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A Raman scattering study of the helix-destabilizing gene-5 protein with adenine-containing nucleotides

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ABSTRACT

Raman spectra of gp5 and complexes of gp5 with poly(rA) and poly(dA) have been determined and analysed. From a fit of the amide I-band with model spectra it follows that the secondary structure of gp5 contains 52% β-sheet, 28% undefined conformation and 19% α-helix. The band at 1032 cm^{-1} due to phenylalanine has an anomalous intensity both in the spectra of the complexes and the free protein. This possibly indicates a stacked structure present in the protein.

Binding of gp5 to poly(rA) and poly(dA) influences the intensity of bands near 1338 and 1480 cm^{-1} which are considered to be marker-bands for the phosphate-sugar-base conformer. A change in conformation of the nucleotides is also reflected by vibrations originating in the phosphate- and sugar-residues of the backbone. In the spectrum of complexed poly(rA) the intensity of the conformation sensitive band at 813 cm^{-1}, which is due to the phosphodiester group, is zero. It seems that gp5 forces poly(rA) and poly(dA) to a similar conformation.

A marker band for stacking interaction in poly(rA) indicates that stacking interactions in the complex have increased.

INTRODUCTION

The gene product 5 (gp5) of the bacteriophage M13 belongs to the class of helix destabilizing proteins. The classification arises from the fact that the protein has a much larger affinity for single stranded DNA than for double stranded DNA. This results in a decrease of the melting temperature of the latter in the presence of the protein.

The gene-5 protein binds to 3 nucleotides in a complex with $(dA)_8$ (1). In the presence of polynucleotides one protein covers 4 to 5 nucleotides (2,3). The cooperative binding parameter is small (≈5) for short nucleotides and much larger for polynucleotides (≈ 50-300) (2). This leads to the simultaneous presence of completely saturated polynucleotides and completely uncomplexed polynucleotides under conditions of excess polynucleotide (2).

Several spectroscopic techniques have been applied for the study of complexes of gp5 with nucleotides, examples are NMR (2), fluorescence spectroscopy (2,1), circular dichroism (CD) (4) and absorption spectroscopy

(4). A model for the poly(dA)-gp5 complex was proposed in (2). It was noted from NMR-data that the adenyl-bases are destacked in the complex. New stacking interactions, however, occur between aromatic amino acid residues and the adenine-bases. From electron microscopy and neutron diffraction experiments it was derived (6) that, taking into account that gp5 binds to 4 nucleotides, about 24 nucleotides are present in a helical pitch of 9 nm. The outside diameter of the complex was estimated to be 10 nm.
From a detailed analysis of the NMR- and NOE-experiments (2) it was proposed that the nucleotide is positioned on the inner side of a cylinder with the H8-proton closest to the protein which, for the larger part, must be on the outside of the cylinder. These data further suggested that the structure of the phosphate-sugar-base conformer of poly(dA) in the complex is somewhat different from that of poly(dA) in the native state.

Scheerhagen et al. used CD and absorption spectroscopy to study complexes of gp32 and gp5 with nucleotides (4). It was noted by these authors that the spectra of a particular nucleotide in both types of complexes corresponded quite well. It was suggested that gp32 and gp5 influenced the nucleotide conformation in a similar manner. A hydrodynamic study of the complex of gp32 with poly(rA) (7) indicated a 50% increase in the phosphate-phosphate distance compared with that in the free polynucleotide. The experimental CD and absorption spectra were also simulated by calculations (5). From this work a detailed model of poly(rA) in complex with gp32 resulted. This model was a refinement of the model mentioned above for poly-dA complexed with gp5. The following aspects of the model are noteworthy: Poly(rA) in complex has an increased base-base distance; a low rotation per basepair; a shift of the bases towards the helix axis and a large tilt of the base molecules so that they are parallel to the helix-axis.

The gp5 protein contains 87 amino acids and has a molecular weight of ≈ 9700 a.m.u. The amino acid sequence is known (8). The protein contains 5 tyrosine and 3 phenylalanine residues. NMR studies of gp5-nucleotide complexes (2) have revealed that 2 tyrosine and 1 phenylalanine are stacked with the adenine-bases.

We have determined Raman spectra of gp5 and gp5 complexed with poly(rA) and poly(dA) to study the polynucleotides in the complex and to compare the spectra of gp5-complexed nucleotides with those of gp32-complexed nucleotides (9). The results of this study will be used to verify some suggestions made in an earlier publication (9) with respect to the interpretation of nucleotide Raman spectra as measured when complexed with proteins.

MATERIALS AND METHODS

Nucleotides: poly(rA) was obtained from SIGMA, poly(dA) was obtained from PL-Biochemicals. The extinction coefficients used are: poly(rA) ϵ = 10000 $M^{-1}cm^{-1}$ and poly(dA) ϵ = 9100 $M^{-1}cm^{-1}$.

Gene-5 protein: gp5 was isolated as described by Garsen et al. (10). For the determination of the protein concentration a molar absorption coefficient of 7100 $M^{-1}cm^{-1}$ at 276 nm was used. The pH of the solution was 7.1. The NaCl concentration was 50 mM. Under these conditions the protein binds stoichiometrically to nucleotides (2). Spectra were taken from solutions containing about 5 mg/ml protein. The E(280)/E(260) ratio was higher than 1.8 and usually ranged between 1.8 and 1.9. The complexes with polynucleotides had a monomer nucleotide/protein ratio of 4. This is equal to the number of nucleotides in the nucleotide-binding site (2) of each protein molecule. There is some dispute about this value in literature. The nucleotide binding site may cover from 4 (2) to 5 (3) nucleotides. Our choice ensures a complete saturation of the polynucleotide. Prior to Raman measurements the samples were routinely centrifuged for 4 minutes at 1000 g. During measurements the sample was thermostated at 15°C. The protein was deuterated by lyophilizing twice from a D_2O-buffer.

