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Surface-Enhanced Raman Spectroscopy of DNA Bases

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A Raman microprobe has been used to measure the surface-enhanced Raman spectra of adenine, guanine, cytosine and thymine. Comparison of the SERS spectrum with solution spectra shows that some line positions are not influenced by the adsorption process while others show large shifts. In the SERS spectrum new lines, not visible in the solution spectrum, appear while some lines visible in the solution spectrum are not enhanced to a detectable level and are therefore not seen in SERS. The relative intensities are changed owing to an apparently vibration-dependent enhancement factor. A line-broadening effect occurs for most lines except carbonyl stretching vibrations in cytosine and thymine. All SERS spectra show increased contributions of bending vibrations and side-chain groups. In particular, amino group vibrations in adenine and cytosine are clearly visible. Comparison of the shape and intensity of the carbonyl stretching vibrations in cytosine, thymine and guanine show important differences. It is hypothesized that these differences indicate differences in the orientation of these groups with respect to the surface.

INTRODUCTION

Raman spectroscopy is widely used in the study of biological materials, e.g. proteins,[1] DNA[2] and chromosomes.[3] With this technique valuable information is obtained about the chemical composition, the secondary structure[4] present in the macromolecules and the chemical surrounding of specific subunits.[2,5] A major disadvantage of the Raman technique, however, is the small scattering cross-section of biological molecules. As a result, high concentrations must be used. Unfortunately, many compounds of biological interest are difficult to prepare in large amounts and they can, therefore, hardly be investigated using normal Raman spectroscopy (RS). Moreover, a high-power laser providing about 300 mW at the sample is needed.

It is now well known that many molecules[6] show an enhanced Raman scattering when adsorbed on metal surfaces or metal particles in solution. This enhancement mechanism is also present for very large[7,8] and complicated molecules.[9] This phenomenon has become known as surface-enhanced Raman scattering (SERS).

Many theories have been developed to explain the large experimental enhancement factors of up to 10^7. It is now widely accepted that a combination of enhancement mechanisms is necessary to explain such a large enhancement factor.

Two mechanisms have been proposed. In one, the electromagnetic theory has been used to describe quantatively the enhanced field strengths near roughened surfaces.[11] This increased field strength is a result of the resonant excitation of collective oscillations in free-electron-like metals (e.g. Ag, Au and Cu) by the incident laser field. In this case the enhancement factor ranges from 10^3 to 10^5 and depends on geometrical factors. The corresponding surface roughness is of the order of 100 nm.

Secondly, the concept of some 'active site' has been used.[12] This concept implies the presence of structures on the surface capable of forming particular molecular configurations. One of the concepts proposed is the adatom.[13] Experimental evidence for the role of these structures in the enhancement process is obtained from the irreversible loss of intensity which occurs when the potential of the working electrode is lowered[14] or the temperature is raised[15] so much that destruction of the special structures takes place. In this model the surface roughness is of an atomic nature. The contribution of this mechanism to the enhancement factor is of the order of $10\text{–}10^3$.[16] In contrast to the electromagnetic model, in which the enhancement mechanism has a long-range character,[11] the 'active site' model invokes an enhancement mechanism limited to the molecular ⟨sp⟩ layer in direct contact with the metal surface. Some kind of physical or chemical bonding between the adsorbate molecule and the surface is assumed in this case.

It has also been suggested[17] that lone pair nitrogen bonding is of importance for the adsorption behaviour. It seems likely that for the bases which we have investigated this mechanism also plays a role. As a consequence of the adsorption process, changes in the molecular vibrational levels and therefore in the detailed structure of the Raman spectra may be expected. Therefore, it is striking that SERS spectra often show good correspondence with RS spectra from the same molecules in solution. However, our spectra of the DNA bases indicate that changes do occur and no model has yet been evolved that explains the influence of the metal surface on the relative intensities, positions and widths of vibrational modes active in the SERS process.

We present in this paper the SERS spectra of the DNA bases and an interpretation in terms of normal mode assignments and molecular orientation with respect to the surface. The spectra form the basis for further investigations.

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MATERIALS AND METHODS

The Raman microprobe, as built in our laboratory, consists of a Nikon Optiphot microscope, a Jobin-Yvon Hg 2S monochromator and a Hamamatsu R 943-02 photomultiplier tube. A PDP-LSI-11 on-line computer was used to control the monochromator and to handle the data. The light source was a Spectra-Physics 15 mW 124B He-Ne laser.

Other instrumental parameters are mentioned in the figure captions. The solutes were dissolved in a buffer containing 100 mM KCl and 1 mM Na_2HPO_4. The measurements were performed in an electrochemical microcell with a volume of 80 μl, details of which have been given previously.[18] The working electrode was an Ag wire fitted in a Perspex holder; the counter electrode was a Pt wire. A saturated calomel electrode (SCE) served as the reference electrode. Between measurements with different samples the cell was rinsed and soaked extensively with distilled water to remove materials absorbed into the Perspex jacket.

Surface roughening of the working electrode was obtained as illustrated in Fig. 1. During the oxidation cycle a 100 mC cm^{-2} charge passed. The oxidation-reduction cycle lasted 45 s. This procedure yielded reproducible high-intensity spectra. All spectra presented in this paper were measured at or close to the potential of zero charge for the electrode system, because in this case the intensities were maximal. Spectra taken at potentials for which the charge of the working electrode was more positive showed striking differences and will be published in a later paper.

With the microprobe arrangement the intensity variations as a function of the position on the electrode surface were measured. The variations were within a factor of 2 and may be due to either inhomogeneous adsorption and/or inhomogeneities in the roughening of the surface. The spatial resolution was limited by the dimension of the illuminating spot, which had a size of 7 μm. Apart from the absolute intensity differences, no other differences between the spectra were observed.

