci, 38700 La Tronche, France
[d] Dipartimento di Chimica, Università di Perugia, Via A. Pascoli, I-06100 Perugia, Italy
[e] NiPS Laboratory, Dipartimento di Fisica, Università di Perugia, Via A. Pascoli, I-06100 Perugia, Italy
[f] Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Università di Perugia, Via del Giochetto, I-06123 Perugia, Italy

HIGHLIGHTS

- The cellular death is investigated by Raman micro-spectroscopy in T-lymphocytes.
- Insights to underlying cellular activities have been found.
- Markers in the membrane modifications induced by cellular death are indicated.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 May 2013
Received in revised form 18 June 2013
Accepted 18 June 2013
Available online 27 June 2013

Keywords:
Raman spectroscopy
Cellular death
Cellular imaging
Apoptosis process
Necrosis process
Jurkat cell

ABSTRACT

Cellular imaging techniques have become powerful tools in cell biology. With respect to others, the techniques based on vibrational spectroscopy present a clear advantage: the molecular composition and the modification of subcellular compartments can be obtained in label-free conditions. In fact, from the evolution of positions, intensities and line widths of Raman and infrared bands in the cell spectra, characteristic information on cellular activities can be achieved, and particularly, cellular death can be investigated. In this work we present the time evolution of the Raman spectra of single live Jurkat cells (T-lymphocyte) by looking at the high frequency part of their Raman spectra, that is the CH stretching region, around 3000 cm^{-1}. In particular, investigation into the composition or rearrangement of CH bounds, markers of cellular membrane fatty acids, can represent an important method to study and to recognize cell death. The experimental procedure we used, together with the analysis of these high frequency vibrational bands, may represent a new, improved and advantageous approach to this kind of study.

* Corresponding author at: Istituto di Biofisica, Consiglio Nazionale delle Ricerche, c/o Fondazione Bruno Kessler, Via alla Cascata 56/C, 38123 Trento, Italy. Tel.: +39 0461 314 628.
E-mail address: silvia.caponi@cnr.it (S. Caponi).

0301-4622/$ – see front matter
http://dx.doi.org/10.1016/j.bpc.2013.06.013

1. Introduction

Among all the available physico-chemical approaches, spectroscopic investigations are more and more successfully applied to complex systems of biological interest. In particular, vibrational FTIR and Raman spectroscopies are label-free, non invasive methods, very useful for the study of cellular systems without staining them. Following the changes of IR and Raman band-shapes in cooling–thawing cycles, information can be obtained about cell behaviour, membrane phase transitions and modification of membrane properties, together with water–ice transitions [1–4]. The coupling of optical microscopy devices to the usual Raman spectroscopic set-up is particularly effective when used to investigate animal and human cellular samples, characterizing different macromolecules inside single living cells and providing optical markers for cytological analysis [5–8].

Cellular death is widely investigated in biomedicine, in order to understand the different mechanisms underlying the biochemical modifications taking place during cellular and tissue consumption in biological samples.

Considering that de-regulation or alteration of cell death processes are the key events in a wide range of human diseases and despite the different mechanisms engaged by nature to lead cellular death, direct monitoring of cells during those processes is desirable to determine their molecular outcome.

One can collect a great number of possible origins of cellular death, referring to the two principal paths, that is necrosis and apoptosis. The differences are well known: necrosis is a death induced by a traumatic external treatment usually associated with inflammation, a sort of "cellular murder", while apoptosis is a programmed cell death process, a sort of "cellular suicide" involved in physiological tissue development. The first can be caused by physical agents, such as temperature stress and ionizing radiation, or can be chemically (by drugs, for example), or biologically (viral agents) induced, while the second one depends on the intrinsic nature of the cells, although it could be also initiated by extracellular processes. Usually apoptosis regards single cells, while necrosis affects groups of neighbouring cells [9]. The two processes can be observed and distinguished in the same cellular samples: the efficacy of cancer therapies, for example, can be evaluated by an estimation of tumour necrosis [10], but at the same time apoptosis can be initiated by chemiotherapeutic agents [11].

It is thus crucial to be able to identify and characterize putative markers for cell death in the case of necrosis and/or apoptosis: different cellular components are in fact involved in the two death processes. Apoptosis usually involves sequential condensation and fragmentation of nuclear DNA that can be followed by treating cells with dyes that link to nucleic acids (ex. Hoechst 33258), while necrotic cells are characterized by the rupture of the cellular membrane, usually visualized by Trypan blue assay.