Raman spectrometer: The Raman spectrometer consisted of a Jobin-Yvon HG2S monochromator and a Coherent Argon-ion laser operating at 514.5 nm. The photomultiplier tube was a Hamamatsu R 943-02 cooled to -20°C. The 632.8 nm (He-Ne) and 514.5 nm lines were used for wavelength calibration. Control of the stepping motors of the monochromator as well as data collection were performed by a LSI-11 computer. The slit widths were adjusted to 4 times 400 µm, giving rise to a spectral resolution of 3.2 cm^{-1}. A laser power of 900 mW was used. No deterioration of the sample occurred as was checked by comparison of successive runs in multi-run experiments. The scanning interval was 2 cm^{-1}. The accuracy of the bandpositions is +/- 2 cm^{-1}.

Curve-fitting:

Preliminary to the curve-fitting procedure the spectral data were treated as follows:

1) The spectra were smoothed by a five point sliding average,
2) The spectra were normalized using the 1004 cm^{-1}-line of phenylalanine,
3) The buffer/background spectrum was subtracted from spectra,
4) A zero-base line was drawn from 1500 to 1750 cm^{-1},
5) The region between 1630 and 1700 cm^{-1} was normalized, so that the

sum of the intensity over all the channels equaled one. Then the actual curve-fitting procedure using multiple linear regression and making use of the reference intensity profiles of (11) was carried out.

The data of the amide III'-band were treated in the following way: points 1) till 3) as above,

4) Subtraction of the protonated protein spectrum from the deuterated protein spectrum.

5) Normalization of the intensity in the interval of 950 to 1005 cm^{-1}. Then curve-fitting using the reference spectra of (12) was performed.

The use of the 1004 cm^{-1}-band of phenylalanine as an internal intensity reference was chosen after comparison of the intensity of this band in gp5 and gp32 (19) with that of the free amino acid. The results indicated that this band is a reliable intensity standard. The intensity of the 1004 cm^{-1}-band was also compared with the intensity of the band at 1446-1452 cm^{-1}. This band, which is due to the CH_2-deformation modes, is often used as an internal intensity reference (13). Also in comparison with this band the 1004 cm^{-1}-band of phenylalanine appeared to be a reliable standard. For a comparison of the intensities of nucleotide vibrations under different conditions the intensity of the band at 1580 cm^{-1} was used (18).

Multiple linear regression: A PASCAL program was written using the method of multiple linear regression (14) to fit a set of known component spectra to the measured spectrum. A short treatment of the mathematical procedure is given below:

The intensity I(y) is measured in N channels, with the wavenumber interval y, running from 1 to N. The intensity in the y-th interval due to the x-th component is represented by R(y,x). S(x) is the concentration by which the number of counts of the x-th component spectrum has to be multiplied. A calculated spectrum is then obtained according to:

$$\sum_{y=1}^{N} I'(y) = \sum_{x=1}^{M} R(y,x) * S(x),$$

in which M is the number of component spectra. The calculated spectrum, I'(y), differs from the measured spectrum I(y) by D(y). Minimalisation of D(y) using a least squares criterium enables one to write in matrix-notation:

$$R^T I = R^T R S$$

For the calculation of S(x) it is therefore necessary to calculate the

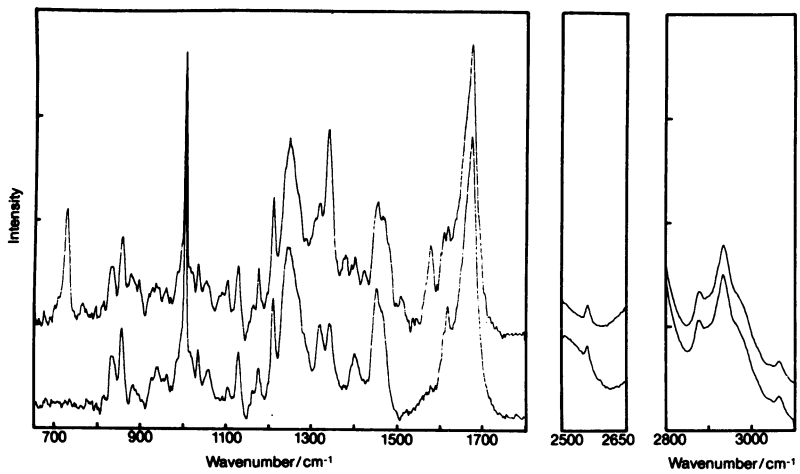

Figure 1: Spectra of gp5 (lower spectrum) and gp5/poly(rA) (upper spectrum). The protein concentration was about 5 mg/ml. The solution contained 50 mM NaCl and the pH was 7.1. All other conditions were as mentioned under Materials and Methods. The spectra are shown from 650-1800 cm^{-1}, 2500-2650 cm^{-1} and 2800-3100 cm^{-1}. The spectrum in the latter interval was measured in D_2O solution. Assignment of the peaks is as given in table 1.

inverse matrix of (R^T R). This was done by the augmented matrix method (15).

RESULTS

Gene-5 protein: The Raman spectrum of gp5 is shown in fig. 1 and summarized in table 1. Some characteristics of the spectrum are:

Amide I and -III': Analysis of both the amide I and amide III'-regions (table 1) using the model spectra from (11,12) reveals that the gene-5 protein contains a high amount of β-sheet structure (table 2). Apart from this β-sheet structure also α-helix and undefined structure are present in the protein.

Phenylalanine: The intensity of the band at 1032 cm^{-1} due to the in-plane C-H deformation which is characteristic for mono-substituted benzenes (16), is in gp5 ≈ 30% weaker than what is expected from the spectrum of the free amino acid.

Tyrosine: An analysis of the intensity ratio of the bands at 834 and 854 cm^{-1} (17) shows three different possibilities for the hydrogen bonding pattern

Table 1 : Positions and assignments of peak maxima in the Raman spectrum of gp5.

