Hydrogen-Deuterium Exchange in Adenosine 5′-Monophosphate Detected by Surface Enhanced Raman Scattering (SERS)

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Z. Naturforsch. **36 c,** 809–812 (1981); recieved March 27/July 9, 1981

Surface Enhanced Raman Scattering, Hydrogen Exchange, Nucleic Acids

The enhanced Raman effect from 5′-AMP adsorbed on the roughened silver surface is used to characterize hydrogen-deuterium exchange. SERS spectra of 5′-AMP present in H_2O and D_2O solutions at rather low concentrations were recorded in the spectral range of 2000 to 3500 cm^{-1}. Surface enhanced Raman bands in this spectral range are found to increase in intensity, relative to bands of normal Raman solution spectra. In addition, one notes that the CH and CD stretching vibrations, almost obscured by H_2O or D_2O scattering, now stand out clearly.

Introduction

Recently the surface enhanced Raman spectra (SERS) of adsorbed nucleic acid components (sugar, nucleic bases, nucleosides and nucleotides), polyriboadenylic acid (poly-A), and DNA (native and denatured) have been studied by us in rather dilute aqueous solutions [1–4]. With all these biologically significant molecules an enormous intensity enhancement was observed in the spectral range of 100 to 1700 cm^{-1}. Spectral frequencies corresponded closely to those of normal Raman spectra of these biomolecules in solution. The ring-breathing modes and the skeletal vibrations produced prominent Raman scattering. In addition strong bands at about 240 cm^{-1} exhibited characteristics of interfacial bands.

The mechanism of hydrogen exchange has shown that DNA is subject to local structural fluctuations, which result in frequent local openings and closing of the structure [5, 6]. Such structural fluctuations may have considerable biological significance in recombination, transcription, and replication processes. A study of the isotopic exchange thus reveals interesting aspects of the kinetics of structural fluctuations of the DNA.

In particular the study of deuterium exchange by means of laser-Raman spectroscopy has shown that the Raman spectrum provides a method for the determination of the exchange rate constant in purine nucleotides [7, 8]. In the normal Raman spectrum the important hydrogen-deuterium exchange at the 8-C ring position of purines is followed through the lines at 1485 cm^{-1} and 1462 cm^{-1}, respectively. From the intensity dependence of these Raman lines pseudo-first order rate constants for the H-D-exchange in 5′-AMP and in poly-A have been determined as a function of the temperature in the range of 20–90 °C. The results indicate rather small structural differences among the mono- and polynucleotides [9]. However, it is impossible to locate directly the C-D stretching frequencies in the range at 2000 to 2800 cm^{-1} because of strong Raman scattering of D_2O which obscures this spectral range.

In this paper the effect of deuterium exchange on the SERS spectra of adenosine 5′-monophosphate in this spectral range is reported. As the adsorbed D_2O has a relatively weak scattering coefficient, the C-H → C-D isotopic exchange can be directly followed.

Experimental

Adenosine 5′-monophosphate was purchased from Boehringer Biochemica, Mannheim, and used without further purification. 5′-AMP (2×10^{-3} M) was dissolved in D_2O solution containing 0.1 M KCl. In this conditions an adsorbed amount of AMP of about 2×10^{14} molecules cm^{-2} can be estimated [10]. After *one day* incubation 5′-AMP solution was introduced into a spectroelectrochemical Raman cell [2].

Surface Raman spectra were recorded with a Spex 14018 spectrometer using 514.5 nm excitation from a Spectra Physics, mod. 164-09, argon ion laser.

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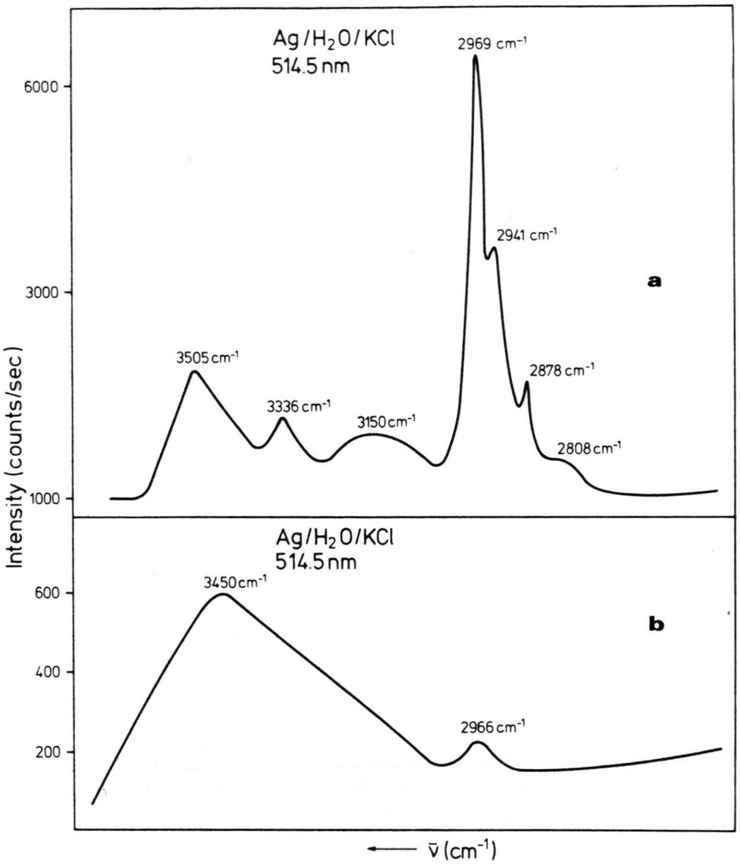

