ULTRASENSITIVE SPECTROSCOPY OF DNA FROM *IN VITRO* GROWN *SOLANUM TUBEROSUM* L. LEAVES

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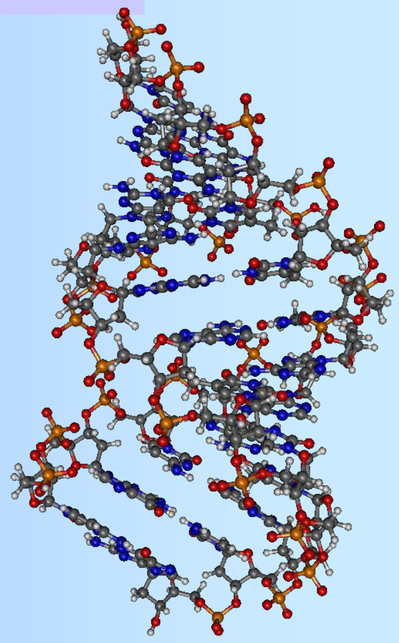

ABSTRACT: In this work surface-enhanced Raman spectra of nucleic acids from *in vitro* grown *Solanum tuberosum* L. cultivars and populations were measured at pH 7 with different laser lines (532 nm, 633 nm, 785 nm). Also, structural changes of genomic DNA from different *in vitro* grown *Solanum tuberosum* L. plants [RFA Roclas Clone 2.2 Ferma, RFA Roclas Clone 2.9 Ferma, RFA Roclas Clone 3.1 Ferma, Blue Congo, population of Purple Potato (Lăzarea), Roclas Clone C] are observed upon changing the acidic pH of DNA-chloride-capped silver nanoparticles (Cl-AgNPs) systems by surface-enhanced Raman spectroscopy (SERS). Binding affinity changes of DNA with Cl-silver nanoparticles are supposed to take place upon lowering the pH. Laser line-dependent SERS profiles of DNA have been found.

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) is emerging as an important characterization technique for biological materials. The detection of specific DNA sequences in human beings, viruses, bacteria and plants has become increasingly important in various fields. Particularly, SERS for sub-micromolar detection of DNA/RNA mononucleotides was reported. Also, increasing surface-enhanced Raman spectroscopy effect of RNA and DNA components by changing the pH of silver colloidal suspensions was observed. Besides, protonated genomic DNA structures were previously reported by Raman spectroscopy. Moreover, SERS spectra of adenine recorded under a broad range of pH values and concentrations, using both silver and gold colloids, provided evidence for the existence of several distinct species. Furthermore, influence of coverage in the surface-enhanced Raman scattering of cytosine and its methyl derivatives on metal colloids, chloride and pH effects were described.

2. Results

SERS spectra of nucleic acids from *in vitro* grown *Solanum tuberosum* L. cultivars and populations were registered at pH 7 using different laser lines (532 nm, 633 nm, 785 nm). For these measurements a Renishaw inVia Reflex Raman spectrometer was used. A dependence of surface-enhanced Raman signals on the laser line has been found. The 532 nm laser line was found the most suitable to study our DNA-colloid systems. Also, for several plant DNAs, the SERS spectra were collected with a DeltaNu system for neutral and different values of acidic pHs.

Binding affinity changes of DNA with chloride-capped silver nanoparticles (Cl-AgNPs) and nucleic acids structural changes are supposed to take place upon lowering the pH.

For RFA Roclas Clone 2.9 Ferma DNA an increase in intensity was found near 1226 cm^{-1} belonging to thymidine in-plane ring - CH_3 stretching, at pH 5 comparatively with pH 7. In the case of RFA Roclas Clone 3.1 Ferma nucleic acids, bands at 335 cm^{-1}, 664 cm^{-1}, 692 cm^{-1}, 800 cm^{-1}, 1048 cm^{-1}, 1170 cm^{-1} were observed to increase in intensity at pH 5 comparatively with pH 7. For Blue Congo DNA vibrations around 330 cm^{-1}, 811 cm^{-1}, 1127 cm^{-1}, 1514 cm^{-1} increased intensities were detected at pH 4B as compared with the other pH values. In the case of Roclas Clone C nucleic acids profiles near 450 cm^{-1}, 487 cm^{-1}, 689 cm^{-1}, 775 cm^{-1} were found to increase at pH 4 comparatively with pH 7. All these spectral changes are a proof of structural modification in the corresponding DNA molecular groups upon lowering the pH.