Position [cm^{-1}]	Assignment	Reference
812	Aliphatic side chains	41
834	Tyr also Gly,Val	39, 38
854	Tyr	39
882	Gly,Ser also C-C-str and Val	39, 38
928	Thr, Val	39, 38
938-944	Val, Leu (CH_3 sym rock,ip), Lys, C-C-str	39, 38
960	Val, Leu (CH_3 sym. rock,op)	39
984	Ile	39
1004	Phe (trigonal ring breathing)	16
1018	unknown	
1032	Phe (in plane C-H def.), Gly,Ser,Val	16, 39
1058	Lys, Glu, Ser	39
1078-1082	Glu, Thr, C-N-str	39, 40
1090	C-N-str	41
1102	Ala, C-N-str	39, 40
1128	Val,Leu,Ile,Glu,Asp,Gly,C-N-str	39, 40
1154	C-N-str	40
1160	Val,Leu (CH_3 asym rock, ip)	39
1176	Val,Leu (CH_3 asym rock, op)	39
1196	unknown	
1208	Tyr (C_6H_4-CH_2-st), Phe (C_6H_5-CH_2-str)	16
1242	amide III	39
1256	Phe,Tyr,amide III	41, 39
1268	amide III	39
1282	amide III	39
1320	CH_2 twist/wag,C_α-H-def	39
1340	CH_2 twist/wag, C_α-H-def. Val,Leu,Ile	39, 16
1374	CH_3 sym def, CH_2 scissor, Gly	39
1400	CO_2-sym st	16
1446-1456	CH_2-sc	39
1464	CH_3 asym def, CH_2 sc	39
1494	His	41
1520	unknown	
1578	amide II	41
1598	Phe	16
1608	Phe, Tyr	16
1614	Tyr, Phe	16
1672	amide I	41
2559	S-H-str	41
2875	aliphatic C-H-str	41
2934	aliphatic C-H-str	41
3064	aromatic C-H-str (Phe,Tyr)	16, 41

Used abbreviations: str: stretch, sym: symmetric, ip: in phase, op: out-of-phase, def: deformation, asym: asymmetric, sc: scissor.

of 5 tyrosine residues in gp5. These possibilities are indicated a, b and c in table 3 where the result of this analyses are presented.

Gp5-nucleotide complex:

A comparison between poly(rA) (at 15°C) and poly(rA) complexed with gp5 has been made. It is well known that poly(rA) exists in an ordered

Table 2 : Percentages of secondary conformations determined by curve fitting of the amide I and amide III'-bands in gp5.

	Amide I	Amide III'
α-helix	19.3	12.3
β-sheet	52.3	48.2
undefined	28.4	39.5

configuration with significant base-base interaction at 15°C. We will henceforth call this conformation the stacked conformation. Complex formation induces several changes in the spectrum of poly(rA) (fig. 2): a) a decrease in the intensity of the ring-vibrations at 1304, 1338, 1378 and 1482 cm^{-1}, b) an increase in the intensity of the vibration at 1420 cm^{-1}, c) the bands

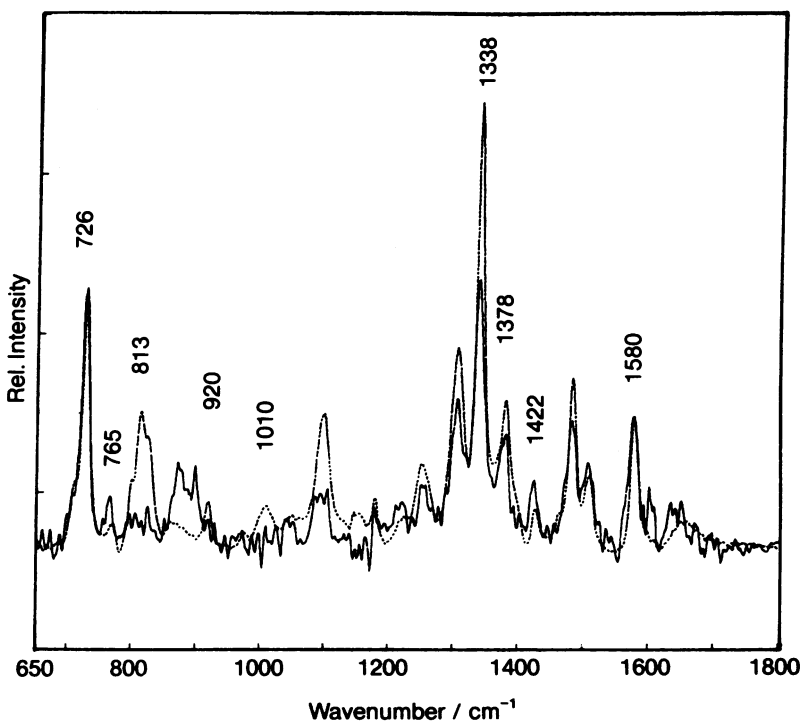

Figure 2: Comparison of the spectra of poly(rA) complexed by gp5 (solid line) and native poly(rA). The first spectrum was obtained by subtraction of the spectrum of gp5 from the spectrum of the complex. The spectrum of native poly(rA) was obtained at neutral pH and at 15°C. The solution contained 150 mM NaCl and 10 mM Na_2SO_4. The spectrum of the buffer is subtracted.

