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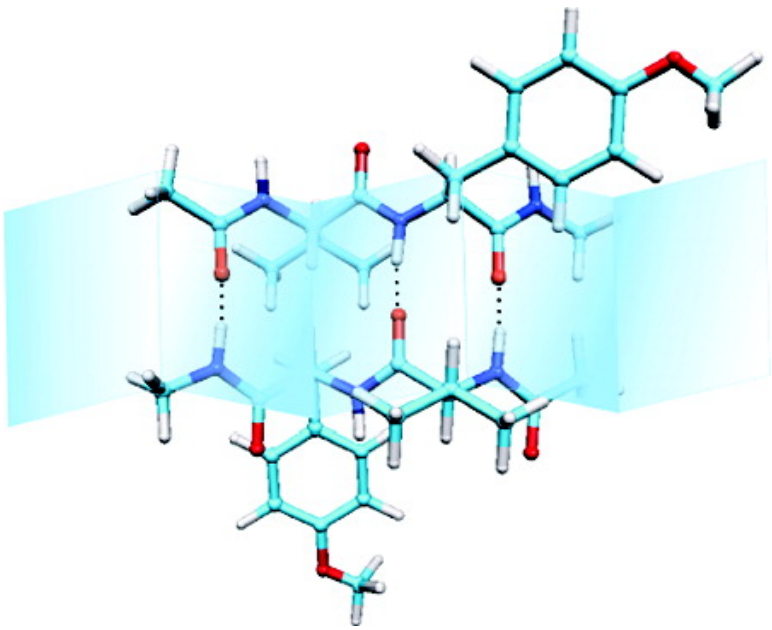

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Investigation of Secondary Structure Elements by IR/UV Double Resonance Spectroscopy: Analysis of an Isolated β-Sheet Model System

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Abstract: An isolated β-sheet model system is investigated in a molecular beam experiment by means of mass- and isomer-selective IR/R2PI double resonance spectroscopy as well as ab initio and DFT calculations. As the exclusive intermolecular assembly, a β-sheet motif is formed by spontaneous dimerization of two isolated peptide molecules. This secondary structure is produced from the tripeptide model Ac-Val-Tyr(Me)-NHMe without any further environment to form the binding motif which is analyzed by *both* the characteristic amide A and I vibrations. The experimental and theoretical investigations yield the assignment to an antiparallel β-sheet model. The result of this detailed spectroscopic analysis on an isolated β-sheet model indicates that there are intrinsic properties of a β-sheet structure which can be formed without a solvent or a peptidic environment.

I. Introduction

Secondary structure elements are fundamental features in protein structures. They largely determine the path of protein folding, impart mechanical stability, create oriented functional group arrays, and dictate interactions with external binding partners. Proteins can be classified according to their relative content of α-helices and β-sheets, and often their biochemical function turns out to be closely related to it. The regular arrangement of two peptide strands in an extended conformation, producing an alternating hydrogen bond donor−acceptor pattern, leads to the arrangement of a β-pleated sheet, which can be parallel or antiparallel with respect to their *N*- and *C*-termini. This arrangement is important for most proteins because it creates rigid, regular areas with all amino acid residues pointing roughly into the same direction. The investigation of factors governing the stability of β-sheet structures is hampered by the fact that solvent effects and the protein environment play a major role and can hardly be separated from the β-sheet's intrinsic stability. As there are many aspects that have to be taken into account, an analysis should be made step by step beginning with small isolated species in order to examine first the intrinsic properties. Therefore, investigations of molecules in supersonic beam expansions should be a very good starting point for further experiments. This has been done first by Levy and co-workers who investigated the jet cooled aromatic amino acids phenylalanine, tryptophan, and tyrosine and some tripeptides by means of resonant multiphoton ionization and fluorescence spectroscopy.[1−4] An important improvement has been made by the application of infrared resonant two photon spectroscopy (IR/R2PI)[5−8] on (unprotected) amino acids[9−11] because direct information is obtained for the structure of the electronic ground state S_0 via analysis of the vibrational pattern which is sensitive for different structural motifs. The IR/R2PI method is isomer and mass selective, that is, for each isomer of an investigated species individual IR spectra can be recorded. With modern nanosecond laser systems (cf. e.g. 12−14), it is possible to cover in the IR analysis the complete region from the fingerprint to the C═O, CH, NH, and OH stretching vibrations (400−4000 cm^{-1}), that is, all important peptide vibrations in the amide I, II, and amide A region can be investigated.

Many IR/UV double resonance investigations have been carried out on the conformers of isolated neutral amino acids and peptides (cf. e.g. refs 15−34), but as far as secondary

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structure binding motifs are addressed, mainly γ-turns,[16,23−25,28−29] β-turns,[21,23−24,30−31] or helical structures[31−33] are discussed. One of the most prominent secondary structures is the β-sheet that can be obtained by the formation of at least a dimer of two peptide strands. It is a very general question if the β-sheet binding motif is formed by itself when two peptide molecules aggregate or if the formation requires a well-defined environment.

