

Ramachandran Plot for Alanine Dipeptide as Determined from Raman Optical Activity

Václav Parchaňský,^{†,‡} Josef Kapitán,[§] Jakub Kaminský,[†] Jaroslav Šebestík,[†] and Petr Bouř^{*,†}

[†]Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo náměstí 2, 16610 Prague, Czech Republic [‡]Department of Analytical Chemistry, Institute of Chemical Technology, Technická 5, 16628 Prague, Czech Republic [§]Department of Optics, Palacký University Olomouc, 17. listopadu 12, 77146 Olomouc, Czech Republic

Supporting Information

ABSTRACT: Accessible values of the φ and ψ torsional angles determining peptide main chain conformation are traditionally displayed in the form of Ramachandran plots. The number of experimental methods making it possible to determine such conformational distribution is limited. In the present study, Raman optical activity (ROA) spectra of Ac-Ala-NHMe were measured and fit by theoretical curves. This revealed the most favored conformers and a large part of the potential energy surface (PES) of this model dipeptide. Such experimental PES compares well to quantum chemical computations, whereas molecular dynamics (MD) modeling reproduces it less faithfully. The surface shape is consistent with the temperature dependence of the spectra, as observed experimentally and predicted by MD. Despite errors associated with spectral modeling and the measurement, the results are likely to facilitate future applications of ROA spectroscopy.



SECTION: Spectroscopy, Photochemistry, and Excited States

P roper understanding of protein structure and folding represents a central point of vast areas of computational chemistry and biology. The work of Ramachandran¹ was an important milestone in this respect because it revealed relatively simple general principles determining the shapes of all peptides and proteins. It pointed out the importance of preferred and forbidden values of the φ and ψ torsional angles. Later, more precise quantitative maps could be determined in terms of probability or free-energy angular dependence (potential energy surface, PES),² yet such behavior of a particular peptide molecule is still difficult to determine experimentally or predict theoretically. Quite often, smaller peptidic molecules exhibit richer conformational flexibility than larger ones.³

Only a limited number of experimental techniques enable a direct monitoring of molecular conformations in solution. Nuclear magnetic resonance, for example, has been utilized as a standard for a long time.^{4–7} Later, optical spectroscopic techniques proved to be useful for this purpose, in particular, 2D infrared spectroscopy⁸ or methods utilizing molecular chirality, that is, different absorption or scattering of left- and right-circularly polarized light.^{9,10} Of those, Raman optical activity (ROA) is perhaps the most complex but also the most promising method. It combines the sign information provided by chiral techniques, a rich and well-resolved spectral pattern inherent to vibrational spectroscopy, and the advantages of Raman scattering. Additionally, it spans a wide range of wavenumbers and is suitable for the natural (aqueous) environment, and the spectrometers can be conveniently constructed with optics available for visible light.¹¹ Owing to

the solid theoretical basis and spectral simulations,¹² many molecular structure problems could be tackled in the past by ROA, including bioorganic complexes,¹³ determination of the absolute configuration of small molecules,¹⁴ isotopically driven chirality,¹⁵ conformation of nucleic acids,¹⁶ proteins,¹⁷ and even geometry of whole viruses.¹⁸

The processes of photon absorption and emission are usually extremely fast so that an ROA spectrum is a sum of subspectra of all species present in the sample. Theoretically, these can be decomposed into theoretical curves to yield conformer ratios. So far, only a limited number of systems with restricted conformational freedom could be treated in this way because of the finite precision of simulations and the experimental noise. For example, in model dipeptides, we proved that conformer ratios determined from ROA and NMR are nearly identical.¹⁹

Such ROA spectral decomposition may be mathematically illdefined because of the limited accuracy of experimental and simulated spectra or when different conformers provide similar spectral response. However, for Ac-Ala-NHMe the decomposition presented below seems to be reasonable even within the full 2D PES. We took advantage of a relatively low noise-tosignal ratio in the experiment and a broad measurable spectral region ($150-1800 \text{ cm}^{-1}$). At the same time, the double-hybrid functionals²⁰ and analytical computational techniques for

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ROA,^{12,21,22} not available until recently, provided us with an accurate theoretical basis.