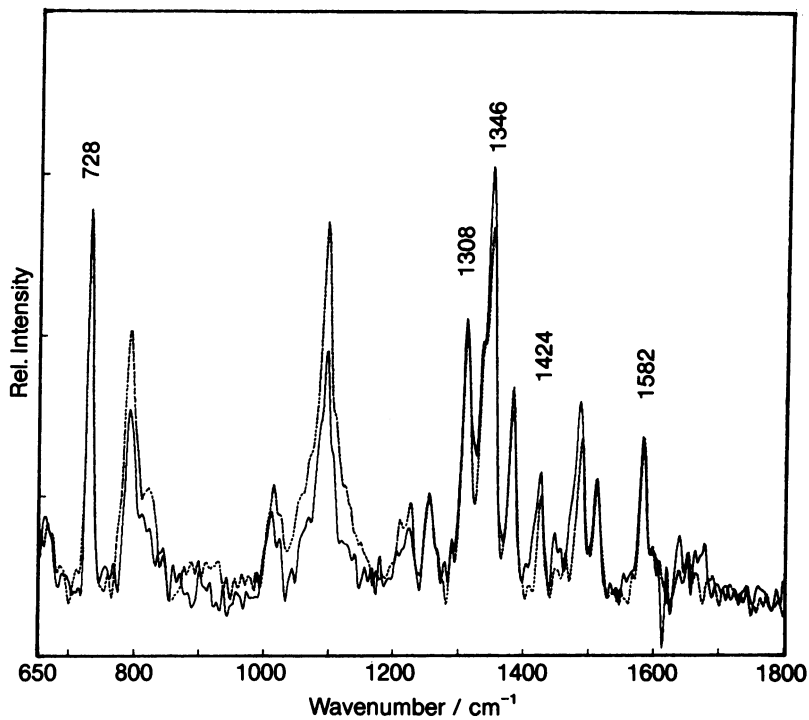

Figure 3: Comparison of the spectra of poly(dA) complexed by gp5 (solid line) and native poly(dA). The procedure to obtain these spectra was as under fig. 2 and the Materials and Methods.

due to the phosphate-sugar backbone have changed strongly. The intensity of the characteristic band for the A-structure of poly(rA) at 813 cm^{-1} is zero in the complex. The band at 1100 cm^{-1} due to the totally symmetric stretch vibration of the phosphate group has suffered a large loss in intensity upon complex formation. Several bands due to the sugar/phosphate-group have changed upon complex formation. In native poly(rA) bands are observed at 1050, 1010, 920, 840 and 765 cm^{-1}. In the spectrum of the complexed poly(rA) bands are observed at 898, 872 cm^{-1} and 760 cm^{-1}.
Two base vibrations do not change: the bands at 726 and 1508 cm^{-1} while the band at 1580 cm^{-1} was taken as an internal intensity reference (18).

A comparison between poly(dA) and poly(dA) complexed with gp5 (fig. 3) reveals changes which are different from those occurring upon complex formation of poly(rA) by gp5. Complex formation induces a small increase in the intensity of the vibrations at 1346, 1424 and 1486 cm^{-1}. The bands at

795 and 1090 cm^{-1}, due to the phosphate group in the backbone decrease in intensity. No or only slight changes can be observed in the vibrations at 728, 1250, 1308, 1380 and 1510 cm^{-1}. The band at 1582 cm^{-1} has been taken as an internal intensity reference (18).

DISCUSSION

Gene-5 protein: In table 1 the positions and assignments of the strongest bands in gp5 are presented. The band at 1004 cm^{-1}, due to the trigonal ring-breathing mode of phenylalanine was used an an internal intensity reference.

Amide-vibrations:

Several methods are known (11,12,19,20) to extract information from Raman spectra about secondary structure of proteins.

The most thoroughly investigated method makes use of the amide I-band envelope. In table 2 the results are presented of the fit of the amide I-band of gp5, using the model spectra of Berjot et al. (11). The fit shows that 52% β-sheet structure is present in the protein. More surprising in the light of previous crystallographic results (21) is the presence of a substantial amount of α-helix structure in the protein. An independent method to check this, was to perform measurements on deuterated protein. The presence of approximately 12% α-helix was confirmed by these measurements. There are, however, several aspects which make the structure determination using the amide III'-band less reliable than the one using the amide I-band. In the first place contributions of other protein vibrations are present in the region from 950-1005 cm^{-1}. In order to correct for these contributions the spectrum of the protonated protein was first subtracted. The assumption is then that this part of the spectrum is not influenced by the deuteration. In protonated protein spectra the contributions in the 950-1005 cm^{-1}-region are due to Val, Leu, Lys, C-C-stretch and Ile and it cannot be excluded that the spectra of these compounds are influenced by deuteration. The second point which makes the fit of the amide III' less reliable than that of the amide I-band is that the model spectra are represented by only 15 points on an interval of 55 cm^{-1}. The fit of the amide I-band was done with 36 datapoints on an interval of 70 cm^{-1}.

Our conclusion is therefore that, since both methods give nearly the same amount of β-sheet secondary structure, about 50% of this structure is present. Furthermore α-helix secondary structure is present. The amount is estimated to be 15 ± 10%. The observation that the protein, both free and in

	gp5 a b c	gp5/nucleotides a b c
acceptor of strong hydrogen bonds (2.5)	1 0 2	1 0 2
donator of strong hydrogen bonds (0.3)	1 0 2	1 0 2
moderate strength acceptor and/or donator (1.25)	3 5 1	3 5 1

Table 3 : Analysis of the hydrogen bonding interactions of the 5 tyrosine residues of gp5 and complexes of gp5 with nucleotides. In the first column the type of interaction and the intensity ratio I(854/834) as presented by Simawiza et al. (17) is given. The analysis results in three possible different distributions, denoted a, b and c, of the three types of hydrogen bond interactions over the 5 tyrosine residues present in gp5.

complex, contains a significant amount of α-helix was not known from previous (21) crystallographic work. The data illustrate one of the advantages Raman spectroscopy has: The aggregation state of the sample is no restriction for the collection of data on protein conformation.

The Raman spectrum showed further that no significant change of secondary protein structure was observed upon nucleotide binding. The conclusion is that the secondary structure of the protein in solution is different from that in a crystal. From the crystallographic data it was concluded that about 80% of the protein exists in the β-sheet structure.