To characterize the (secondary) structure of peptides, a set of torsional angles is used.[35] The orientation of the backbone is described by the torsional angles ϕ ($C'-N^i-C_\alpha-C'^{+1}$) and ψ ($N^i-C_\alpha-C'^{+1}-N^{i+1}$). Depending on the values of ϕ and ψ, nine different conformations can be obtained for each residue in the so-called Ramachandran-plot. The amide group NH−C═O itself, defined by the angle ω, can have a syn (0°) or anti (180°) configuration. Calculations show that the latter is more stable. Finally the most important angle χ_1 to describe the side-chain has three positions with distinct minima of the potential energy: gauche-plus (g+, 60°), anti (a, 180°), and gauche-minus (g-, −60°). In β-sheets, the strands adopt an extended geometry ($\phi \approx -(110-150°)$, $\psi \approx 110-150°$) and are connected via intermolecular hydrogen bonds between N−H and C═O groups. In a parallel β-sheet, the same termini (C−C and N−N) are facing each other, whereas in an antiparallel β-sheet, one monomer is turned by 180°. As a consequence of the different binding schemes, antiparallel β-sheets form via (intermolecular) hydrogen bonds alternately 10 and 14 membered rings, whereas parallel β-sheets contain only 12 membered rings (cf. Figure 1).

The smallest model system for a β-sheet is a dimer of two N/C-protected amino acids. In order to mimic a small part of a large protein where the distant polar end groups have less influence on the overall structure the amino acids are protected at both termini. In preliminary experiments, we have investigated the model systems $(Ac\text{-}Phe\text{-}OMe)_2$ and $(Ac\text{-}Phe\text{-}NHMe)_2$ in molecular beam experiments by application of R2PI- and IR/R2PI-spectroscopy.[15,28] In combination with ab initio and DFT calculations, the structures have been assigned. Both molecules form β-sheet like dimers with two intermolecular hydrogen bonds. The dimer of Ac-Phe-OMe has a 10 membered ring, whereas the dimer of Ac-Phe-NHMe has a 14 membered ring. Due to the aforementioned binding schemes, both are examples for antiparallel β-sheets.

However, a more realistic unit cell of an antiparallel β-sheet contains both binding schemes, a 10 *and* 14 membered ring. A real polypeptide can then be obtained by just adding these unit cells. In order to study such a unit cell, one has to investigate at least the dimer of a tripeptide model. An ideal solution is a dipeptide carrying an N-acetyl group and a C-terminal N-methylamide; both protecting groups are genuine peptidic extensions and *in toto* represent a third truncated amino acid (glycine). Each monomer has three amide groups that can interact in many ways to form different aggregates. As a consequence, the IR spectroscopy can give a very good hint on the number and strengths of hydrogen bonds that are red-shifted and have usually broadened absorption bands with respect to the non-hydrogen-bonded amide groups.

In order to find out binding motifs for a β-sheet unit cell, we present in this paper IR/R2PI investigations on the dimer of the tripeptide model Ac-Val-Tyr(Me)-NHMe both in the spectral region of the amide A and amide I transitions. It is a great advantage of gas-phase spectroscopy on isolated peptides that structural information cannot only be taken from amide I (and II) regions but also from the very structure sensitive amide A region leading to a detailed description of backbone structures. The monomer has already been investigated by IR/R2PI spectroscopy in our group.[22] The aromatic residue tyrosine is methylated at the OH position of the phenolic side chain to protect this polar group. This strategy should avoid a binding scheme like the one in the dimer of Ac-Trp-OMe[34] via the polar side chains. Thus the dimer of Ac-Val-Tyr(Me)-NHMe can represent a model for a β-sheet binding motif.

II. Experimental Section

The experimental setup has been described elsewhere (cf. ref 15). Thus, only a short description is given: The R2PI and IR/R2PI spectra were measured in a vacuum system consisting of a differentially pumped linear time-of-flight mass spectrometer and a pulsed valve (General Valve Iota One, 500 μm orifice) for skimmed jet expansion (X/D=130). A frequency-doubled dye laser (Lumonics HD 300), pumped by a Nd:YAG laser (Lumonics HY 400), was used for excitation to the S_1 state and for ionization. The IR light in the region of 2.84−3.08 μm (3250−3520 cm^{-1}) was generated with a $LiNbO_3$ crystal by difference frequency mixing of the fundamental (1064 nm) of a seeded Nd:YAG laser (Spectra-Physics PRO-230) and the output of a dye laser (Sirah, Precision Scan) pumped by the second harmonic (532 nm) of the same Nd:YAG laser. The IR output is amplified by an optical parametric