Figures 2-4 show the spectra of cytosine, thymine and adenine. SERS spectra are compared with normal Raman solution spectra obtained in a conventional arrangement. In Fig. 5 the SERS spectrum of guanine is shown together with the solution spectrum of *d*-GMP.

RESULTS AND DISCUSSION

Very little is known about the actual behaviour of molecules adsorbed on a metal surface. SERS combines the sensitivity for internal molecular forces of the Raman technique with an enhancement mechanism that is large enough to avoid intensity problems due to the very low concentration of molecules at a surface. Comparison of the SERS spectra of adenine, guanine, cytosine and thymine with the spectra of these molecules in aqueous solution shows significant differences. Line shifts occur for some lines in addition to changes in the relative intensities and linewidths. From the voltammetric measurements no oxidation or reduction of the molecules can be observed. The covalent structure of the molecules is assumed to remain intact.

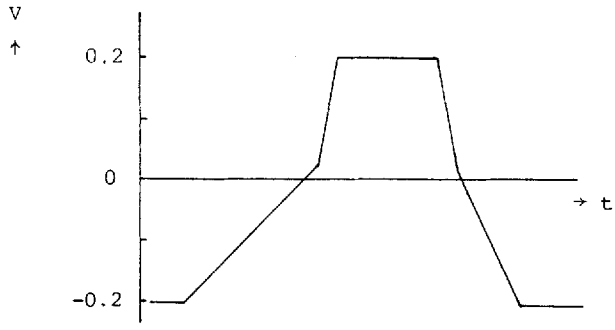

Figure 1. Oxidation–reduction cycle used to prepare the Ag electrode surface. The potentials are referred to a saturated calomel electrode. The maximum current during the oxidation step is 25 μA.

In the SERS spectrum no contributions from the bases in solution can be seen. This is due to the limited depth of focus in a microprobe arrangement and because the molecules in solution lack the extra enhancement mechanism active for molecules adsorbed on the surface.[16] No theory has yet been developed which describes in detail the influence of the metal surface on the vibrational dynamics of adsorbed molecules. This influence is, however, directly measured with SERS although a correct assignment of the observed lines is necessary for this purpose. Normal mode calculations have previously[19] been performed for these molecules (with the exception of thymine, for which we have used the data for uracil) and are used for the assignments. For additional data on thymine, Raman spectra of solid thymine[20] have been used. A good correlation was obtained between measured SERS lines and the calculated spectra, although the assignment remains tentative in case large line shifts occur. A few lines in SERS could not be correlated with any calculated line.

Before describing the spectra in more detail, some general features will be mentioned. The strongest line in all the spectra can be assigned to the in-phase ring stretching vibration. It is only slightly shifted in the case of adenine and cytosine, from 724 to 732 cm^{-1} and from 790 to 796 cm^{-1}, respectively. More significant shifts occur for guanine and thymine, from 680 to 656 cm^{-1} and from 750 to 776 cm^{-1}, respectively.

In the spectra increased contributions can be seen from the ring bending vibrations and from external groups of the ring system. An increased linewidth is observed for almost all lines in the spectrum. Exceptions are the carbonyl stretching modes in cytosine and thymine, for which a significant decrease in linewidth occurs. The width of the carbonyl stretching mode in guanine, which is only very weak, does not seem to have become smaller.

Remarkable in the case of adenine and guanine is the almost complete absence of lines in the double bond stretching region. In spite of the lack of exact information on the real concentrations of the molecules contributing to the surface-enhanced signal we have calculated enhancement factors. The surface covered by adenine and thymine at the mercury-solution interface has been obtained[21,22] and was about 55 $Å^2$. We have assumed that all DNA bases occupy the same surface area at the silver electrode. In the calculations no increase in the available surface as a result of the electrochemical roughening was supposed. Further, we

