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RESEARCH ARTICLE

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Raman optical activity of the antibiotic vancomycin bound to its biological target

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Abstract

The glycopeptide vancomycin has an antimicrobial effect by halting the synthesis of the bacterial cell-wall by binding the D-Ala-D-Ala peptide motif of the cell-wall precursor Lipid II. This interaction has been extensively investigated using techniques such as XRD and NMR. Here, vancomycin complexed with Ala-γGlu-Lys-D-Ala-D-Ala (the pentapeptide part of Lipid II) is studied by means of Raman and Raman optical activity (ROA) spectroscopy. Where for conventional structural elucidation techniques, the direct study of a drug-biological target complex is routine, it is not the case for the ROA technique. Through experimental recordings and DFT-level spectral calculations of the complex, it was found that ROA specifically probes the conformation of vancomycin, even in the presence of the biological target. As such, it could be determined that no noticeable conformational changes are involved when binding Ala-γGlu-Lys-D-Ala-D-Ala. This work showcases the interest of directly examining interacting systems conformationally by means of ROA, potentially complementing and/or confirming findings from other studies, and eventually contributing to a more complete understanding of the structure–activity relationship of a drug compound.

KEYWORDS

antibiotic, intermolecular interactions, Raman optical activity, vancomycin

1 | INTRODUCTION

Since the 1940s, marked as the start of the golden era of antibiotics, there have been sizeable advances in the discovery, characterization and clinical usage of antibiotics.[1,2] This led to a world-wide increase in quality of life, as infectious diseases ceased to pose major health threats. Bacteria, however, have the capacity to build resistance toward antibiotics that are abundantly used. Through various inherent mechanisms, for example, the molecular structure that is targeted (the biological target) by the antibiotic alters, preventing the latter to function properly.[3] Fortunately, certain biological targets are less easily chemically modified, and therefore, antibiotic agents that act on these are favorable. In this light, it was found that targeting Lipid II, a bacterial cell-wall precursor, is a clever strategy and moreover is a strategy that is already adopted by nature itself.[4]

One of the most known agents that act on Lipid II is vancomycin (the molecular structure is given in Figure 1a), the main representative of the glycopeptide antibiotic category. Vancomycin has been discovered in

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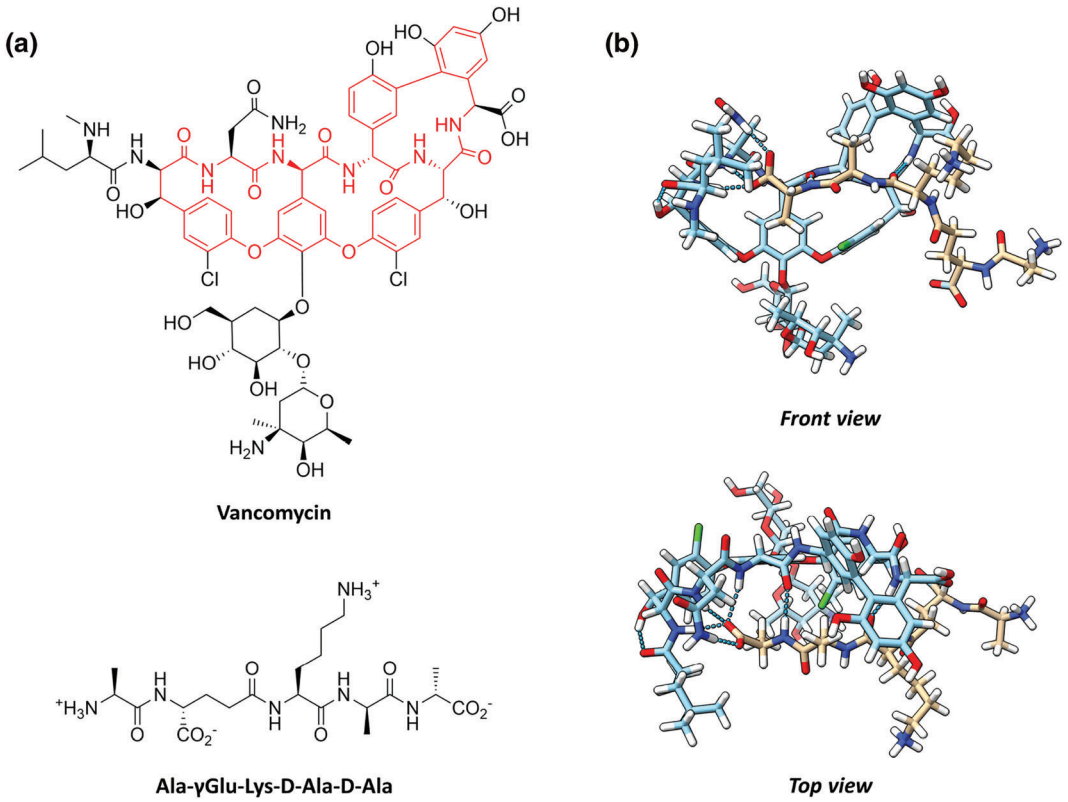