The Ac-Ala-NHMe alanine "dipeptide" molecule (Figure 1) is traditionally used as a convenient model to study the



Figure 1. φ and ψ torsional angles in *N*-acetyl-L-alanine-*N'*-methylamide (Ac-L-Ala-NHMe, the alanine "dipeptide").

structure and interactions of the amide linkage. It is small, features a strong amide—amide interaction, and is accessible by accurate experimental and computational approaches.^{23–39} The vibrational optical activity, in the form of either vibrational circular dichroism (VCD) or ROA, has also been recognized as being capable of providing valuable information about the structure of small peptides including Ac-Ala-NHMe.^{40–43} The early spectral analyses, however, were not particularly successful in reproducing the measured spectral patterns.⁴⁰ It appeared that the solvent effect and molecular flexibility had to be included in a realistic way to properly simulate both the PES and spectral properties.⁴⁴

Small-molecule Ramanchandran plots are perhaps not directly applicable to large peptides and proteins in a quantitative way. Previous experience indicates that PES or potential of mean force (PMF) contours obtained for short peptides may resemble those obtained by a statistical analysis of X-ray peptide structures fairly well.^{36,45} We thus hope that our study not only documents the possibilities of ROA methodology but also sheds more light on the conformational behavior of larger molecules.^{7,46}

As shown in detail in the Supporting Information (SI), we measured both enantiomers of the dipeptide, in H_2O and D_2O , to develop a feeling for the stability of the results. The D-form was synthesized, whereas the L-enantiomer was obtained commercially. By fitting the ROA spectrometer with a temperature cell we were able to verify the theoretical PES via comparing the theoretical and experimental temperature-induced changes in the spectra.

The theoretical spectra (see SI for details) were obtained for a 2D grid of the (φ, ψ) torsion angles using the double-hybrid mPWPLYPD²⁰ method with the 6-311++G** basis set and the SMD⁴⁷ solvent model. Raman and ROA intensity tensors¹¹ were calculated²² at the B3LYP level, all within the Gaussian⁴⁸ program suite. For each grid point (400 conformers, corresponding to 18° angular steps) the mPWPLYPD harmonic force field was scaled in internal coordinates⁴⁹ or via relative masses to adjust positions of most intense Raman bands to the experiment. Results for the internal coordinate



Figure 2. (A,B) Free-energy plots obtained by the decomposition of the experimental Ac-Ala-NHMe ROA spectra in H_2O and D_2O solutions, respectively, and (C) the PMF/ff03 and (D) mPWPLYPD/6-311++G**/SMD theoretical free-energy surfaces. For panel D, the two lowest-energy conformers are indicated.

scaling that appeared slightly more flexible are shown. We understand the scaling as an empirical correction of the most obvious computational errors and thus do not try to interpret the scaling constants (Table S1, SI) in any other way.

The experimental intensity $S(\omega)$ at frequency ω was then decomposed into the theoretical curves $S_i(\omega)$, $S(\omega) = \sum_i p_i S_i(\omega)$, where the coefficients $p_i = \exp(-\Delta G(\varphi_i, \psi_i)/RT)$, $\Delta G(\varphi_i, \psi_i)$ is the free energy, R is the gas constant, and T is temperature. A square-root integral deviation between the experiment and theory was minimized directly with respect to the free energy using the conjugate gradient^{50,51} method and an arbitrary Fourier expansion.

The PES $(\Delta G(\varphi_i, \psi_i))$ obtained from the fit could be compared with the theoretical one, for example, that predicted by the mPWPLYPD method. As an alternate model, the PMF was calculated using the weighted histogram analysis method (WHAM)⁵² implemented in the Amber software package.⁵³

The free-energy surfaces obtained by the decomposition of ROA spectra measured in H₂O and D₂O are plotted in Figure 2A,B, respectively. The theoretical PMF (part C) and mPWPLYPD (part D) surfaces are shown as well. Most probably, not all features of the surfaces obtained by the decomposition are real. In particular, the "H₂O" surface (part A) provides shallow minima ((φ, ψ) \approx (55°, -60°), (-150°, -130°), etc.) not seen in D₂O or in the theory. We should note that we do not expect the isotopic effect of the $H_2O \rightarrow D_2O$ exchange to have any significant influence on peptide conformation. For example, if simulated at the harmonic oscillator level (Figure S1 in the SI), free-energy differences smaller than 0.05 kcal/mol near PES minima would be explicable by the exchange only. Therefore, the main differences between the A and B panels could be caused only by inaccuracies in simulated spectra and the experimental precision.