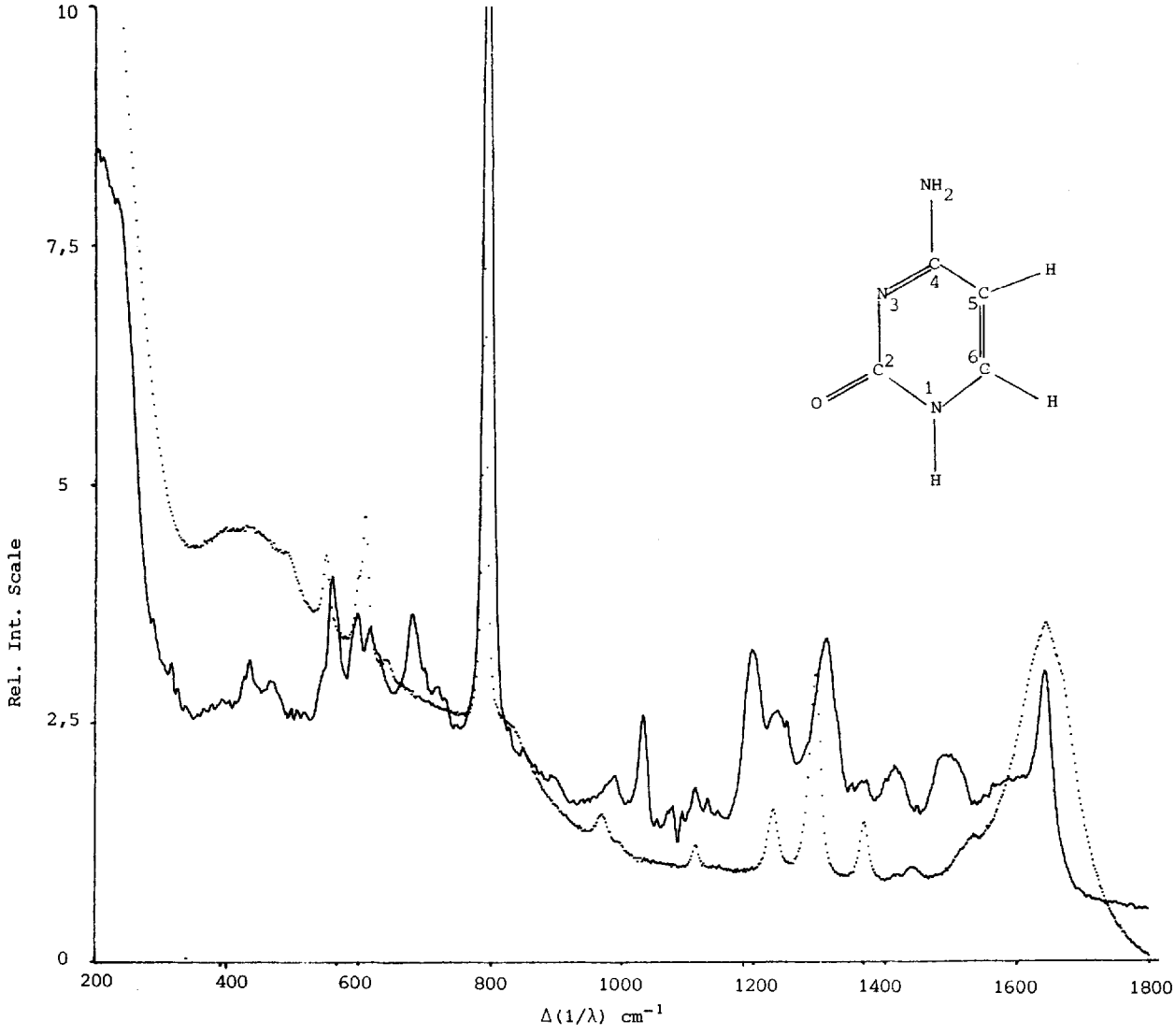

Figure 2. RS (dotted line) and SERS (solid line) spectra of cytosine. The instrumental parameters are as follows: power at the sample, 1.9 mW; spot diameter, 7 μm; irradiance, 4.9×10^7 Wm^{-2}; objective, 40×; numerical aperture, 0.65; slit widths, 4×400 μm; spectral resolution, 3.2 cm^{-1}; interval, 2 cm^{-1}; integration time, 4 s. The relative intensity in Figs 2–5 relates to the SERS spectra only.

have assumed that one monolayer contributes to the signal. Owing to these assumptions, the calculated enhancement factor is an upper limit. The product of peak height times full width at half maximum has been taken as a measure of the intensity.

Cytosine

Results for cytosine are given in Table 1. Considerable changes occur throughout the spectrum when cytosine is adsorbed on the silver electrode. Six lines can be seen in the ring bending mode region, two of which (at 558 and 598 cm^{-1}) coincide with lines in the RS. The lines at 616 and 690 cm^{-1} have not previously been noted in the RS, but can be assigned to bending vibrations which are calculated to occur at 617 and 646 cm^{-1}.

The weak bump at 470 cm^{-1} can also be assigned to a bending vibration calculated at 454 cm^{-1}. No assignment could be made for the line at 430 cm^{-1}. It should be mentioned that neither line may have been visible in the RS because of their weakness and the presence of solvent scattering around 450 cm^{-1} which is absent in the SERS. Prominent are two vibrations at 1020 and 1196 cm^{-1}, which reveal the presence of the amino group in cytosine. Their calculated values are 1037 and 1212 cm^{-1}, respectively. Other lines in this region at 986 and 1118 cm^{-1} coincide with lines present in the RS. A weak line at 1140 cm^{-1}, not previously noted in the RS, can be assigned. In the single bend stretching region there is good agreement between the SERS spectrum and the solution spectrum. An extra contribution is present at 1482 cm^{-1}. It is resolved from the line at 1508 cm^{-1}, which has made a large shift from 1538 cm^{-1}. In the double bond stretching regions one clear line can be seen at 1640 cm^{-1} which seems to be superimposed on a weak, broad band. The line at 1640 cm^{-1} is assigned to the carbonyl stretching mode. It is shifted to lower frequency by 15 cm^{-1} when compared with its spectrum from D_2O solution.[23] Such a shift for the carbonyl group has also been noted in other systems.[24] Electron donation of this group to the surface would decrease the bond order, resulting in a decrease in the vibrational frequency.