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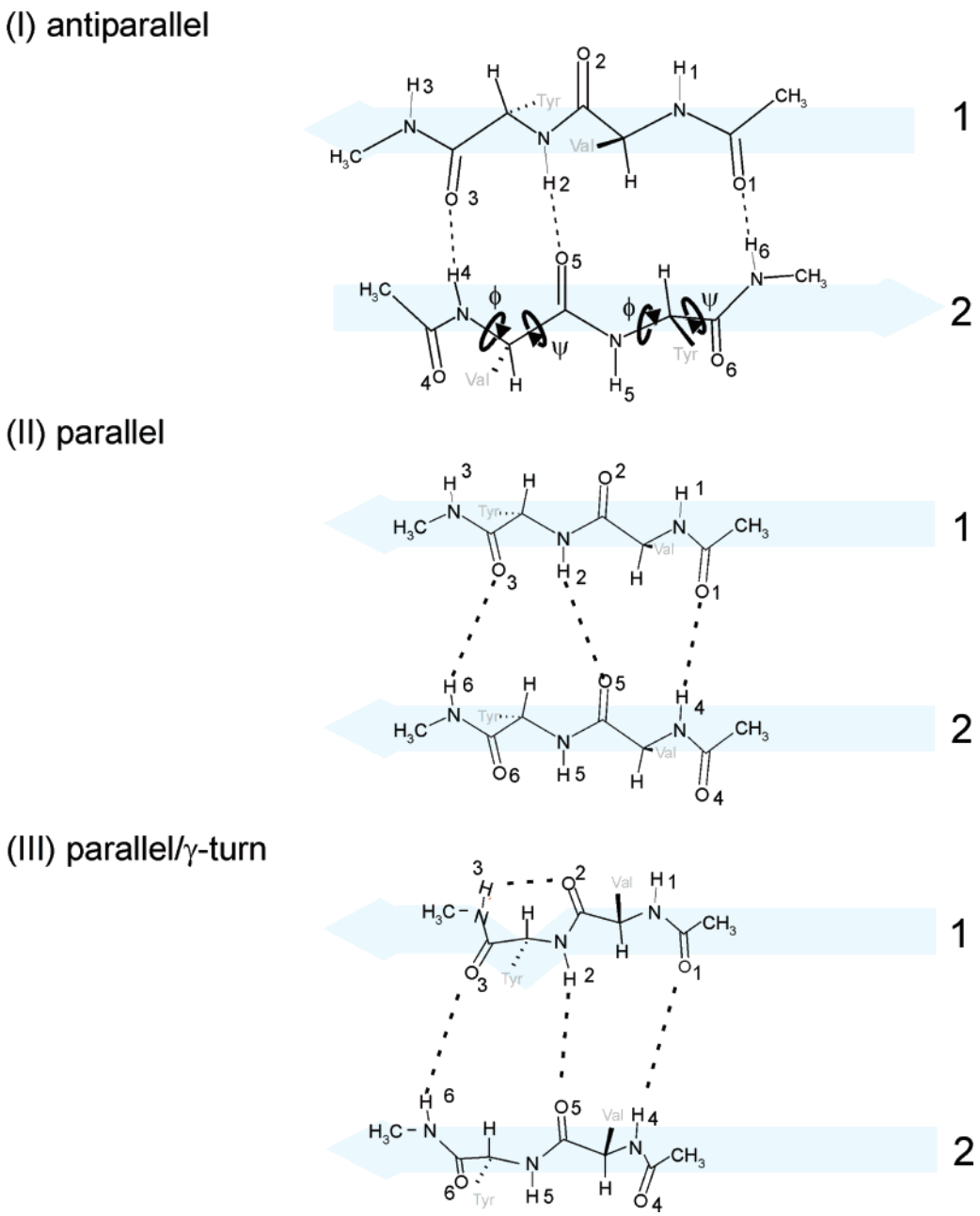

Figure 1. Schematic secondary structure motifs for different β-sheet arrangements of the Ac-Val-Tyr(Me)-NHMe dimer. The dihedral angles ϕ and ψ, describing the backbone of an amino acid within a peptide, are depicted in the upper trace (I). The two monomers are indicated by 1 and 2. By considering the intermolecular hydrogen-bonds (dotted lines) 10 and 14 membered "rings" can be described for structure (I) and 12 membered "rings" for structures (II) and (III), see text. The arrows show the direction of the backbone form the N-terminus to the C-terminus.

amplification (in a $LiNbO_3$ crystal) of the output of the IR laser (2.84−3.08 μm) and the fundamental of the Nd:YAG laser.[15a)]

IR light in the region of 6 μm is generated by a third nonlinear process.[12,15b] Signal (1.79−1.82 μm, 5500−5600 cm^{-1}) and idler (2.56−2.63 μm, 3800−3900 cm^{-1}) of the OPA process are the input of a difference frequency mixing process in an $AgGaSe_2$ crystal leading to the IR light of 1600−1800 cm^{-1}.

Since the time delay chosen for the two lasers is not longer than 100 ns, all lasers have been spatially overlapped. In order to obtain IR/R2PI spectra, the IR laser is fired 60 ns prior to the UV laser. At least 20 IR/R2PI spectra are averaged and the depletion resulting from a vibrational transition must be observed in every single IR/R2PI spectrum. Furthermore in each single IR spectrum each data point is averaged by 40 laser shots. Substance (Ac−Val−Tyr(Me)−NHMe) and valve are heated to 180 °C. Helium was used as carrier gas (2000 mbar). The synthesis of Ac-Val-Tyr(Me)-NHMe is described elsewhere.[22]