Fig. 1a. SERS spectrum of 5′-AMP in H_2O after one cycle of electrochemical activation from E_s −0.1 V to 0.2 V and back. – b) The background surface spectrum taken before the activation cycle, 2×10^{-3} M 5′-AMP in 0.1 M KCl + 10^{-3} M Na_2HPO_4, pH 5.2, silver electrode, surface area 0.2 cm^2.

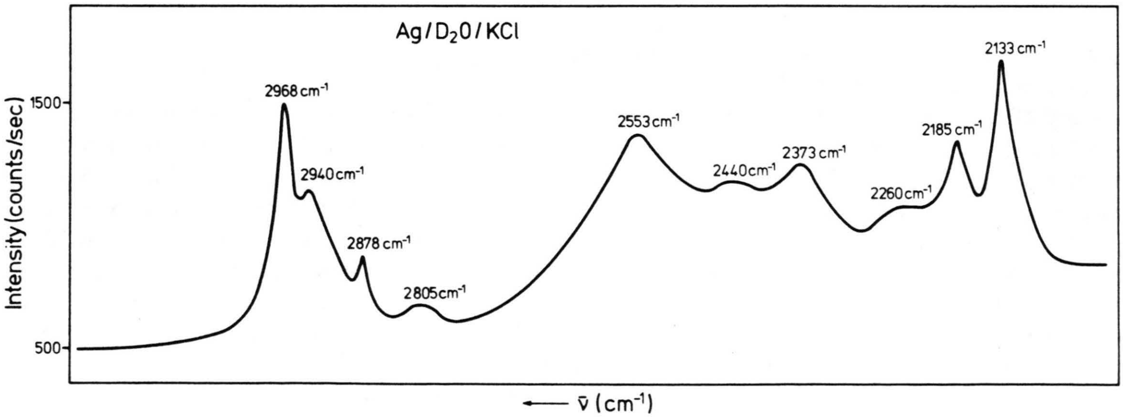

Fig. 2. SERS spectrum of 5′-AMP in D_2O medium after one cycle of electrochemical activation from E_s −0.1 V to 0.2 V and back. 2×10^{-3} M 5′-AMP in 0.1 M KCl + 10^{-3} M Na_2HPO_4, pD 5.1.

Further details of Raman and electrochemical instrumentation are given elsewhere [2].

Results

The SERS effect for adenosine 5′-monophosphate on Ag is illustrated in Fig. 1 by comparing the normal Raman spectrum of 5′-AMP in H_2O solution (Fig. 1 b) and in the adsorbed state (SERS) at the silver electrode after electrochemical roughening (Fig. 1 a). Both spectra are recorded unter the same experimental conditions. The electrochemical roughening procedure, which is an essential part of this method, involves a single oxidation-reduction cycle between -0.1 V and $+0.2$ V.

Fig. 2 shows the SERS spectrum of 5′-AMP after partial exchange of the hydrogen from C-H, N-H, and O-H groups by deuteration.

Discussion

The most important observation in the comparison of the Normal Raman Scattering (NRS) and the Surface Enhanced Raman Scattering (SERS) of 5′-AMP in the C-H vibration range is, that SERS spectra are many times more intense than the NRS spectra. The frequency shifts between these two spectra are small (ca. 2–30 $\mathrm{cm^{-1}}$) indicating that the molecular and electronic structure of adsorbed 5′-AMP is not dramatically perturbed by the electric field in the compact adsorption layer. Therefore, we can compare the already assigned NRS bands [9] in the region 2800–3500 $\mathrm{cm^{-1}}$ due to C-H, N-H and O-H stretching vibrations with the SERS bands (*cf.* Table I).

SERS bands which are due to the different C-H stretching vibrations of ribose are very intense and give well-resolved Raman lines at 2808, 2878, 2941 and 2969 $\mathrm{cm^{-1}}$.

In the NRS spectrum only appear two C-H stretching vibrations at 2945 and 2885 $\mathrm{cm^{-1}}$.