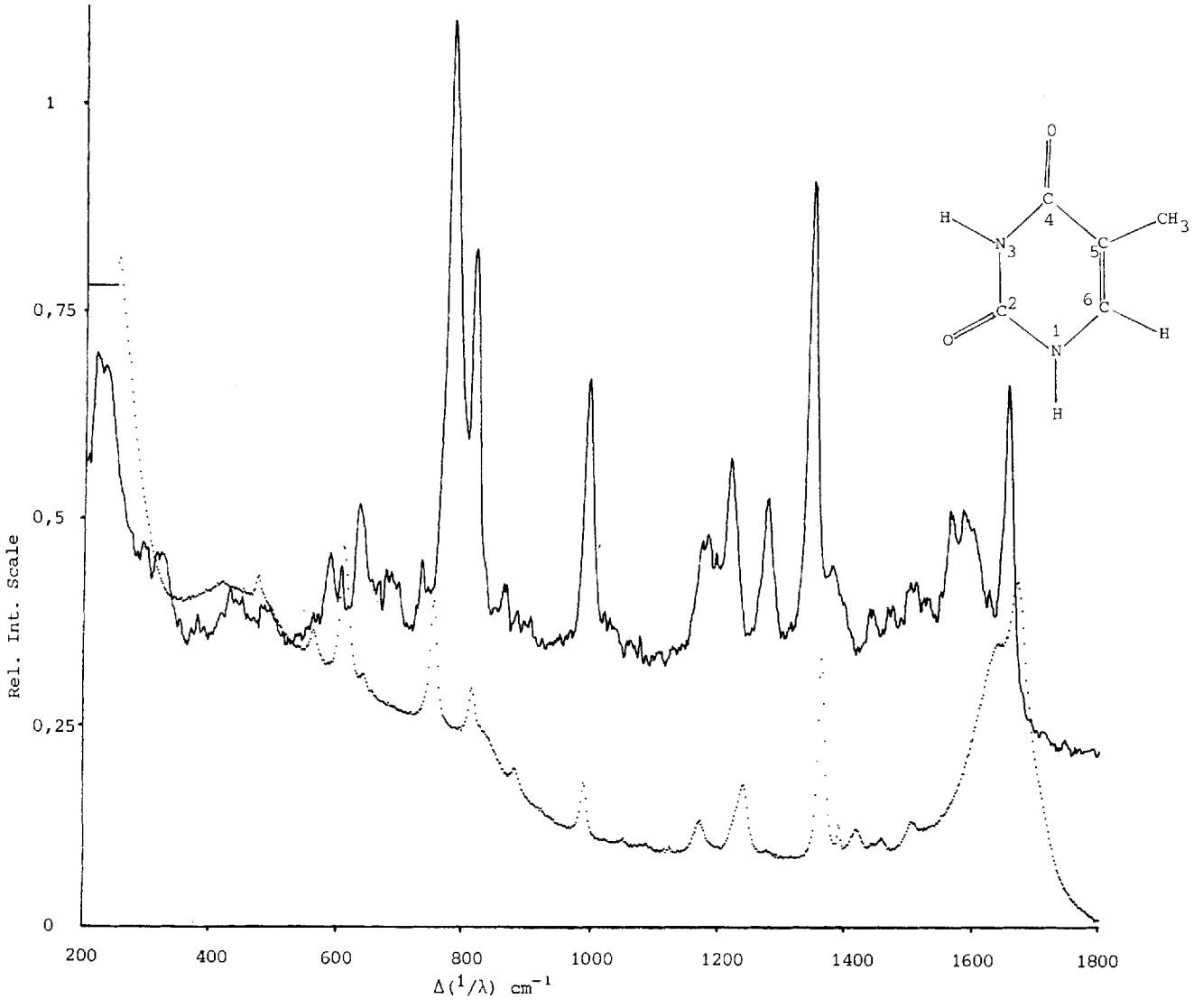

Figure 3. RS (dotted line) and SERS (solid line) spectra of thymine. Integration time, 2 s; other instrumental parameters as in Fig. 2.

Thymine

Results for thymine are given in Table 2. In the ring-bending mode region three lines in the RS-spectrum at 430, 470 and 565 cm^{-1} can be found in the SERS spectrum at 430, 490 and 586 cm^{-1}. Two new lines can be seen at 314 and 632 cm^{-1}. Calculations predict lines at 343 and 614 cm^{-1}. An almost one-to-one correspondence can also be deduced for the rest of the spectrum. The upward shift of the totally symmetric ring stretching vibration from 750 to 776 cm^{-1} is particularly striking since it is known from RS that this mode, common to all pyrimidines, is particularly insensitive to changes in the chemical surroundings.[2]

In the SERS spectrum a strong line appears at 1275 cm^{-1}, which is only very weakly present in the RS spectrum and which can be assigned to a combination of bending and stretching modes, i.e. ring stretch + CH bending.[20] Only weak lines can be seen in the single bond stretching region at 1442, 1472 and 1504 cm^{-1}.

In the double bond stretching region three lines are observed at 1562, 1584 and 1652 cm^{-1}. The line at 1584 cm^{-1} cannot be seen in the RS owing to the solvent interference. Calculations show that this line can be assigned to the $N_3C_4(s)+N_1C_2(s)+C_6C_5(s)$—$N_1C_6(s)$ vibration. The line at 1562 cm^{-1} cannot be identified. The line at 1652 cm^{-1} is assigned to a carbonyl stretching mode. It is interesting that whereas in the RS two lines at 1691 and 1657 cm^{-1} are attributed to the C_2O and C_4O stretching modes, respectively, in the SERS spectrum only one line at 1652 cm^{-1} can be seen. The different positions of the carbonyl stretching modes in the RS occurs because the π-electrons of the C_2-carbonyl band are isolated from ring π-orbitals so no electron delocalization takes place, in contrast with the situation for the C_4-carbonyl group. The occurrence of a single line in the SERS spectrum is either because both carbonyl-stretching modes vibrate with equal frequencies, or because only one vibration can be seen. If both modes occur at the same position it is the C_2O group which has shifted by 39 cm^{-1} and it is likely that this group is coordinated to the surface. In the case that the 1652 cm^{-1} line arises from one mode only, this will most likely be the C_4O mode. Both possibilities suggest that the orienta-