III. Results and Discussion

(a) Structure of (Ac-Val-Tyr(Me)-NHMe)$_2$: Theoretical Predictions. In order to support the vibrational assignments force field, ab initio (Hartree−Fock, HF) and DFT (B3LYP functional) calculations have been performed using the Discover,[36] Gaussian 03,[37] and Turbomole[38] programs. To generate starting geometries for ab initio calculations, a preselection via molecular dynamics using the CFF force field[36,39] has been done

(36) *CFF Consistent Force Field and DISCOVER Molecular Simulation Program,* Version 2000; Accelrys Inc.: San Diego, CA, 2000.
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by using the same procedure as the one described in our publications on the dimer of Ac-Phe-NHMe[28] and Ac-Val-Tyr-(Me)-NHMe[22] monomer. Briefly, molecular dynamics have been performed at a temperature of 750 K and with a time step of 1 fs. After 1000 steps, the structure has been minimized. This structure has been used to continue the dynamics in the same manner. The following minimized structure has been compared to the first via its relative energy to discriminate between different minima. With this technique, 30 000 structures have been minimized and compared to each other. The dynamics have been repeated several times to explore the potential energy surface (PES).

The preselection using the CFF force field shows that β-sheet structures turn out to be by far the most stable conformers. The possibility to form three intermolecular hydrogen-bonds without any tension yields a preference for either an antiparallel or a parallel β-sheet model as the one described in Figure 1a,b. Nearly all structures up to 2000 cm^{-1} (23.9 kJ) relative energy are either antiparallel (**I**) or parallel (**II**) β-sheet models, they differ only with respect to different side-chain conformations of the amino acids. The remaining few structures within the first 2000 cm^{-1} of relative stability form parallel structures including an intramolecular hydrogen-bond (γ-turn) (**III**) as depicted in Figure 1c. Thus, already the simple force field calculations indicate that the chosen model system, the Ac-Val-Tyr(Me)-NHMe dimer seems to be an ideal candidate to investigate a β-sheet model that can be treated as unit cell of a large β-sheet. The most stable β-sheet structures obtained from the force field calculations are used as basis for further ab initio and DFT calculations. In a series of earlier publications,[15,17,28] we have shown that already simple HF calculations using the small 3-21G(d) basis set are appropriate to predict vibrational frequencies of protected amino acids, peptides and aggregates. Of course, these frequencies are scaled but the chosen factors for the amide A and amide I vibrations are the same for all investigated species. Thus, HF/3-21G(d) calculations have also been performed for the dimer of Ac-Val-Tyr(Me)-NHMe, which makes it possible to calculate the frequencies of a large number of structures predicted from the force field calculations. In order to achieve a more realistic description of the relative stabilization energies and to verify the frequency calculations performed at the HF level, DFT calculations using the B3LYP functional and the cc-pVDZ basis set have been performed, too. Two structures with binding motif (**III**) (parallel/γ-turn) and (**I**) (antiparallel) turn out to be the most stable arrangements which are close in energy by about 220 cm^{-1}, whereas the most stable parallel arrangement (**II**) is less stable by 1130 cm^{-1} (including zero point energy and BSSE[40] corrections). The stabilization energies of structures (**I**), (**II**), and (**III**) with respect to two isolated monomers are 47, 36, and 49 kJ/mol, respectively. These structures consisting of monomers (1) and (2) (cf. Figure 1) have the following side-chain orientations:

(**I**): Val(a)Tyr(a)/Val(a)Tyr(g+)

(**II**): Val(g−)Tyr(a)/Val(a)Tyr(g+)

and

(**III**): Val(a)Tyr(g−)/Val(g−)Tyr(a)

Table 1. Inter- and Intramolecular Distances of Hydrogen Bonds Calculated at the B3LYP/cc-pVDZ Level of Theory

(I) antiparallel		(II) parallel	(III) parallel/γ-turn
Intermolecular distances [pm]			
Val−NH^4···O^3C−Tyr	198	Val−NH^4···O^1C−Acetyl	200	203
Tyr−NH^2···O^5C−Val	196	Tyr-NH^2···O^5C−Val	204	176
NMe−NH^6···O^1C−Acetyl	191	NMe−NH^6···O^3C−Tyr	195	197
Intramolecular distances				
NMe−NH^3−O^2C−Val				222

Table 2. Experimental and Calculated Vibrations at the B3LYP/cc-pVDZ Level of Theory

	exp.	(I)antiparallel	(II)parallel	(III)parallel/γ-turn
NMe−NH^3	**3488**	**3486(32)**	3480(56)	3376(89)
Val−NH^1	**3455**	**3452(80)**	3451(68)	3469(27)
Tyr−NH^2	**3279**	**3293(444)**	3348(289)	3276(563)
NMe−NH^6	**3357**	**3341(511)**	3390(464)	3355(373)
Val−NH^4	**3376**	**3387(444)**	3374(389)	3369(374)
Tyr−NH^5	**3442**	**3445(64)**	3465(33)	3462(51)
Acetyl−CO^1		1672(63)	1663(77)	1692(713)
Val−CO^2	**1656**	**1645(630)**	1660(1224)	1675(89)
Tyr−CO^3	**1692**	**1692(492)**	1685(492)	1687(316)
Acetyl−CO^4		**1713(176)**	1708(156)	1705(89)
Val−CO^5	**1669**	**1658(657)**	1674(59)	1660(552)
Tyr−CO^6		1698(96)	1701(67)	1713(132)

[a] Intensities in km/mol are given in brackets. The frequencies have been scaled by factors 0.9648 (NH) and 0.9716 (C═O). Atoms are numbered with respect to Figure 1.