Apparently, a part of the protein changes from, probably β-sheet structure in the crystal to α-helix structure in the solution.

Tyrosine:

Siamwiza et al. (17) have obtained a quantitative relationship between the ratio of the intensity of the tyrosine vibrations at 854 and 830 cm^{-1} and the type of hydrogen bonding at the phenolic OH-group. The intensity distribution of these bands is the result of a Fermi-resonance between a totally symmetric ring breathing mode near 830 cm^{-1} and the overtone of an out-of-plane vibration near 410 cm^{-1} (17). Siamwiza et al. could distinguish four different types of hydrogen bonding. The characterization and the intensity ratio to which they give rise are collected in table 3. The analysis of the Raman data of the 5 tyrosine residues in gp5 was as follows: In the first place one may neglect the possibility that a fully deprotonated tyrosine residue is present at pH=7.1 as the pKz of the phenolic group is

10.1. A best fit of the data is therefore obtained using only the remaining types of hydrogen bonding patterns. The fit of the intensity profile in gp5 and complexed gp5 could not discriminate between three possibilities, labeled a, b and c in table 3. In case of gp5 additional information comes from chemical modification studies (22) where it was shown that three tyrosine residues were accessible for nitration, indicating that these three residues are in contact with the bulk-solution. For tyrosine residues in contact with the solution it is expected that they participate in moderate strength hydrogen bond donating and/or accepting interactions. This makes possibility c for gp5 unlikely. It is, however, not possible to discriminate any further between the possibilities in table 3. Consequently it cannot be concluded that a change in the tyrosine residues takes place upon complex formation, since the same possibilities for the hydrogen bonding was found in free and complexed gp5.

Phenylalanine:

Our measurements show an anomalous small intensity in the phenylalanine band at 1032 cm^{-1}. Upon nucleotide binding this band does not change. McPherson et al. (21) suggested from crystallographic data that one of the phenylalanine residues is in a stacked configuration with two tyrosine residues. It may be that this configuration leads to a decrease of the intensity of the phenylalanine mode at 1032 cm^{-1}.

S-H-stretch:

One cysteine residue is present in gp5 giving rise to an easily distinguishable signal at 2559 cm^{-1} (fig. 1). Although cross-linking of this residue with a thymine residue in DNA is possible (23) no indication of an interaction of this group with adenine molecules could be obtained by Raman spectroscopy (fig. 1).

C-H-stretch:

The C-H-stretch vibrations in gp5 are not sensitive for complex formation with nucleotides. The spectra obtained for the interval 2800-3100 cm^{-1} are those measured for the deuterated protein and its complex. In case of deuterated protein there is a larger spectral separation between protein and solution contributions in this region.

Nucleotides:

In polynucleotides several types of interaction can be distinguished. In general these interactions are simultaneously present in a polynucleotide strand. It is therefore necessary to disentangle the influence that each interaction may have on the intensity and position of the Raman vibrations.

Table 4 : Assignment of the Raman bands of adenine.†

Poly(rA) [cm^{-1}]	Poly(dA) [cm^{-1}]	Assignment
726	728	$-C_4N_3^s-C_6N_{12}^s-N_9R^s-C_4N_9^s$
1304	1308	$N_9C_8^s+N_3C_2^s+C_8H^b-C_2H^b$
1338	1346	$-N_7C_5^s+C_8N_7^s$
1378	1380	$C_8N_9^s+C_2N_3^s$
1422	1424	$-N_1C_6^s+C_6N_{12}^s$
1482	1486	$C_2H^b-N_1C_2^s-+N_3C_2^s$
1508	1510	ν_s: corresponding with e_{1u} of Benzene (25)
1580	1582	$C_5C_4^s-C_4N_3^s$

† Used abbreviations: s: stretch, b: bend.
Assignments according to ref. 24, 25 and 26.

Our approach to this problem has been to compare the Raman spectra of poly(rA) and poly(dA) both at high and low temperature. As a result of the temperature increase the interactions change which is revealed by changes in certain bands in the Raman spectrum. We have furthermore considered data from the literature on adenine-containing polynucleotides and have been able to assign certain bands of adenine as marker bands for stacking interactions and other bands of adenine as marker bands for the phosphate-sugar-base conformer. We will explain these conclusions shortly.

The assignment of the normal modes of adenine according to Hirakawa et al. (24), Tsuboi et al. (25) and Majoube (26) are summarized in table 4 for those bands which are important in this discussion.

Earlier publications (27,28,29,9) have revealed the importance of: stacking (27) (i.e. interactions between successive bases), hydrogen bonding of the bases (28,29) and the interaction of the base residues with the phosphate-sugar group in the phosphate-sugar-base conformer (9).

Stacking:

According to the theory of the Raman hyperchromic effect (27) an increase in the intensity of the ring vibrations of the adenine base is expected upon destacking. It was suggested that this increase is proportional to the extinction coefficient of the nearest electronic transition. The absorption hyperchromism of this transition at ≈ 260 nm, observed upon melting of the poly(rA), correlated with the Raman hyperchromism observed for the bands at 726, 1304, 1378, 1422 and 1508 cm^{-1} (27) in Raman spectroscopy. Because the Raman hyperchromism is most pronounced for the bands at 1304 and 1508 cm^{-1} these bands were considered as marker bands for stacking interactions in poly(rA). The 1510 cm^{-1}-band in poly(dA) is the band which increases most in intensity when destacking occurs it is therefore