The aromatic C-H stretching frequencies of the 2-CH and 8-CH groups of the adenine ring are centered at 3053 $\mathrm{cm^{-1}}$ and 3120 $\mathrm{cm^{-1}}$ in the NRS spectrum [8]. These bands are broad and not resolved in the SERS spectrum. The O-H stretching vibrations of the hydroxyl groups of ribose appear in the SERS spectrum at 3336 $\mathrm{cm^{-1}}$.

Before electrochemical activation of the silver electrode, one observes the broad and intense O-H stretching mode of water. At rather low concentration of 5′-AMP (2×10^{-3} M), this O-H scattering masks the aromatic CH stretching vibrations in the NRS spectrum (*cf.* Fig. 1 b). After activation the intensity of the 5′-AMP vibrations is many times larger than that of the water vibrations (*cf.* Fig. 1 a). This indicates that the SERS of the O-H-stretching vibrations of adsorbed water are very weak.

When D_2O is the solvent for 5′-AMP the isotopic hydrogen exchange between protons of the 5′-AMP

Table I. Raman bands [a] of Adenosine 5′-monophosphate in different media.

NRS			SERS			
5′-AMP in H_2O	Assignment [b]	Ag/5′-AMP in H_2O	Assignment [c]	Ag/5′-AMP in D_2O	Assignment [c]	$\frac{\nu(H)}{\nu(D)}$
		3505	H_2O str	2553	D_2O str	1.37
		3336	R (OH str)	2440	R (OD str)	1.37
3140	Overtone					
3120	8 CH str	3150	2- and 8-CH str	2373	2- and 8-CD str	1.35
3053	2 CH str					
2945	R (CH str)	2969	R (CH str)	2968, 2260	R (CH str) R (CD str)	1.31
		2941	R (CH str)	2940	R (CH str)	
2885	R (CH str)	2878	R (CH str)	2878, 2185	R (CH str) R (CD str)	1.32
		2808	R (CH str)	2805, 2133	R (CH str) R (CD str)	1.32

[a] Frequency in $\mathrm{cm^{-1}}$.
[b] Frequencies and assignment based on Ref. 9.
[c] Abbreviations: str, stretching; R, ribose group.

and deuterons of the solvent takes place. The SERS bands of 5′-AMP in D_2O solution are sensitive more or less to the kinetics of hydrogen-deuterium exchange in the adenine ring and in the ribose. The spectral effect produced by this deuteration is shown in Fig. 2.

The SERS spectra in the region around 2000 to 3500 cm^{-1} are changed by incubation. The occurrence of SERS bands in the region 2000–2600 cm^{-1} is accompanied by disappearance of SERS bands in the region 3000–3500 cm^{-1}.

All O-H stretching vibrations of water molecules at the Ag surface are shifted from 3505 cm^{-1} to 2553 cm^{-1}. The SERS vibrations of D_2O at 2553 cm^{-1} are weak and the scattering does not obscure the surface enhanced Raman scattering signals of 5′-AMP molecules. This permits the observation of hydrogen-deuterium exchange of 5′-AMP in this spectral range.

The vibration bands of hydroxyl groups of ribose are removed by converting them into the OD form. The SERS of OH at 3336 cm^{-1} is shifted to the SERS of OD at 2440 cm^{-1}. All hydroxyl groups are converted into the OD form (*cf.* Fig. 2).

The aromatic C-H stretching frequencies in 5′-AMP of 2-CH and 8-CH groups of the adenine ring are shifted from 3150 cm^{-1} for non-exchanged 2- and 8-CH to 2373 cm^{-1} of the 2- and 8-CD formed by isotopic exchange. The deuterated SERS bands are not masked by the scattering of the OD vibrations of D_2O. All 2- and 8-CH groups are in the 2- or 8-CD form. Fig. 2 indicates that the CH and CD forms of ribose are most easily distinguished in the SERS spectrum by their intense and well-resolved lines at 2968, 2940, 2878 and 2805 cm^{-1} (CH form) whereas they appear for the deuterated form at 2260, 2185 and 2133 cm^{-1}. In D_2O solution of 5′-AMP the CH groups of ribose are exchanged slowly compared with the exchange rate of OH and the 2- and 8-CH groups. Therefore one can see in the SERS spectrum of deuterated adenosine 5′-monophosphate as well the CH as the CD frequencies of the ribose group (*cf.* Table I). These SERS lines are also well amenable to precise intensity measurements for the determination of the exchange kinetics.

In conlusion, the SERS approach to H-D exchange studies of 5′-AMP has shown that three classes of sites can be probed directly; *i. e.* in the ribose OH and CH and in the adenine base the 2- and 8-CH.

At present the kinetic parameters of H-D-exchange at these three sites in 5′-AMP, poly-A and native DNA as a function of temperature are under investigation. The data will provide useful information about structural fluctuations of DNA outside the melting temperature range.

Acknowledgement

The authors are indebted to Prof. Dr. H. W. Nürnberg for his continuous encouragement and critical reading of the manuscript.

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