FIGURE 1 The molecular structure of vancomycin and Ala-γGlu-Lys-D-Ala-D-Ala (a). The portion of vancomycin that can be conformationally studied using ROA carries a red color.[7] A 3D visualization of the interaction between vancomycin (blue) and Ala-γGlu-Lys-D-Ala-D-Ala (brown), part of the Lipid II cell-wall precursor (b). The complex was optimized starting from a NMR structure (PDB entry 1GAC[8]; see Section 2.2) using DFT at the B3PW91/6-31G++(d,p) level of theory. The five hydrogen bonds that contribute to the complex formation are indicated, as well as two additionally found in the optimized structure.[5] Visualization and hydrogen bond determination were performed using the UCSF ChimeraX program[9] [Colour figure can be viewed at wileyonlinelibrary.com]

Streptomyces orientalis soil bacteria and approved for clinical use in the 1950s.[5,6] Today, the compound is still actively administrated to fight various infections and is considered as a last-resort antibiotic. It functions by binding Lipid II, inhibiting the transglyosylation and transpeptidation steps during the cell-wall formation cycle.[5] This leads to the weakening of the bacterial cell-wall and eventually bacterial cell death. Understanding the interactions of vancomycin and Lipid II on a molecular level is of importance for defining the structure-activity of the compound, allowing the development of more efficacious and favorable drugs that have a similar working principle.

Techniques such as XRD and NMR are capable of directly studying the interactions between molecular systems. As such, the interactions between vancomycin and Lipid II too have been extensively investigated, and it was found that vancomycin has a strong binding affinity for the D-Ala-D-Ala sequence at the end of the pentapeptide portion (Ala-γGlu-Lys-D-Ala-D-Ala, Figure 1a) of Lipid II (a 3D visualization thereof is given in Figure 1b).[8,10-15] Besides XRD and NMR, vibrational spectroscopic techniques are suitable for conformational analyses and dynamics of compounds. Raman optical activity (ROA) is a spectroscopic technique that has been successfully employed to elucidate the in-solution structure of vancomycin.[7] Hitherto, however, ROA analyses in general have always been limited to the study of the bioactive agent free in solution, rather than probing the interaction of the pharmaceutical compound with its biological target, unlike commonly performed using XRD and NMR. Herein, for the first time, to the best of our knowledge, an ROA study of a drug-biological target complex is reported. Specifically, this work contributes to a better understanding of the ROA spectral responses displayed by the antibiotic glycopeptide molecular class.