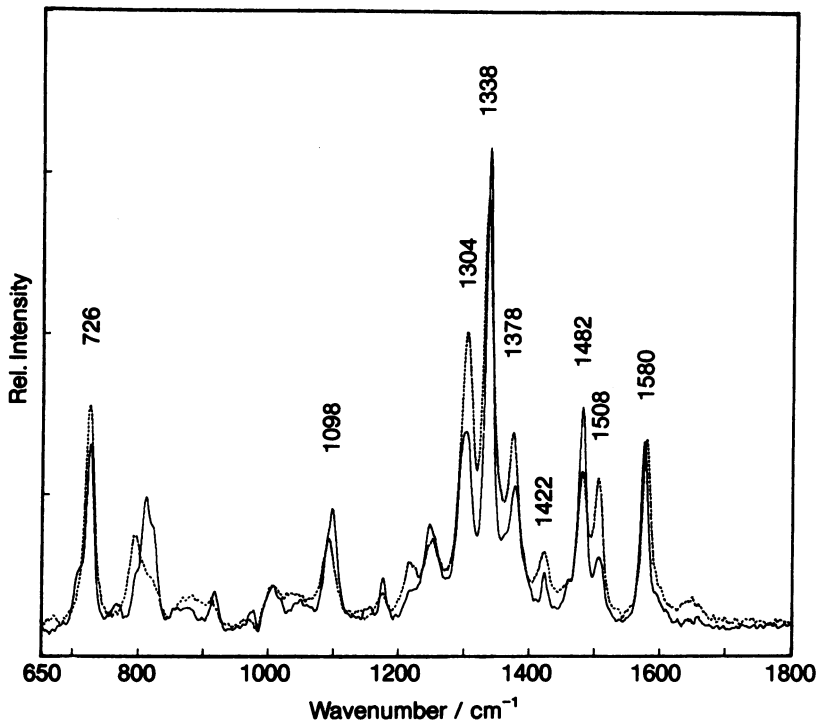

Figure 4: Comparison of the spectra of poly(rA) at 15°C (solid line) and at 85°C (dotted line). At these temperatures poly(rA) is in respectively a stacked and destacked conformation. Conditions as under fig. 2.

considered to be a marker band for stacking interactions in poly(dA). Resonant Raman spectroscopy (30,31) showed that also the vibrations at 1338 and 1482 cm^{-1} were resonantly enhanced when excited at 260 nm. It was concluded that the 1338 cm^{-1}-band obtains its intensity (at least partly) from vibronic transitions in the 260 nm region. Studying the Raman hyperchromic effect, using visible light revealed a hypochromic effect of this band (fig. 4) upon melting of poly(rA). This indicates that other interactions are also important for the intensity of this vibration.

Phosphate-sugar-base conformer:

Poly(rA) and poly(dA) have different conformations in solution. A comparison of the Raman spectra of both compounds at 15°C (stacked configuration) and 85°C (destacked configuration) are given in fig. 4 and 5 suggests that it is the structure of the phosphate-sugar-base conformer which is important for the intensity of the 1338 cm^{-1} band (in poly(rA))

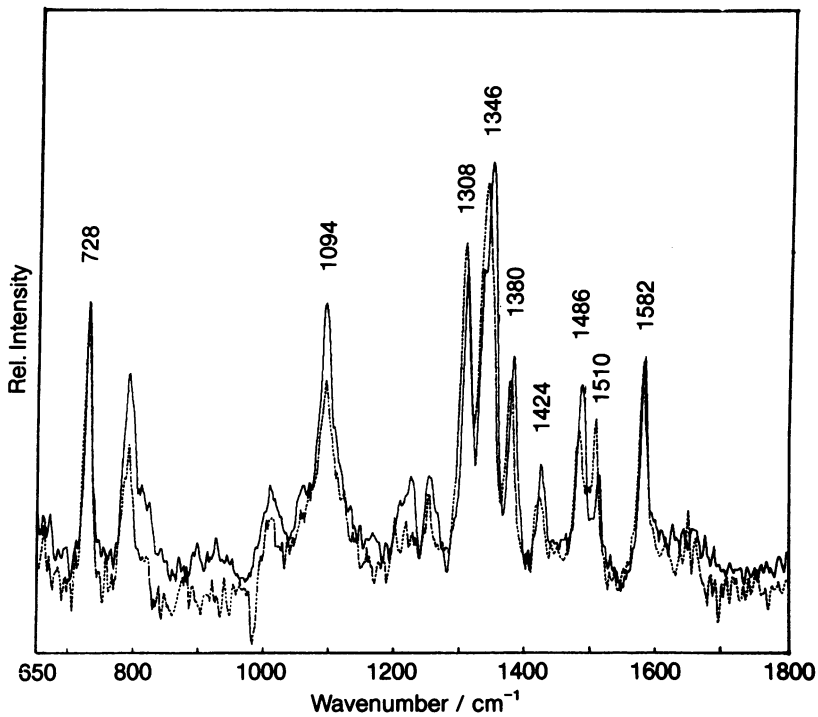

Figure 5: Comparison of the spectra of poly(dA) at 15°C (solid line) and at 85°C (dotted line). See under fig. 2 and 4 for the conditions.

and the 1346 cm^{-1} band (in poly(dA)). This is also concluded for the band at 1482 cm^{-1} in poly(rA) and at 1486 cm^{-1} in poly(dA).

Hydrogen bonding:

Hydrogen bonding interactions also have a definite influence on the intensity of the band at 1338 cm^{-1} in poly(rA) as can be concluded from a comparison of the Raman spectra of poly(rA), poly(rA).poly(rU) (29) and poly(rA-rU).poly(rA-rU). Watson and Crick type hydrogen bonding apparently gives rise to a decrease in the intensity of the vibration at 1338 cm^{-1}(28). As formation of hydrogen bonds is one of the modes of interaction which may be expected to occur between proteins and nucleotides this interaction may very well also contribute to the decrease in intensity of the vibration at 1338 cm^{-1} found upon complex formation of poly(rA) with gp5. It is assumed here that hydrogen-bonding does not play an important role in these complexes.