The frequencies calculated at the HF/3-21G(d) level are scaled with the factors *f*(amideA) = 0.9076, *f*(amide I)= 0.914 obtained from the investigation on Ac-Phe-OMe,[15] Ac-Phe-NHMe,[28] and Ac-Val-Phe-OMe.[17] For the DFT calculations, the factors *f*(amideA) = 0.9648, *f*(amide I)= 0.9716 are chosen. These values result from the comparison of the experimentally observed frequencies[15] with the frequencies calculated for the CO and NH stretching frequencies of Ac-Phe-OMe at the B3LYP/cc-pVdZ level of theory.

In order to get a description of (intermolecular) hydrogen bonds, the corresponding bond-lengths are of interest. The bond lengths obtained at the B3LYP/cc-pVDZ level for the structures (**I**), (**II**), and (**III**) are listed in Table 1.[41] It is interesting to note that in the case of the antiparallel arrangement (**I**) all intermolecular bond-lengths (N−H···O) are close together and below 200 pm, whereas in the parallel arrangement the Tyr-N−H^2···O bond length is higher than the "outer" hydrogen-bonds and in the parallel/γ-turn geometry (**III**) the "inner" Tyr-N−H^2···O bond-length decreases strongly to 176 pm. These structural differences lead to a characteristic vibrational pattern for each β-sheet type. Thus, an unambiguous structural assignment with respect to the type of secondary structure binding motif is possible if both amide A and amide I vibrations are taken into account (see Table 2, next section).

(b) Structure of (Ac-Val-Tyr(Me)-NHMe)$_2$: Spectroscopic Results. The R2PI spectrum of (*Ac-Val-Tyr(Me)-NHMe)$_2$* recorded between 35 300 and 35 800 cm^{-1} exhibits a broadened unstructured electronic transition with a maximum at 35 644 cm^{-1}.This band is assigned to one isomer according to the identical IR/R2PI spectra obtained at different positions of the

(40) Boys, S. F.; Bernardi, D. *Mol. Phys.*, **1970**, *19*, 553.
(41) More structural parameter and a list of all calculated structures can be received from the authors on request.

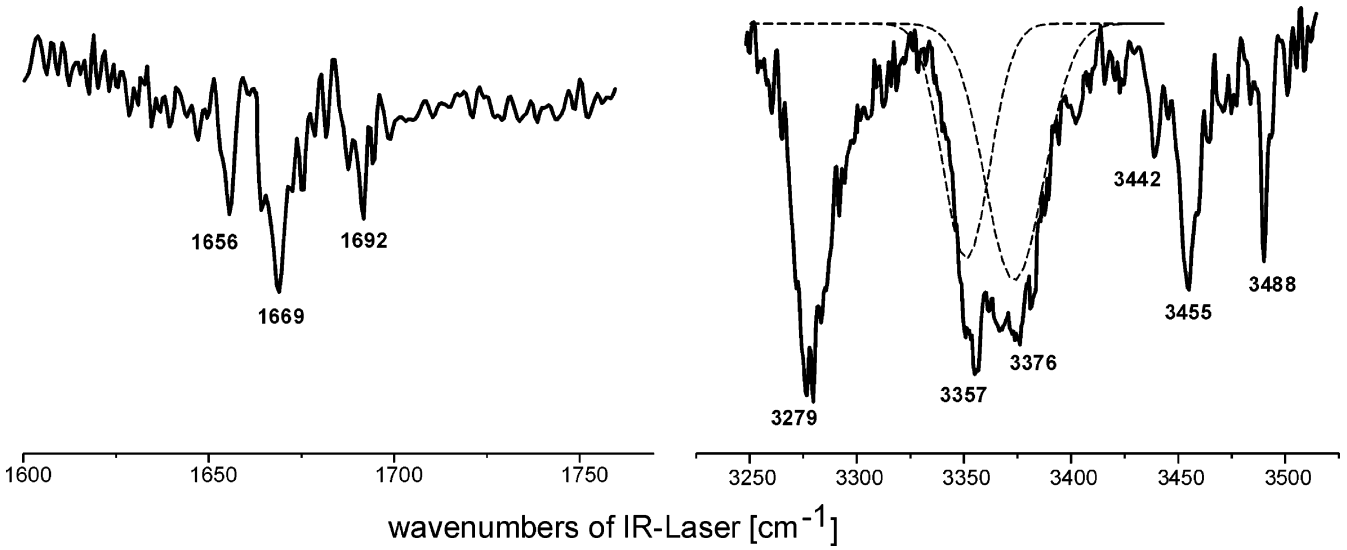

Figure 2. IR/R2PI-spectra of (Ac-Val-Tyr(Me)-NHMe)$_2$ in the range from 1600 to 1760 and 3250 to 3520 cm^{-1} recorded via the electronic transition at 35 644 cm^{-1}. In the region of the amide A frequencies, the dotted line indicates a band contour analysis for the overlapping bands at 3357 and 3376 cm^{-1} derived from a Gaussian fitting procedure.