2 | METHODOLOGY

2.1 | Raman and ROA measurements

Vancomycin and Ala-γGlu-Lys-D-Ala-D-Ala (pentapeptide) were purchased from Ambeed and Pepmic,

respectively, and were used without further purification. An acetate buffer was used as solvent system, adjusting and retaining a pH of 3.6, to ensure adequate solubility. The samples where the pentapeptide and vancomycin were measured alone in solution had a concentration of ~82 mM. Then, 1:1 and 1:2 vancomycin:pentapeptide molar ratio samples were prepared. All samples readily dissolved after a couple of minutes of manual shaking. This sample preparation, albeit in a higher concentration, is in line with those used in NMR[8] and ECD[11] studies, for which a complex formation occurred invariably. All Raman and ROA spectra were recorded under ambient conditions using the ChiralRAMAN-2X scattered circular polarization (SCP) ROA instrument (BioTools, Inc.), equipped with a 532 nm excitation laser.[16] The laser power for the samples pentapeptide, vancomycin and the complex were respectively 800, 250, and 250 mW at the source. The total measurement time ranged from 2 to 5 days. The illumination time of a single scan was set to 2.2 seconds. The Raman spectrum was solvent subtracted and subsequently baseline corrected using the Boelens et al. procedure.[17] A Savitsky-Golay filter has been used for ROA baseline correction (order 5, framelength 401) and ROA spectrum smoothing (order 3, framelength 9).

2.2 | Raman and ROA calculations

The geometry of a single vancomycin-pentapeptide complex was extracted from the protein data bank entry 1GAC (an NMR determined ensemble; modified to form vancomycin as in our previous work[7]).[8] Two subsequent density functional theory (DFT) geometry optimization steps were performed using the Gaussian 16, rev. C.01 program[18] on the B3PW91/6-31G(d,p) and B3PW91/6-31++G(d,p) levels of theory, respectively. The Hessian was calculated on the corresponding level of theory, and the geometry was confirmed to be located in a minimum of the potential energy surface. The Hessian, Raman and ROA tensors were then calculated using the B3PW91/6-31++G(d,p) level of theory. For simulating the Raman and ROA intensities the excitation wavelength was set to 532 nm, in accordance with the experiment. The obtained Raman and ROA intensities were temperature corrected (298 K) and Lorentzian line broadened with an full width at half maximum of 20 cm^{-1}.[19] The water solvent was implicitly taken into account using the integral equation formalism model (IEFPCM) as incorporated in Gaussian 16. The calculation happened on a computer node with two 32-core AMD Epyc 7542 generation CPUs with a RAM memory of 256 GB (220 GB was requested in the Gaussian input file). The geometry optimization step took 3 days 13 h 10 min (real time). The subsequent frequency and ROA intensity calculations required 2 days 2 h and 30 min.

3 | RESULTS AND DISCUSSION

Prior to the presentation and analysis of the Raman and ROA spectra of the vancomycin:pentapeptide complex, the measurements of the two compounds separately are treated. First, consider the Raman and ROA spectra of the pentapeptide, given as black spectra in Figure 2. The Raman and ROA measurement of the pentapeptide has no precedence, as it contains a γGlu and two D-Ala residues, and are in that sense unique. A prominent Raman signal can be found at ~1440 cm^{-1}, with a shoulder at half intensity around ~1460 cm^{-1}. This feature is attributable to the CH_2 scissoring deformation of Lys.[20] An intense Raman band is also typically found for D-Ala-D-Ala (or L-Ala-L-Ala), which is instead typically centered around ~1460 cm^{-1}.[21,22] Probably, the three Ala residues too contribute to the shoulder that is observed in our spectrum. Furthermore, the CH_2 scissoring deformations from γGlu also yield Raman intensity in that region.[23,24] Next, two broad Raman bands are present in the amide I region, a region governed by the C=O stretch vibrations, at 1670 and 1719 cm^{-1}. Moving on to the ROA spectrum (Figure 2), a feature-rich spectrum was obtained. Several features appear to show resemblances with earlier (empirical) studies of Ala and Lys containing peptides.[20-22] A full comparison is, however, not found (nor for the Raman spectrum), which might not come as a surprise given that the pentapeptide here contains unnatural amino acids, and might, as a consequence thereof, display conformational behavior unlike that of peptides with natural residues. A deeper analysis of the normal modes and the displayed Raman and ROA intensities, besides the few relevant elements touched upon earlier, is outside the scope of the current article.