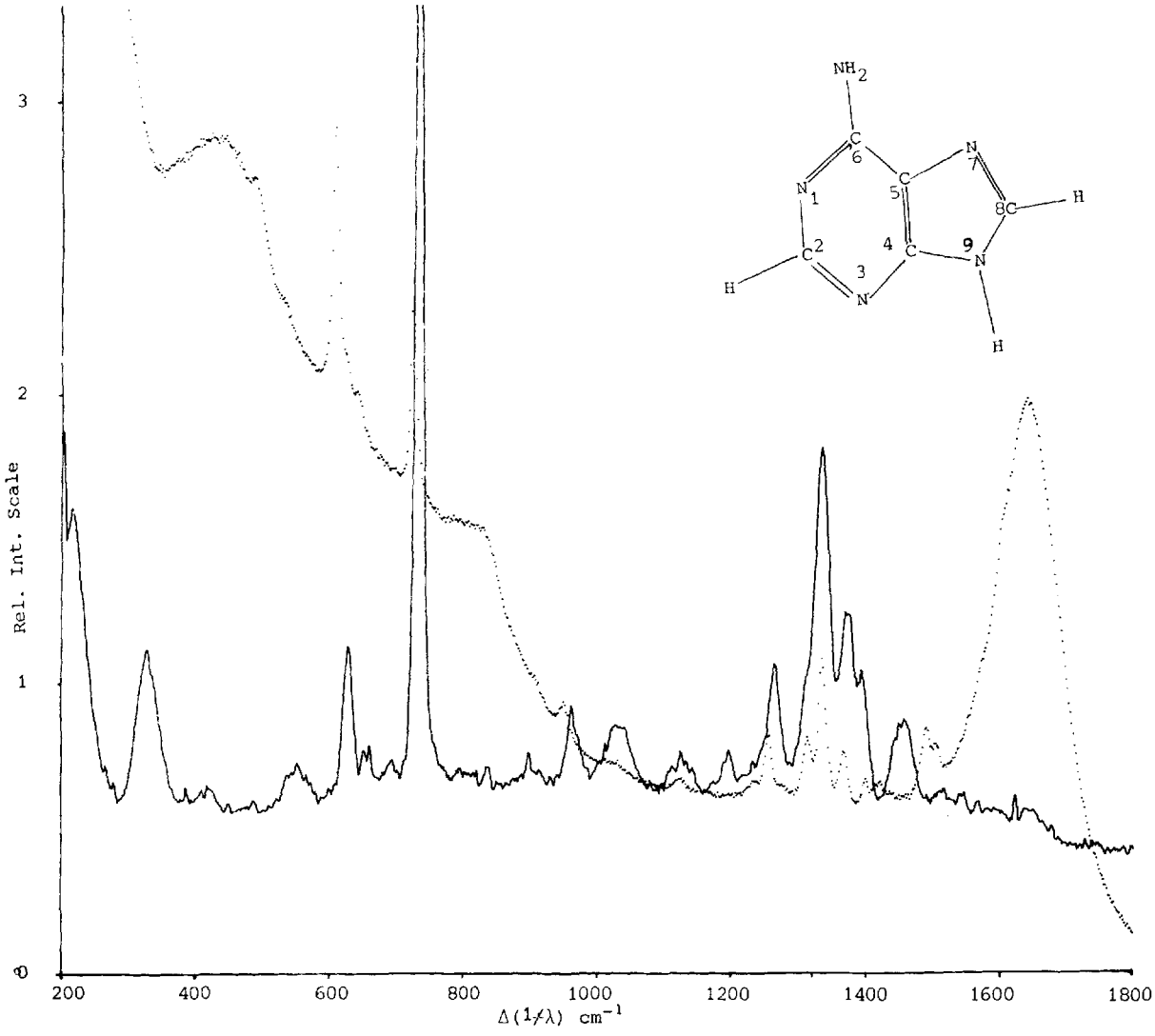

Figure 4. RS (dotted line) and SERS (solid line) spectra of adenine. Integration time, 2 s; other instrumental parameters as in Fig. 2.

tion of the molecular plane is perpendicular to the surface instead of being parallel to it, as has been previously suggested.[25]

If the 1652 cm^{-1} line is due to the C_4O mode, then the absence of the C_2O vibration near 1691 cm^{-1} gives support to the idea that only those carbonyl groups which are clearly visible are coordinated to the metal. As was the case for the carbonyl stretching mode in cytosine, the line width is much smaller than in the solution spectrum.

Adenine

Results for adenine are given in Table 3. The SERS spectrum shows five lines which are not or only very weakly visible in the normal Raman spectrum. Three of these lines can be found in the ring-bending mode region at 326, 548 and 626 cm^{-1}. In the RS lines can be found at 312, 540 and 628 cm^{-1}. The calculations give lines at 540 and 623 cm^{-1} but no line around 326 cm^{-1}. A possibility is the neighbouring line calculated at 361 cm^{-1} which is assigned to the $-C_6N_{12}(b) + N_9R(b)$ vibration. The other two lines new from SERS are found at 1028 and 1194 cm^{-1}; the calculations placed them at 1018 and 1174 cm^{-1}. It can be seen from Table 3 that contributions are made by the external amino group to several of these lines, viz. 326, 626, 1028 and 1194 cm^{-1}, as well as to the line at 960 cm^{-1}. It is striking that no lines are detected above 1500 cm^{-1}. In normal Raman spectroscopy two lines are found in this double bond region, at 1515 and near 1585 cm^{-1}. These vibrations are closely connected to the central and most rigid part of the molecule (23), i.e. the $C_4{=}C_5{-}C_6$ group.

In the single bond stretching region, a one-to-one correspondence with the solution spectrum is observed, although the line at 1310 cm^{-1} in the RS can only be seen as a shoulder from the 1334 cm^{-1} line in the SERS. A large line shift occurs for the line at 1460 cm^{-1} if correspondence is assumed with the line at 1488 cm^{-1} in the RS. Calculations, however, indicate various other vibrations in that region.[19]

Guanine

Results for guanine are given in Table 4. The SERS spectrum closely resembles the RS spectrum. However,