electronic transition. In Figure 2, the IR/R2PI spectra both in the region of the NH stretching vibrations (amide A, 3250−3520 cm^{-1}) as well as in the region of the amide I vibrations (1600−1760 cm^{-1}) recorded via the electronic transition at 35644 cm^{-1} are shown. According to previous spectroscopic results, usually "free" NH-stretching modes are located above 3400 cm^{-1}, whereas hydrogen-bonded NH groups lead to vibrational frequencies below 3400 cm^{-1}. The spectrum shown in Figure 2 clearly indicates the existence of three hydrogen-bonded vibrational frequencies (at 3279, 3357, and 3376 cm^{-1}) and three free NH stretching modes at 3442, 3455, and 3488 cm^{-1}. This result ideally fits to a formation of a β-sheet arrangement as predicted by the ab initio and DFT calculations.

The experimental spectra and the spectra calculated at the B3LYP/cc-pVDZ level of theory for the most stable antiparallel (**I**), parallel (**II**) and parallel/γ-turn (**III**) structures are shown in Figures 3 and 4. It is evident that the antiparallel β-sheet arrangement yields a very good agreement between the experimental and calculated vibrational frequencies. The predicted vibrations correlate with respect to their frequencies and their relative intensities to the experimentally observed transitions. This holds both for the amide A (NH stretching) region (Figure 3) and for the amide I (mainly C═O stretching) region (Figure 4). The detailed assignment of all vibrations to the different amide (NH and C═O) vibrations is given in Table 2. In contrast to the assignments obtained for the antiparallel arrangement (**I**), no strong red-shifted vibration is expected for the parallel arrangement (**II**) in the NH stretching region. Furthermore, only one intense vibration in the region of the amide I vibrations (around 1660 cm^{-1}) should be observed for structure (**II**). In the case of the combined parallel/γ-turn (**III**) structure, four hydrogen-bonded NH stretching modes (below or nearly at 3400 cm^{-1}) are expected. This is not observed and additionally the amide I vibrations are located at slightly higher frequencies as the ones observed experimentally. As mentioned above, only the vibrations of the most stable structures with respect to antiparallel, parallel and combined parallel/γ-turn binding motifs are shown in Figures 3 and 4, but it can be pointed out generally, that the spectra observed for other structures of these binding motifs are very similar, i.e., the shift of NH and C═O stretching modes with respect to different side-chain arrangements leads to similar vibrational spectra in the amide A and amide I regions.

As further discussed above already, the vibrations calculated at the quite low HF/3-21G(d) level are useful to predict vibrational spectra of peptides and peptide aggregates. In Figures 3 and 4, the vibrational frequencies obtained at the HF/3-21G(d) level for an antiparallel, parallel, and parallel/γ-turn structure are given, too. It is obvious that the agreement with an antiparallel β-sheet arrangement (**I**) is very good, especially for the amide A vibrations, confirming the results obtained at the DFT level.

It is the topic of this paper to find out if a dimer of peptides formed in the gas phase is able to form a β-sheet arrangement and it can clearly be concluded that the answer is positive. Experimental evidence has been produced for the fact, that a peptide with sterically demanding amino acid residues can spontaneously form a β-sheet arrangement in the gas phase, confirming the intrinsic stability of this fundamental secondary structure in the gas phase. Even in the absence of solvent or any other chemical environment, that is, under conditions that rely solely on hydrogen bonds, the β-sheet is formed. Furthermore for the investigatied species no other isomer is observed.

A second classical tacit assumption is the superior intrinsic stability of an antiparallel β-sheet arrangement over the related parallel orientation.[42] Our experiment, which strips the peptide cluster off all solvent molecules and reduces the peptide size to a trimer model, is an example which supports this consideration. Nevertheless, more data are required to answer this fundamental question. Most likely, the nonlinearity and increased length of all involved hydrogen bonds in the parallel case are responsible for its inferior stability. Interestingly, the antiparallel arrangement is also by far the most abundant species found in Nature.

Since the relative red-shift of the hydrogen-bonded NH stretching vibrations (NH_{Tyr}: 3279 cm^{-1}, NH_{NMe}: 3357 cm^{-1},