Despite these inconsistencies, the decomposition does reveal credible information about the dipeptide. The most favored conformer is predicted by both experiments at $(\varphi, \psi) \approx (-70^{\circ}, 130^{\circ})$ and corresponds to a form traditionally referred to as $\varepsilon_{\rm L}$. The " $\alpha_{\rm L}$ " form appears at $\sim (-75^{\circ}, -20^{\circ})$ for H₂O and $(-60^{\circ}, -40^{\circ})$ for D₂O. Note that at (φ, ψ) regions with low conformer populations the decomposition is less stable than that around high-populated areas. For example, the position of the " $\beta_{\rm L}$ " minimum at $(-150^{\circ}, -130^{\circ})$ found for H₂O only is somewhat dependent on decomposition parameters (e.g., Figures S2 and S3 in the SI).

Note that the classification used for the small peptide conformers used in the literature^{30,54} is often vague. For example, $\alpha_{\rm L}$ may be encountered also as $\alpha_{\rm R}$ and so on. More importantly, these conformations can be related to peptide and protein secondary structures. Then, the $\varepsilon_{\rm L}$ form would correspond to the polyproline II conformation with canonical (φ, ψ) angles of $(-78^{\circ}, 149^{\circ})$, and $\alpha_{\rm L}$ can generate α - and 3_{10} -helices with canonical angles of $(-57^{\circ}, -47^{\circ})$ and $(-60^{\circ}, -30^{\circ})$, respectively.⁵⁵ Both the polyproline II and helical conformations are also adopted by alanine-like polypeptides.⁵⁶

At present, we can only speculate why the two (H_2O, D_2O) experiments treated in the same experimental and theoretical ways gave slightly different energy maps. However, the overall better agreement of the D_2O spectra with the modeling strongly suggests an interference of aqueous vibrations, including stretching of the hydrogen bonds. Indeed, in D_2O , the overall downshift of vibrational frequencies can limit the

coupling and make the continuous solvent model that had to be adopted in this study more realistic.

The theoretical PMF and ab initio surfaces (Figure 2C,D) are similar in that both of them predict the $\alpha_{\rm L}$, $\beta_{\rm L}$, and $\varepsilon_{\rm L}$ minima at approximately the same angular values. The $\alpha_{\rm L}$ and $\boldsymbol{\varepsilon}_{\mathrm{L}}$ theoretical geometries very closely correspond to the decomposition values, while $\beta_{\rm L}$ calculated at ~(-150°, 160°) is shifted from the H₂O decomposition angles (~ -150° , -130°). However, the PMF computation favors $\alpha_{\rm L}$, whereas mPWPLYPD suggests a dominance of the $\boldsymbol{\varepsilon}_{\mathrm{L}}$ conformers. Because the dominance of $\boldsymbol{\varepsilon}_{L}$ is predicted by both H₂O and D₂O experiments, we may suppose that the mPWPLYPD modeling is closer to reality in this case than the PMF one. The experimental and theoretical well widths determining molecular flexibility are rather consistent as well; considering the Boltzmann factor $kT \approx 0.6$ kcal/mol and width of the minima of PES we can estimate that the angles vary within about 10° around the equilibrium positions at 293 K.