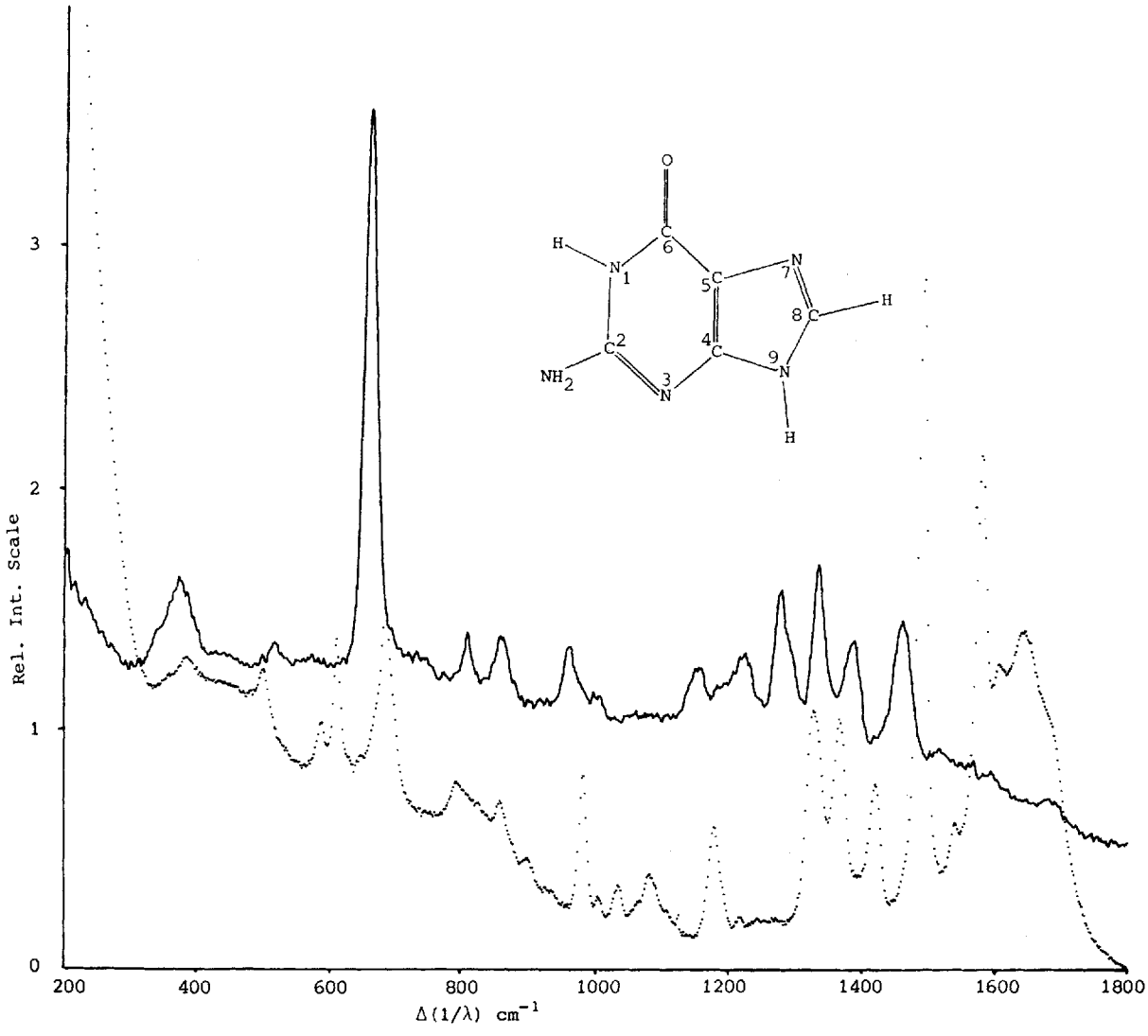

Figure 5. RS (dotted line) and SERS (solid line) spectra of guanine. Integration time, 10 s; other instrumental parameters as in Fig. 2.

a downward shift is seen to occur for most of the lines. This is also the case for the line at 680 cm^{-1} in the RS, which is seen at 656 cm^{-1} in the SERS. This line is the totally symmetric stretching vibration of the six-membered ring and corresponds with a line common to all purines near 725 cm^{-1}. Guanine forms an exception in this respect, in that this line is found at a much lower frequency. It has been suggested[2] that this may reflect a difference in covalent structure compared with other purines. The occurrence of a shift in the case of guanine and its absence in the case of adenine may also reflect this difference.

Extra lines can be found at 804 and 1222 cm^{-1}. The line at 1222 cm^{-1} has been mentioned before[23] but was assigned a zero intensity. No line corresponding to the line at 804 cm^{-1} can be found in the RS spectrum. From infrared studies[26] it is known that a line occurs at 789 cm^{-1} which shifts to higher wavenumber, i.e. 800 cm^{-1}, when a sugar group is connected to position 9. The presence of the metal surface may also cause this line to shift to higher wavenumbers.

A noteworthy effect is the strong and broad line at 370 cm^{-1} which is appreciably shifted from the calculated value at 405 cm^{-1}. It is assigned to the composition of the bending vibration of the external nitrogen atom relative to the ring system and of the bending of the hydrogen atom at position 9. Again, as was the case for adenine, only weak lines can be seen in the double bond stretching region. A weak bump coincides with the carbonyl stretching vibration at 1680 cm^{-1}. It is striking that the intensity is very different from the carbonyl stretching vibration in cytosine and thymine. This may be caused by a different orientation of this group, i.e. not coordinated with the surface, with respect to the surface.

It is satisfying that most of the lines in the SERS spectrum can be assigned using the results of normal mode calculations performed for the case of a free molecule. A spectrum can be seen from the molecules adsorbed on the surface and not a solution spectrum. This is evident because of the absence of the strong totally symmetric stretch vibration of these molecules on the position where they are found in solution.