The tests on living cells have some important limits: first of all, it is not possible to use the same biological sample after the test; second, they need target molecules to be labelled to give information about the involved molecular mechanisms. During cryopreservation to build cell banking used in cell-based therapies of particular cells and tissues, the so called cryoinjury of single cells in the cooling–thawing cycles is the principal cause of cellular death (necrosis, in this case) that can prejudice the whole banking process and the efficacy of the final clinical use of the cells [12]. Even if the macroscopic effect of cooling–thawing is well known, the available information about the detailed processes inside the biological samples is insufficient for their comprehension at a molecular level. The optimization of the cooling–thawing procedure, together with the choice of the best cryoprotectants, depends on how deep the knowledge is on cell necrosis at a molecular level.

MicroRaman spectroscopy has been used in explorative measurements to study both apoptosis and necrosis processes, in different cellular samples [13–16]: The observed modifications of the recorded spectra have been associated with biochemical changes in cell samples, without any definitive conclusions about the details of the mechanism, due to the scarcity of the systematic dedicated series of measurements even if the spectra are manipulated by fitting procedures and statistical treatments [17].

Up to now, the available vibrational spectra, IR and Raman ones, usually regard the region under 1800 cm^{-1}, where it is possible to individuate nucleic-acid backbones, DNA, RNA, proteins and lipid vibrations. About IR spectra, the high intensity of OH stretching vibrations interferes with the lower intense CH_2 peaks that cannot be easily recorded in absorption experiments. As the first attempt to overcome the above poverty of indications, the present paper reports microRaman measurements of T-lymphocites at different hydration conditions and as a function of time, that allow individuating the modifications associated to cellular membrane through the spectral modifications in the CH stretching region.

The band shape analysis of Raman spectra that we propose, collected with a particular experimental procedure, suggests the choosing of the CH_2 stretching signals as band markers, in order to follow efficaciously the biochemical modifications associated the membrane status linked to the death process.

2. Materials and methods

2.1. Cell culture

Jurkat cells are a T-lymphocyte immortalized human cell line used to study T-cell leukaemia, chemokine expression receptors susceptible to viral entry and T-cell signaling [18]. They have been proven as a suitable model not only for the study of the T-cell receptor machinery but also for apoptosis [19]. In fact, several well-defined physiological, pharmacological, and pathological triggers are able to induce apoptotic effects on Jurkat cells.

Jurkat are non-adherent cells and cell culture was maintained in DMEM (Dulbecco's modified eagle medium) supplemented with 10% (v/v) heat-inactivated foetal calf serum, 100 U/ml penicillin and 100 U/ml streptomycin at 37 °C under 5% CO_2 and 95% air. Prior to use in experiments, 6 ml at 2×10^6 cells/ml was pelleted and prepared by 2 sequential centrifugations at 800 ×*g*, 5 min, the first one to remove the medium and the second one in 5 ml of PBS (Phosphate Buffered Saline buffer) to wash the cells and eliminate any residual medium contamination. After an addition of 20 μl of PBS the resultant pellet of cells was transferred in an eppendorf tube ready for Raman analysis.

2.2. Raman experiments and data treatment

The Raman spectra are obtained using a micro-Raman setup (Horiba Jobin-Yvon, model LabRam Aramis), equipped with a He–Ne laser of $\lambda = 632.8$ nm with a 10 mW power, an edge filter with O.D. higher than 8 which prevents the acquisition of Raman signal below 100 cm^{-1} from the laser line, a 1200 grooves/mm grating for a resolution of about 2 cm^{-1} as measured by fitting the Rayleigh line, and a Peltier cooled CCD detector (1024 × 256 Pixels) which, in the present conditions, allows the simultaneous acquisition of about 700 cm^{-1}. The experimental apparatus is equipped with different objectives: 10× and 50× plan apochromat air objectives and a water immersion UPLSAPO 60XW from Olympus. In the first used configuration, the used exciting source was focused onto the sample through a 50× objective with a 100 μm working distance. In this case the pellet was deposited onto a metallic slide under the microscope-objective. Evaporation leads to a low hydration level, and subsequent cellular death in a few minutes of waiting time. To reduce the evaporation a second configuration was adopted: the pellet of cells in suspension was deposited onto a metallic slide and it was covered by a coverslip glass in order to maintain a high hydration level. In this case a water immersion objective is used. In fact thanks to its high numerical apertures, N.A. = 1.2, a working distance of 280 μm and its correction collar which allows adjustment to accommodate cover glass