The Raman and ROA spectra of vancomycin alone in aqueous solution are given in blue in Figure 2. Although the concentration used in the recordings here was more than doubled with respect to recordings in our previous work, no spectral effects were observed.[7] This shows that the conformation of vancomycin is not affected by an eventual concentration effect. The normal mode analysis and the conformational behavior of vancomycin has previously been extensively described supported by DFT spectral calculations, and can be consulted in Aerts et al.[7]

The Raman and ROA spectra of the vancomycin:pentapeptide are depicted in Figure 2 (red color for the 1:1 molar ratio, brown color for the 1:2 molar ratio). The large majority of the Raman spectrum remains identical

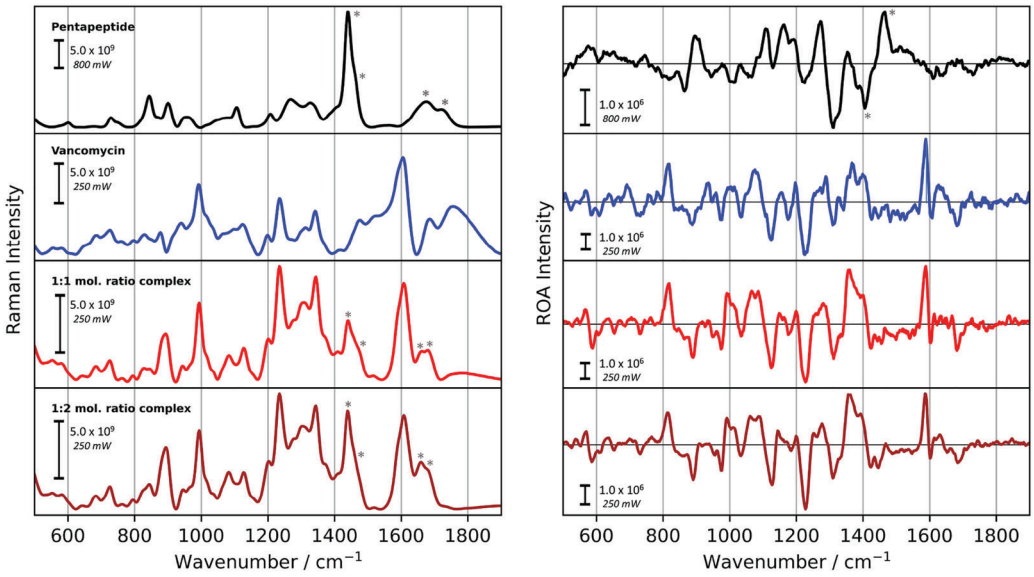

FIGURE 2 Experimental Raman (left) and Raman optical activity (ROA; right) spectra of Ala-γGlu-Lys-D-Ala-D-Ala (black; pentapeptide), vancomycin (blue) and Ala-γGlu-Lys-D-Ala-D-Ala bound to vancomycin (red: equimolar concentration; brown: 1:2 vancomycin:Ala-γGlu-Lys-D-Ala-D-Ala molar ratio). The signals specifically mentioned in the text are indicated with a gray asterisk. Measurement time normalized y-scales have been included in each panel, as well as the used laser power for the measurement [Colour figure can be viewed at wileyonlinelibrary.com]

to that of vancomycin alone. The most noticeable discrepancy is the occurrence of the strong Raman band at around ~1440 cm^{-1}, which is originating from pentapeptide, as discussed earlier. This is confirmed by the increase in Raman intensity for the 1:2 molar ratio measurement. The second difference can be found in the amide I region, where two bands are identified around 1657 and 1680 cm^{-1}, instead of a single band for vancomycin at 1680 cm^{-1}. The sharp signal at 1657 cm^{-1} is new with respect to the Raman spectra of the two compounds separately. It appears that the ratio between the two amide I bands changes when increasing the molar concentration of the pentapeptide, however, one should be careful not to over-interpret this element. What the structural rationale behind the amide I observations precisely is remains speculative, but is probably attributable to the change in the vibrational stretch mode of either of the carbonyl functional groups that is involved during the complexation, from either vancomycin, the pentapeptide, or both simultaneously.

