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NEW TRENDS IN RAMAN SPECTROSCOPY OF BIOMOLECULES

The outlines of Raman spectroscopy has been introduced several years ago. During last years, a great number of papers dealing with new applications and methodical advantages have appeared [5, 15].

Briefly, Raman spectroscopy analyzes scattered light (from a laser) after its collisions with molecules of sample. Elastic collisions do not change the energy of used monochromatic light and are responsible only for its scattering(according to Rayleigh). On the other hand, inelastic collisions lead to loss or gain of energy due to vibrational excitation of a sample molecule or to a transfer of vibrational energy to a Raman photon from already excited molecule. Thus, the differences between frequencies (wavenumbers) of lines in analyzed scattered light and freqency of incident monochromatic laser light correspond to different vibronic levels of sample molecule and are expressed as Raman shift (in cm^{-1}). The term "resonance Raman effect" expresses a strong increase of intensity of certain lines in the Raman spectrum which is observed when the wavelenght of incident laser light corresponds to the wavelenght of an absorption band in electronic spectrum of a sample. This is the chromophore which is responsible for the absorption which also gives enhanced lines in the vibrational resonance Raman spectrum.

In the field of classical (non-resonance) Raman, greatest effort has been directed to understanding how is the conformation or secondary structure reflected in the Raman spectrum. The vibrations of β-turns have been found and added to the Raman diagnostical tests of pretein secondary structure. Recent methods based on analysis of peptide bond vibrations(amide bonds) in Raman spectra give estimates at least as good as the best ones using circular dichroism [18]. For the study of biomembranes, a knowledge of phospholipid conformation is of crucial importance, Raman spectroscopy is now able to give reasonable answers also to this ques-

tion [1], as it has been demonstrated e.g. in the study of state modifications of thymocyte plasma membrane proteins and lipids by concanavalin A [16].

Selective resonance Raman enhancement of porphyrin vibrational modes allowed biochemists to draw structural informations from Raman spectra of hemoproteins. Using a reasoning proposed by S p i r o [13], we were able to decide between hypothesis of iron penta- or hexacoordination in ferric high spin cytochrome P-450 [2]. Resonance Raman spectra gave data in favor of heme iron pentacoordination and, moreover, a reasonable estimate of porphyrin ring radius in this hemoprotein was obtained. From the low-frequency part of resonance Raman spectra, information about the nature of the bonds between heme iron and axial ligands can be drawn out [8, 11]. A detailed analysis of the resonance Raman spectra of deoxyhemoglobins gave evidence to the protein-heme interaction which is reflected in changed affinity to oxygen.

For studying labile compounds and intermediates, time-resolved Raman spectroscopy is uniquely suitable. Whereas one source of radiation generates labile species, second one (most frequently tunable laser) serves as a source of monochromatic light for obtaining Raman spectrum. This technique implies also an improvement in detection. An optical multichannel detection systems consisting of intensified photodiode array or vidicon (intensified TV tube) detector and analyser (small computer) together with pulse generator are able to detect spectra of short-lived intermediates in nanosecond time-scale. This advantage of multichannel detection enabled use of resonance Raman spectroscopy for study of intermediates involved in the action of visual pigments and, on the other hand, as a non-invasive technique for clinical applications [10, 17].

Another powerful technique based on combination of Raman spectroscopy with microscope, is the Raman microprobe. By choosing appropriate characteristic frequency line in the scattered light, the surface distribution of a given substance can be obtained. Sample is iluminated by laser and Raman lines of different substances from the sample are then selected [7]. The Raman microprobe with multichannel detection is a rapid, non-destructive technique which have been used for in situ study of biological samples [3].

The last experimental approach which should be mentioned here is the use of resonance Raman labels [6]. It is, in principle, an extension of generally used labelling technique as it is used in fluorescence or EPR spectroscopy. The resonance Raman label is a small molecule which gives resonance Raman spectrum and which can interact with highly specialized environment in a biomolecule. This method can be used to study the vast variety of problems, i.e. drug-receptor, antibody-hapten or enzyme-substrate interactions. The use of time-resolved approach to e.g. the last example offers an unique possibility for studying the most intimate secrets of biochemical reactions.

The invasion of Raman techniques into biochemisty during last years was possible mainly thanks to their versatility. Raman spectra of highly purified preparations and, on the other hand, of whole particles like mitochondria or ribosomes were obtained. In the near future, an extension of Raman spectroscopy e.g. to study of optically active substances by measuring small differences in the intensity of Raman scattered light from chiral molecules using circurally polarized light can be expected [4]. A number of interesting applications of Raman spectroscopy to biomedical and pathological problems have occured, too [9, 14].

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NOWE KIERUNKI W ZASTOSOWANIU SPEKTROSKOPII RAMANA DO BADANIA CZĄSTECZEK BIOLOGICZNYCH

Streszczenie

W pracy dokonano krótkiego przeglądu najnowszych zastosowań spektroskopii Ramana w badaniach biologicznych. Zastosowania te obejmują m.in. badania błon biologicznych, hemoprotein i intermediatów biochemicznych. Ponadto omówiono metodę mikrosond i znaczników ramanowskich.

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