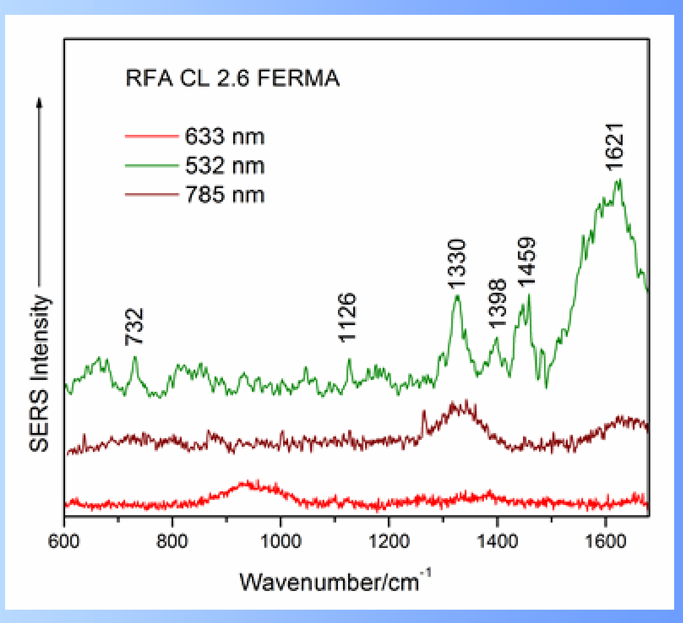

Figure 1. Surface-enhanced Raman spectra of *Solanum tuberosum* L. DNAs isolated from RFA Roclas Clone 2.6 Ferma, at pH 7. Renishaw inVia Reflex Raman spectrometer with excitation laser lines 532 nm, 633 nm and 785 nm, respectively, were used.

Table 1. SERS wavenumbers (cm^{-1}) and tentative assignments of genomic DNAs extracted from *in vitro* grown *Solanum tuberosum* L. leaves, depending on the pH value of the mixture between silver colloid and DNA solution.

Sample	pH	Wavenumber (cm^{-1})	Tentative assignments
RFA Roclas Clone 2.2 Ferma	7	488	stretching deoxyribose
		558	dA (C2-H, N9-H wagging)
		701	dA (ring breathing)
	6	457	stretching deoxyribose
	5	457	stretching deoxyribose
		558	dA (C2-H, N9-H wagging)
	4A	481	stretching deoxyribose
	4B	457	stretching deoxyribose
		558	dA (C2-H, N9-H wagging)
RFA Roclas Clone 2.9 Ferma	6	876	deoxyribose C3'-*endo*
		941	dA/dC/dG (NH_2 rocking)
	5	355	dG, dT, dA stretching
		469	stretching deoxyribose
		940	dA/dC/dG (NH_2 rocking)
		1202	dT (in-plane ring - CH_3 stretching)
		1226	dT (in-plane ring - CH_3 stretching)
		1393	dT (NH deformation / CH_3 deformation), dA
		1590	dG, dA (C2=N3 of guanine)
		1625	dT, $\delta(H_2O)$ (C=O stretching, C=C stretching)
	4	480	stretching deoxyribose
		702	dA (ring breathing)
		1208	dT (in-plane ring - CH_3 stretching)
		1395	dT (NH deformation / CH_3 deformation), dA
		1423	deoxyribose C2'H_2 scissoring
		1590	dG, dA (C2=N3 of guanine)
		1623	dT, $\delta(H_2O)$ (C=O stretching, C=C stretching)
	3	360	dG, dT, dA stretching
		488	stretching deoxyribose
		1129	dA (C8-N9 stretching, N9-H, C8-H deformation)

3. Conclusions

In this work surface-enhanced Raman spectra of nucleic acids from *in vitro* grown *Solanum tuberosum* L. cultivars and populations [RFA Roclas Clone 2.6 Ferma, Blue Congo, Buzau population, Patraque d'Auvergne, Vitelotte Negresse, population of Purple Potato (Lăzarea), Roclas Clone C] were measured at different laser lines (532 nm, 633 nm, 785 nm) at pH 7. The 532 nm laser line was found the most suited to study our DNA-colloid systems.

Also, nucleic acids from RFA Roclas Clone 2.2 Ferma, RFA Roclas Clone 2.9 Ferma, RFA Roclas Clone 3.1 Ferma, Blue Congo, population of Purple Potato (Lăzarea) and Roclas Clone C plants were analyzed at acidic pH, respectively. Modified SERS intensities of nucleic acids bands were observed upon lowering the pH, being a proof of binding affinity changes of DNA with chloride-capped silver nanoparticles (Cl-AgNPs) and of structural changes induced at acidic pH in DNA molecular groups.

Laser line-dependent SERS profiles of DNA have been found.

Acknowledgements

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