The influence of complex formation of gp5 and poly(rA) upon the marker

bands for stacking interaction at 1304 and 1508 cm^{-1} is different (fig. 2). The intensity of the band at 1508 cm^{-1} does not change upon complex formation. The intensity of the band at 1304 cm^{-1} decreases which would point to increased stacking interactions. An intensity decrease of the 1304 cm^{-1}-band in poly(rA) was also observed when it was complexed by gp32 (9). We explain these results by suggesting that in case of gp5 complex formation of poly(rA) stacking interactions between the aromatic amino acid(s) of the protein and the bases of the polynucleotide occurs, similar to what was found in poly(rA) upon complex formation with gp32. This point will be discussed below in relation to a comparison between gp5 and gp32 in their interaction with polynucleotides.

The 1510 cm^{-1}-band in poly(dA) does not change when poly(dA) is complexed by gp5. This is identical to what was found with poly(rA). The 1508 cm^{-1} behaves, both in poly(rA) and poly(dA), quite differently upon temperature increase and on gp5-binding. We conclude from these observations that the conformational change brought about by interaction of the melting protein gp5 with poly(rA) and poly(dA) is not equal to that ocurring upon an increase in temperature.

Both marker bands for the phosphate-sugar-base conformer of poly(rA) (1338 cm^{-1}, 1482 cm^{-1}) in the complex with gp5 have decreased in intensity. The very large decrease of the 1338 cm^{-1} with approximately 38% indicates a large change of the phosphate-sugar-base conformer. The phosphate-sugar-base conformer in uncomplexed poly(rA) can be characterized by a C_3'-endo sugar puckering and an "anti"-conformation. In poly(dA) both marker bands for the phosphate-sugar-base conformer have increased in intensity (fig. 3) indicating that also in this case a change in the conformer has taken place. In uncomplexed poly(dA) the sugar has a C_2'-endo puckering and the sugar-base orientation is "anti". Comparing the intensity of the 1338 cm^{-1} (1346 cm^{-1})-band in complexed- and uncomplexed poly(rA) and poly(dA) shows that the structure of the phosphate-sugar conformer is much more alike in the two complexed polynucleotides than in the native polynucleotides. This leads to the interesting conclusion that the binding site of the protein cannot accomodate a variety of nucleotides with different secondary structures. It seems to be the case that gp5 forces polynucleotides into a common structure. Again there is a large correspondence with the influence gp32 has on these polynucleotides. Also in that case (9) the spectra of the complexed polynucleotides were much more alike than the spectra of the native nucleotides. The remaining differences between the spectra of the

complexed poly(rA) and poly(dA) near 1340 cm^{-1} may then be due to the chemical difference between the sugar-residues.

Changes in other base vibrations:

Complexation of poly(rA) with gp5 leads to a decrease in intensity of the band at 1378 cm^{-1}. This band is assigned to the ($C_8N_9^s+C_2N_3^s$)-vibration. It may be influenced by the phosphate-sugar-base configuration and changes herein as the sugar-group is attached to the 9-position of the adenine-ring. The intensity of the band at 1422 cm^{-1} increases upon complex formation of poly(rA). This band is also increased in intensity in gp5-complexed poly(dA) (fig. 3). It is assigned (table 4) to the ($-N_1C_6^s+C_6N_{12}^s$)-vibration. Changes in the intensity may therefore be due to an interaction of the external aminogroup on the 6-position of the ring-system with residues of the protein.

Changes in the sugar-phosphate vibrations:

The vibration near 1100 cm^{-1} is attributed to the totally symmetric vibration of the PO_2^--group. In the spectra of gp5-complexed poly(rA) and poly(dA) a large decrease is observed in the intensity of this vibration. It is known that ionic interactions of positively charged ions with the negatively charged phosphate group cause a large decrease in the intensity of the PO_2^-- vibration (32). It is likely that also positively charged groups of the gp5 protein (like lysine- and arginine-residues) which interact with the PO_2^--group, can cause an intensity decrease in the PO_2^--vibration. It is significant to realize, in this respect, that the main contribution to the binding constant between gp5 and nucleotides is of an ionic nature (2). Furthermore evidence was obtained from crystallographic experiments (21) and NMR-spectroscopy (33) that, two lysyl and three arginyl residues are involved in the protein/nucleotide interaction. Our observation of an intensity decrease of the PO_2^--group vibration gives direct spectroscopic evidence of this ionic interaction.

The band at 813 cm^{-1} in the Raman spectrum of poly(rA) is due to the phosphodiester stretch vibration. This band position is characteristic for a C_3'-endo puckered furanose ring, in combination with an "anti"-orientation of the base and base-base stacking interactions in poly(rA). For this band it was concluded that the intensity and the position are very sensitive for the conformation, in particular the bond angles, of the C-O-P-O-C-sequence in the backbone of single stranded nucleotides like poly(rA) (34). This sensitivity is for instance displayed when the temperature of a solution of poly(rA) is increased from 15°C to 85°C. The intensity of the

phosphodiester-vibration at 813 cm^{-1} decreases to zero while a new band with a lower intensity arises at 795 cm^{-1} (34). Complex formation of poly(rA) by gp5 decreases the intensity of the band at 813 cm^{-1} also to zero. Apparently, when complexed the C-O-P-O-C sequence has changed drastically. This may for instance arise from a stretching of the backbone (5).