It has been reported previously for the case of *trans*-2-Butene[16] and pyridine[6] adsorbed on a silver electrode that only the spectrum of the first monolayer differs from

Table 1. Results for cytosine[a]

Band positions/cm^{-1}						
SERS	RS	Assignment	Calculated /cm^{-1}	Linewidth ratio, $\Gamma_{SERS}/\Gamma_{RS}$	Line shift compared with RS	Enhancement factor ($\times 10^{-8}$)
210		Ag^0-Cl^-				
430						
470		$C_2N_1C_6^b+N_3C_4C_5^b$	454			
558	546	$-N_1C_2N_3^b+C_2N_3C_4^b$	566	1.6	+12	0.98
598	590				+8	
616		$C_2{=}O^b+N_1R^b+C_4N_{11}^b$	617			
690		$C_5C_4^b-N_3C_4^b+[N_1C_2O^b-N_3C_2O^b]$	646			
796	787	Ring breathing	793	1.4	+9	0.38
986	975	C_5H^b	979		+11	
1020		$NH_2^r+C_6H^b$	1037			
1118	1116	$N_1R^s-C_2N_3^s$	1122	1.0	+2	0.45
1140		$-N_1R^s+C_6H^b$	1153			
1196		$C_6H^b+C_4N_{11}$	1212			
1230	1230				0	
1306	1294	$N_1C_6^s+C_5C_6^s$	1320	2.0	+12	0.52
1360	1368	$C_4N^s-C_5C_6^s$	1367		−8	
1422	1420					
	1442	$C_4N_{11}^s+N_1C_2^s$	1443			
1482		$N_1C_6^s+N_3C_4^s$	1474			
1508	1536	$-N_3C_4^s-N_1C_2^s$	1538		−26	
1582		$C_4C_5^s-C_5C_6^s$	1523			
1640	1655	$C_2{=}O^s-C_2N_3^s$	1663	0.5[b]	−15[b]	

[a] Abbreviations used: r=rocking; s=stretching; b=bending. Use has been made of Ref. 19.
[b] Data obtained from Ref. 2.

the solution spectrum. Subsequent layers were seen to generate the solution Raman spectrum. The change in the spectrum of the first layer is a result of the direct interaction between the metal and the molecule. The effect that this interaction has on the vibrational spectrum of the molecule is seen in the SERS spectrum. It can be expected that the SERS spectrum reflects the way in which the molecules are adsorbed on the metal surface.

It is known from other electrochemical studies[27] that adenine may adopt a configuration in which the amino group is coordinated to the metal surface and the plane

Table 2. Results for Thymine[a]

Band positions/cm^{-1}						
SERS	RS	Assignment	Calculated /cm^{-1}	Linewidth ratio, $\Gamma_{SERS}/\Gamma_{RS}$	Line shift compared with RS	Enhancement factor ($\times 10^{-8}$)
230		$A_g^0-Cl^-$				
314		$C_2N_1R^b-C_6N_1R^b$	343			
430	430					
490	470	$C_2N_1C_6^b+N_3C_4C_5^b$	470		+20	
586	565	$N_1C_2N_3^b-C_2N_3C_4^b$			+21	0.8
632		$N_1C_2O^b+N_3C_4O^b$	614			
776	750	Ring breathing	795	1.6	+26	1.1
812	811	$N_1C_2^s+N_1R^s+C_5C_4^s+N_1C_6^s+N_3C_4^s$		1.5	+1	1.7
	879					
992	986	C_5-Me^{r*}		1.8	+6	1.0
1184	1169	$-C_6^bH+C_2N_3^s$	1200	1.4	+15	0.7
1216	1214	$Ring^s+C_5-Me^{s*}$		1.0		0.4
1275	1238	$Ring^s+CH^{b*}$		+37		
1344	1360	$N_3H^b-C_4{=}O^s$	1375	1.7	−16	0.3
1372	1388	C_5-Me^{b*}			−16	
	1418	NH^{b*}				
1442	1456	C_5-Me^{b*}			−14	
1472		$-N_1C_2^s+C_2N_3^s$	1491			
1504	1503	NH^{b*}			+1	
1562						
1582		$N_3C_4+N_1C_2^s+C_6C_5^s-N_1C_6^s$	1562			
1652	1657	$C_4{=}O^s+C_s-C_6^s$		0.4	−16	
	1691	$C_2{=}O^s$				

[a] Abbreviations as in Table 1. For the assignments use has been made of the calculated data in Ref. 19 in the case of uracil. Assignments indicated with asterisks are from Ref. 20.

Table 3. Results for adenine[a]

Band positions/cm^{-1}						
SERS	RS	Assignment	Calculated /cm^{-1}	Linewidth ratio, $\Gamma_{SERS}/\Gamma_{RS}$	Line shift compared with RS	Enhancement factor ($\times 10^{-8}$)
214		A_g^0—Cl^-				
326	312	$-C_6N_{12}^b+N_9R^b$	361		+14	
548	540	$C_5C_4N^b-C_2N_1C_6^b$	540		+8	
626	628	$N_9R^b+C_6N_{12}^b-N_7C_5C_6^b$	623		−2	
732	724	Ring stretching	718	1.1	+8	5.0
960	948	$NH_2^r+N_1C_6^s$	966		+12	
1028		$NH_2^r+N_9R^s$	1081		−2	
1122	1124	$-N_3C_2^s+N_9R^s$	1119		−2	
1194		$C_8N_7^s+C_6N_{12}H^b-C_1N_2^s$	1174			
1264	1254	$N_1C_2^s+C_2H^b+N_9C_8^s+C_8N_7^s$	1259	1.4	+10	1.8
	1310					
1334	1332	$-N_7C_5^s+C_8N_7^s$	1329	1.9	+2	2.5
1370	1362	$C_8N_9^s+C_2N_3^s$	1354	1.6	+8	3.7
1390	1398	$-N_1C_6^s+C_6N_{12}^s$	1424		−8	
	1420					
1460	1488	$C_2H^b-N_1C_2^s+N_3C_2^s$	1463		−28	
	1515					
	1585					

[a] Abbreviations as in Table 1.

of the molecule is perpendicular to this surface. Comparison of the SERS spectrum with the RS spectrum reveals that many new features are connected with the vibrational motions of the amino group. This may mean that the presence of the metal enhances and influences in particular the movement of that part of the molecule which is most close to it. When the amino group is involved in the adsorption process, coordination to the surface will probably arise through its lone pairs[17] but it has also been proposed[27] that coordination may occur by means of the hydrogen atoms.