The PMF surface is nearly identical to that computed in a recent study;³² other force fields, however, exhibit larger variations.^{31,33,57} Main features of the mPWPLYPD surface agree with those obtained with simpler quantum chemical models,³⁵ including ab initio molecular dynamics (MD).³⁶

Some models previously reported are less compatible with the experimental decompositions. The integral equation approach³⁷ seems to overestimate the population of the $\alpha_{\rm L}$ conformer. The "C₇-axial" conformation predicted³⁹ at about (70°, -50°) is approximately reproduced at (55°, -60°) by the decomposition for the H₂O experiment only. Other force fields predict a π -helical like conformer with (φ, ψ) \approx (-75°, -40°), very close to $\alpha_{\rm L}$.⁵⁴

Comparison of theoretical and experimental spectra in Figure 3 documents a multicomponent (mutli-conformational) character of the dipeptide ROA response. Spectra of the two highest-populated conformers, $\varepsilon_{\rm L}$ and $\alpha_{\rm L}$, are compared with the whole grid fit and experiment. For example, more components are needed to reproduce the double-negative ROA signal at 348/399 cm⁻¹ or the couplet centered around 1300 cm⁻¹. Clearly, the fit, to a large part combination of the two grid points, is needed to reasonably reproduce the experiment.

One might think that a combination of the 400 subspectra can give a better fit than that in Figure 3. However, values of the decomposition coefficients are strongly limited by the constraints requiring them to be positive and summed up to one. This limits the final agreement but gives the decomposition physical meaning and makes the algorithm more stable.

The stability and reliability of the decomposition is obviously one of the main concerns in this kind of PES determination from ROA spectra. However, we did not observe any significant instability of the mathematical procedure. A direct decomposition based on the Lagrange multipliers used as the initial guess (Figure S4 in the Supporting Information), for example, provided the same lowest-energy minima as the refined conjugate-gradient fit in Figure 2, albeit the Lagrange surface is flatter and provided worse spectral fit. Thus, apart from experimental noise, the error of the simulated spectra appears to be the main limiting factor. Better solvent models, functionals, anharmonic correction, and so on are topics offering themselves for improvement in future studies.

The temperature dependence of ROA spectra provides information about the validity of the model potential-energy



Figure 3. Ac-L-Ala-NHMe ROA spectra, calculated for $(\varphi, \psi) = (-59^{\circ}, 144^{\circ})$ (A, conformer $\varepsilon_{\rm L}$) and $(-77^{\circ}, -15^{\circ})$ (B, conformer $\alpha_{\rm L}$), the decomposition fit using all grid spectra (C), and the experiment (D). Selected peak wavenumbers are indicated, the intensity scale is in atomic (A,B) and arbitrary (C,D for normalized spectra) units.

surfaces. Unfortunately, the spectral variations caused by the temperature are rather small and a detailed scan is not possible because of the long accumulation times. For 293 and 363 K, however, the experimental spectra could be measured. The difference is compared to simulations using Boltzmann statistics and the mPWPLYPD and PMF energy landscapes in Figure 4. Both theoretical models provide principle features observed experimentally. Overall, the PMF difference (B) seems to be more faithful than the double-hybrid (A) one, for example,



Figure 4. ROA spectral temperature differences ("I(293 K) - I(363 K)") for Ac-L-Ala-NH-Me simulated using the (A) mPWPLYPD/6-311++G**/SMD PES, (B) MD PMF, and (C) the experiment.

better reproducing the predominantly positive difference signal around 900–950 cm⁻¹, the largest relative intensity changes within 1300-1450 cm⁻¹, and the predominantly negative difference around 1500 cm⁻¹.

In summary, the decomposition of the alanine dipeptide ROA experimental spectra into theoretical components provided a wealth of information about the structure and flexibility of the lowest-energy conformers. The overall character of the PES including the well widths was consistent with the theoretical simulations, although the decompositions, especially for the H_2O solution, also yielded minor artifact minima. The limited precision of the ROA free-energy landscape is mostly due to experimental noise and approximations in the currently available simulation techniques, both of which can nevertheless be rectified in the future. The results also document how the information obtained from the spectra including the temperature dependence is enhanced by the theoretical modeling.

ASSOCIATED CONTENT

Supporting Information

Experimental and computational details and all measured spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: bour@uochb.cas.cz.

Notes

The authors declare no competing financial interest.

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The Journal of Physical Chemistry Letters

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