Several other changes in sugar-phosphate vibrations were observed. Most of the bands involved belong to C-O-stretch or C-C-stretch vibrations of the sugar-ring (35). The influence of phosphate groups on the 5'- and 3'-position of the sugar can significantly, change the position and intensity of these bands (35,36,37). It is therefore quite likely that at least some of these vibrations are sensitive to the conformation of the phosphate-sugar-backbone. There is hardly any literature on the behaviour of these bands in protein/nucleotide complexes because of the weakness of these bands. Also from our spectra (fig. 2 and 3) no quantitative conclusions can be drawn because of the low signal/noise ratio. Some qualitative remarks can be made however. The band at 1050 cm^{-1} (C-O-stretch) in native poly-rA does not shift or change in intensity upon complex formation. The relatively strong bands at 1010 cm^{-1} (C-O-stretch) and 920 cm^{-1} (sugar-phosphate) are absent in the complexed spectrum. The band around 870 cm^{-1} has been observed in both 3'- and 5' deoxyribose-base molecules, where it was assigned to the sugar/phosphate group (36), and in ribose. A tentative assignment of this band in case of gp5-complexed poly(rA) is therefore to ascribe it to the ribose/phosphate group in this complex. The band at 765 cm^{-1} in native poly(rA), for which no assignment is available, shifts to lower wavenumber (760 cm^{-1}) and increases in intensity.

Our main conclusion from all these observations is that the sugar/phosphate structure in poly(rA) (i.e. C_3'-endo pucker) is drastically changed by the gp5-complexation. It is difficult to determine the new sugar/phosphate conformation from the Raman data. A comparison of the gp5-induced changes with those induced by a temperature increase can be made however. The band at 1010 cm^{-1} does not change at all. The band at 765 cm^{-1} has virtually disappeared. Protein-binding and temperature increase have comparable effects on the band at 920 cm^{-1} which shifts to a lower wavenumber. Furthermore an increase in intensity takes place at 870 cm^{-1}. Again we conclude that the influence of the binding of the "melting" protein gp5 is quite different from the "melting" of poly(rA) upon an increase of temperature.

Comparison between gp5 and gp32 complexation:

It is possible that the interaction of different helix destabilizing proteins with poly(rA) and poly(dA) show common features. We will therefore compare the effects which complex formation with gp5 and gp32 (a phage T4 h.d.p.) have on these polynucleotides.

The $(-N_7C_5^s + C_8N_7^s)$-vibration:

This vibration yields for poly(rA) a band at 1338 cm^{-1} which is a marker band for the conformation of the phosphate-sugar-adenine group (9). Its intensity decreases both by complex formation with gp5 and gp32. The intensity of the corresponding vibration in poly(dA) at 1346 cm^{-1} increases upon complex formation with gp5 and gp32.

From the similarity of the changes induced in the spectra of poly(rA) and poly(dA) in complex with gp5 and gp32 we conclude that the main features of the interaction of these proteins with poly(rA) and poly(dA) are the same.

The $(N_9C_8^s + N_3C_2^s + C_8H^b - C_2H^b)$-vibration:

This vibration leads to a band at 1304 cm^{-1} which marks stacking interactions in poly(rA). From its intensity decrease upon complex formation of poly(rA) with gp5 respectively gp32 we conclude that the stacking interactions are stronger in these complexes. In our opinion this must be an indication of the stacking interactions of aromatic aminoacids with the adenine-bases.

The totally symmetric phophodiester stretch vibration:

The position of this band in the spectrum of poly(rA) complexed by gp32 (9) has shifted to about 795 cm^{-1} which resembles the effect which a thermally induced destacking has on the spectrum of poly(rA). The absence of the band at 813 cm^{-1} both in complexes with gp5 and gp32 learns that the conformation of complexed poly(rA) is different from that of poly(rA) at low temperatures.

Our analysis of the gp5 complexed poly(rA) spectrum indicates however, that the conformation of complexed poly(rA) is also not equal to that of the high temperature conformation of poly(rA). From these similarities we conclude that both gp5 and gp32 change the structure of poly(rA) in a similar way. In case of the gp32 complexed poly(rA) we cannot rule out the possibility that the conformation of the phosphate/sugar backbone is like that of poly(rA) at high temperature. There is also an indication that small differences exist in the phosphate/sugar group. Also we have to keep in account the possibility that in case of similar conformations, specific

protein/sugar or protein/phosphate interactions may influence the vibrational spectra.

CONCLUSIONS

1) Using the 1338 and 1482 cm^{-1} bands in poly(rA) and the corresponding bands in poly(dA) at 1346 and 1486 cm^{-1} as marker bands for the phosphate-sugar-base conformation it is concluded that both poly(rA) and poly(dA) undergo a change in conformation upon gp5-binding. The difference in intensity of the band at 1338 cm^{-1} (1346 cm^{-1}) is much smaller for the complexed polynucleotides than in the free polynucleotides. This suggests that the conformations of poly(rA) and poly(dA) are more alike in the complexed form.
2) The stacking marker band at 1304 cm^{-1} in poly(rA) indicates an increase in stacking interaction upon gp5-binding. The stacking marker band at 1508 cm^{-1} in poly(rA) and the corresponding one in poly(dA) does not change in intensity. This indicates that the destacking of adenine by gp5 is compensated for by new stacking interactions with the aromatic amino acids.
3) The large decrease in intensity of the PO_2^--vibration in poly(rA) at 1098 cm^{-1} and in poly(dA) at 1092 cm^{-1} upon gp5-binding is due to ionic interactions of basic amino acid residues with the PO_2^--groups in the backbone.
4) From the spectrum of complexed poly(rA) it is observed that the structure of the phosphate-sugar group has changed. The changes in the spectra are partly identical and partly different from changes occurring in the same bands upon thermal destacking of poly(rA).
5) The influence of complexation of gp5 and gp32 on the marker band of the phosphate-sugar-base conformer at 1338 cm^{-1} in poly(rA) and 1346 cm^{-1} in poly(dA) is similar. The interaction of these helix destabilizing proteins with poly(rA) is also comparable with respect to the changes in a marker band for stacking at 1304 cm^{-1}. The stacking interactions of the bases is larger in the complex than in the free polynucleotide.
6) To enable more accurate assignments and a more detailed interpretation of the Raman spectra accurate model compound studies together with reliable normal mode calculations are necessary.

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