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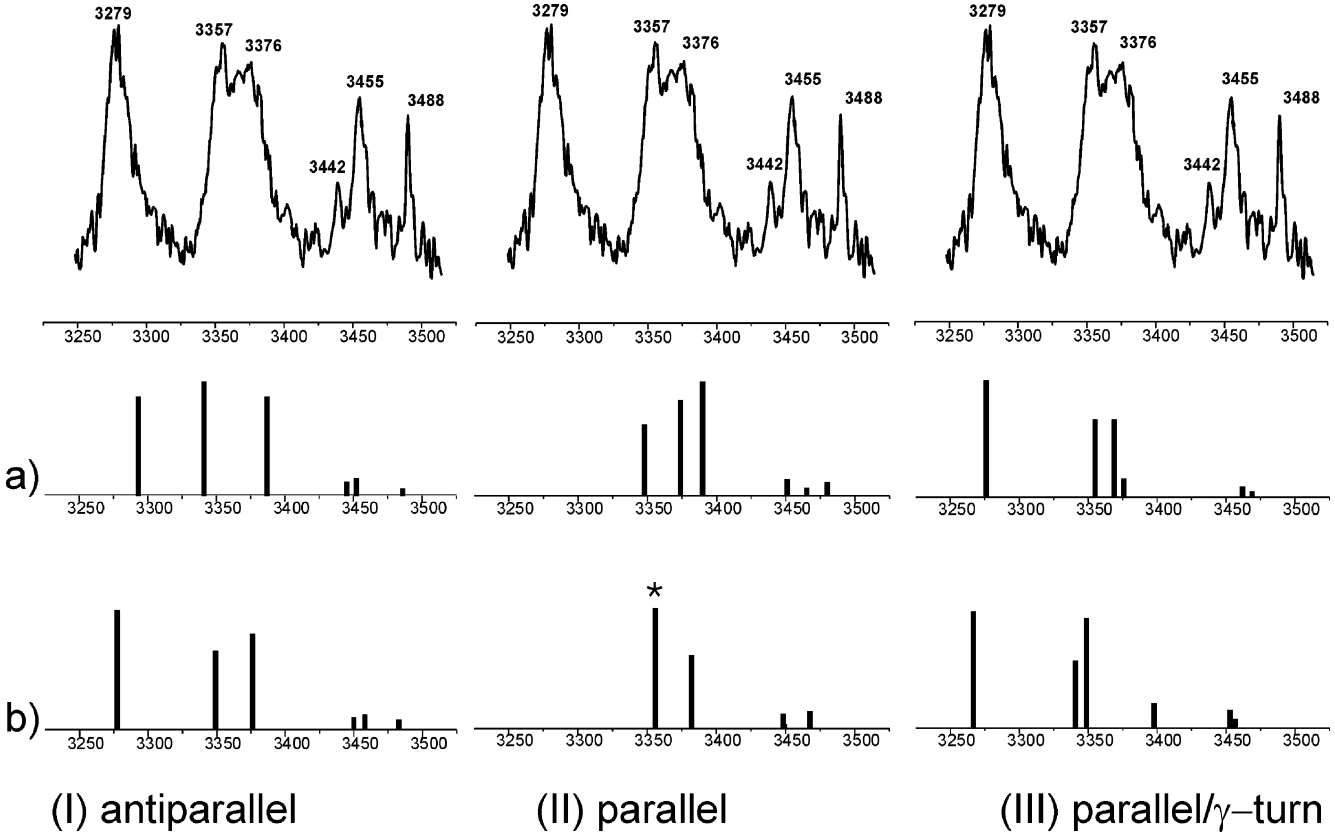

Figure 3. Experimental and theoretical results in the region of amide A vibrations. The calculated stick spectra represent the most stable types of antiparallel (I), parallel (II), and parallel/γ-turn (III) conformers at the (a) B3LYP/cc-pVDZ and (b) HF/3-21G(d) level of theory. The asterix in trace (b) of the parallel structure (II) at 3350 cm^{-1} indicates two overlapping bands.