The spectra of cytosine and guanine also show new contributions arising from the amino group, suggesting a strong enhancement and a disturbed motion for this group. We cannot say, however, that this can only occur when the molecule is attached to the surface by means of the amino group. It may be that other orientations of this group also give rise to the effects we have noted.

Table 4. Results for guanine[a]

Band positions/cm^{-1}						
SERS	RS	Assignment	Calculated /cm^{-1}	Linewidth ratio, $\Gamma_{SERS}/\Gamma_{RS}$	Line shift compared with RS	Enhancement factor ($\times 10^{-8}$)
370	382	$C_2N_{14}^b+N_9R^b$	405		−12	
512	498	$C_2N_1C_6^b+N_9R^b-C_5C_4N^b$	558		+14	
	586					
656	680	In-phase ring stretching of the six-membered ring except C_4C_5	671	1.0	−24	1.3
	742					
	788					
804						
852	854	$-N_7C_5^s-N_1C_2N_3^b$	838			
960		$-N_9R^s+N_3C_2^s$	965			
1000	1002					
	1032					
	1080					
	1106					
1154		$-C_8N_7^s+N_9R^s-C_4N_3^s$	1172	1.5	−21	0.20
	1176					
1222	1214	$-C_8H^b+C_8N_7^s$	1198			
1277	1324	$-C_8N_7^s-N_1C_6^s+N_7C_5^s$	1290	1.0	−47	0.27
1332	1364	$C_8N_9^s-N_7C_8^s$	1347	1.0	−32	0.30
1384	1418	$C_2N_3^s-C_2(ND_2)^s$	1426	1.0	−34	0.27
1458	1488	$N_1C_2^s-N_1C_6^s$	1460	2.0	−30	0.17
1514	1538	$C_4C_5^s-C_4N_9^s$	1555		−24	
1582	1576	$N_3C_4^s-C_4C_5^s$	1581			
	1604					
1680	1680	$C_6=O^s-C_5C_6^s$	1673		0	

[a] Abbreviations as in Table 1.

A comparison of the behaviour of the carbonyl group in cytosine, thymine and guanine is also of importance. In all cases the carbonyl group is attached to the pyrimidine ring. The spectra of cytosine and thymine show a sharp and strong carbonyl stretching mode. In thymine two carbonyl stretching modes are expected, but only one is seen. As has been argued previously, it is possible that the carbonyl group on C_2 is not seen. The presence of a carbonyl group is not always clearly revealed in the spectrum, as can be seen from the spectrum of guanine. The carbonyl stretching mode is very weak and it is not shifted from its position in solution. From this it may be concluded that the intensity and the width of this particular vibrational mode are sensitive to its orientation with respect to the surface. It seems likely that not all three molecules are oriented parallel to the surface, because in that case there would be no difference between the orientation of the different carbonyl groups. It has been proposed that the linewidth of the carbonyl group of these molecules in solution in the RS is broadened as a result of hydrogen bonding with the solvent molecules. We note that the linewidth of the visible carbonyl groups in cytosine and thymine has decreased by a factor of two compared with the solution spectra. This may indicate that the carbonyl groups are not hydrogen bonded, possibly because steric hindrance prevents the solvent molecules from approaching the carbonyl group close enough for bonding, when cytosine and thymine are adsorbed to the surface. Another possibility, however, is that it is not only the absence of hydrogen bonding but also same specific metal-carbonyl group interaction which leads to the decreased linewidth. Both possibilities point to a direct involvement of the carbonyl group in the adsorption process on the surface.

A comparison of the relative enhancement factors (REF) of the totally symmetric stretching vibration for the purines and pyrimidines reveals that in the case of adenine and guanine the REF of this vibration is very much larger than that of all other lines. This is in contrast to the situation for the pyrimidines. In the case of cytosine the REF of this vibration is even the smallest one. This may also be taken as an indication that the orientation of the pyrimidines, attached by means of the carbonyl groups, is different from that of the purines.

CONCLUSIONS

We have shown that the use of a low-power He–Ne laser in combination with a Raman microprobe is sufficient for obtaining good micro-SERS spectra. With the Raman microprobe high irradiances can be achieved together with effective sampling of the scattered radiation.

In normal Raman spectroscopy large parts of the bending mode region (*ca* 450 cm^{-1}) and the double bond region (>1580 cm^{-1}) show scattering of water. This may be circumvented by using D_2O. Using SERS a direct examination can be made of the bending mode and double bond region since solvent scattering is negligible. The spectra we have obtained are due to molecules adsorbed directly on the metal because of the absence of an undisturbed solvent Raman spectrum. Although electromagnetic field enhancement is of importance, because it increases the scattered radiation to a detectable level, it will be only partly responsible for the disturbance of the vibrational spectra we have measured, because the electromagnetic enhancement is independent of the particular vibration. The spectra we have measured must be regarded as a direct examination of the influence that the Ag surface has on the vibrational dynamics of the molecules directly attached to the surface.

The SERs spectra show new lines not visible in the RS spectra, while other lines are absent. With the help of vibrational calculations it was possible to identify almost all the lines visible, including the lines in the SERS spectra. It turns out that in all spectra increased contributions can be seen from the side-chain vibrations, while in the spectra of the purines the vibrations in the double bond stretching region are absent.

Although a theoretical approach towards the interpretation of the spectra has not yet been developed, we have taken the large contribution of amino group vibrations in the case of adenine as an indication that adsorption takes place through this group. The comparison of the carbonyl vibrational modes in cytosine, thymine and guanine reveals differences in intensity and linewidth. It is concluded that the orientation of the carbonyl group with respect to the surface is not the same in the three cases. With some care, it may be concluded that cytosine and thymine are attached to the surface with a carbonyl group and that guanine is either π-electron bonded or coordinated through its amino group.

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