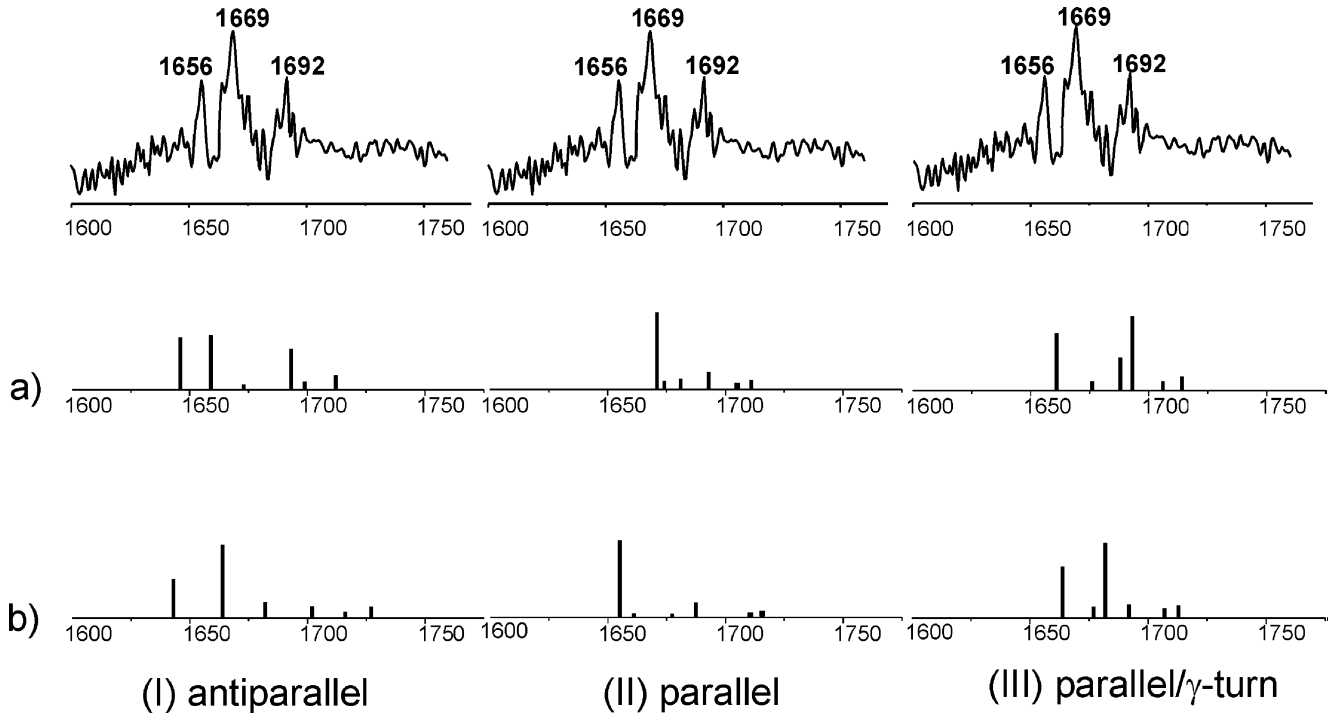

Figure 4. Experimental and theoretical results in the region of amide I vibrations from 1600 to 1760 cm^{-1}. The calculated stick spectra represent the most stable types of antiparallel (I), parallel (II), and parallel/γ-turn (III) conformers at the (a) B3LYP/cc-pVDZ and (b) HF/3-21G(d) level of theory.

NH_{Val}: 3376 cm^{-1}) with respect to the free NH stretching modes (NH_{Tyr}: 3442 cm^{-1}, NH_{NMe}: 3488 cm^{-1}, NH_{Val}: 3455 cm^{-1}) corresponds to the hydrogen bond-strength, the following order of stabilities is obtained: Tyr-NH···O > NMe−NH···O > Val-NH···O due to the red-shifts of 163, 131, and 79 cm^{-1}, respectively (cf. Table 2).

It is of further interest to note that vibrational frequencies of subunits have nearly the same value independent of the size of

the system as far as they belong to the same structural environment, e.g., the NH stretching frequency in a β_L arrangement of the backbone is around 3440−3460 cm^{-1}. In the case of the NH−NMe protecting group the NH stretching frequency increases to 3460−3490 cm^{-1}. NH groups involved in β-turns have frequencies about 3400 cm^{-1} or slightly below.[21,23−24,30−31] If a γ-turn arrangement is formed the frequency decreases significantly below 3400 cm^{-1} usually in the range from 3250 to 3380 cm^{-1}.[16,23−25,28−29] As shown above a similar or even stronger red-shift is also observed for the NH stretching frequencies of the intermolecular hydrogen-bonded groups depending on the strength of the H-bond.

These results obtained for the amide A vibrations are very valuable hints for analyzing larger systems, that is, the strategy to analyze larger model systems is not only based on calculations (which become very expensive for large systems due to the enormous number of isomers which have to be taken into account) but also on the experience obtained for the smaller building blocks. In this context, it is interesting to note that all intense vibrations in the amide I region are above 1650 cm^{-1}. For protein measurements, it is often pointed out that β-sheet structures yield amide I vibrations around 1630 cm^{-1},[42] although there are also other investigations predicting higher values above 1650 cm^{-1}.[43−45] The experimentally observed frequency is also a function of the environment. Our experimental results clearly indicate that a simple classification of a β-sheet structure with respect to the CO stretching (amide I) vibrations is not possible, at least if isolated β-sheet structure elements with no further environment are investigated. The detailed vibrational analysis obtained from our experimental strategy allows a deeper insight in an isolated model system. By increasing the size of the model system step by step and by adding a biomimetic environment (other peptides or solvent molecules) in the same way, it should become possible to understand in a deductive way the driving forces for the development of β-sheets.

IV. Conclusion

The dimer of Ac-Val-Tyr(Me)-NHMe is an ideal model of an isolated peptide that forms an antiparallel β-sheet; its exclusive formation in the gas phase without any directing influence of solvent or peptidic environment provides experimental evidence for the fundamental importance and intrinsic stability of this relatively complicated secondary structure motif. The investigated cluster is one of the largest aggregates investigated up to now with the mass and isomer selective IR/R2PI method. This method applied both for the structure sensitive amide I *and* amide A vibrations yields in combination with ab initio and DFT calculations a detailed analysis on the structure of the peptide backbones. The investigated peptide is a kind of β-sheet unit cell, since a successive application of this binding motif leads to the formation of a complete β-sheet. It is the strategy to find out the driving force to form β-sheet formations as a function of different amino acids and aggregation partners. Thus larger systems will be investigated and with a successive aggregation of water molecules an environment will be introduced indicating if and how a peptide conformer changes due to a hydration shell.

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Supporting Information Available: Complete ref 37. This material is available free of charge via the Internet at http://pubs.acs.org.

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