In the case of the ROA spectra no evident differences can be observed. Based on the strong presence of the Raman band of the pentapeptide at ~1440 cm^{-1}, one might expect the presence of the corresponding ROA -/+ couplet too in the ROA spectrum of the complex, especially in the 1:2 vancomycin:pentapeptide molar ratio. This is, however, not observed experimentally. The ROA signals in the amide I regions are weak, both for the pentapeptide, vancomycin, and the complex recordings, upon which no conclusions can be drawn. A subtraction of the ROA spectra of the complex with the spectrum of vancomycin, as performed for instance by Mensch et al. during the identification of the glycan moiety in RNase B,[25] is not usable here, as the signal intensities of the difference ROA spectra lay in the same order of magnitude as the inherent noise of the individual ROA spectra (see Figure S1).

The spectral results are of great importance for painting a more complete ROA spectral response picture of vancomycin, and with extension the antibiotic glycopeptide molecular class. During the analysis of the ROA spectrum of vancomycin in previous work it became evident that the aromatic portion dominates the spectrum, followed by a moderate contribution from its peptidic part, and barely any contribution from the carbohydrates.[7] Here, when adding the pentapeptide in the sample, no difference in the ROA intensities is observed, which is in accordance with the previous observations for vancomycin. To further evidence the indifferent response by ROA, simulated spectral calculations of the complex were performed, depicted in Figure 3. When comparing the Raman and ROA spectra of the complex with that of vancomycin alone, no drastic spectral differences occur. The most prominent difference is the positive ROA contribution around ~1100 cm^{-1} for the complex.

We delved deeper into the computational spectrum of the complex by assigning each of the normal modes to being vibrations located in vancomycin alone, the

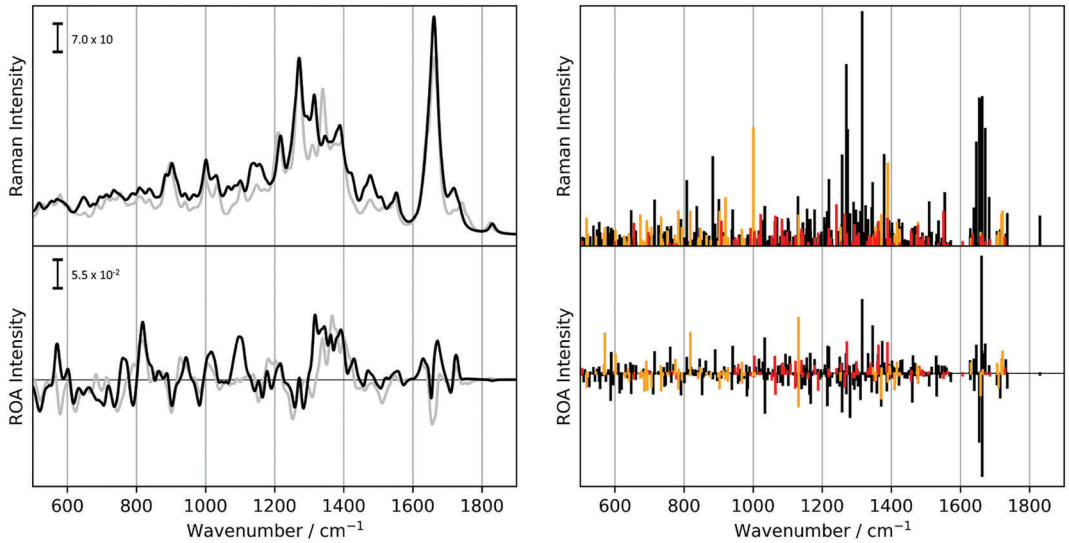

FIGURE 3 Calculated Raman and ROA spectra (left) of vancomycin alone (gray) and vancomycin bound to Ala-γGlu-Lys-D-Ala-D-Ala (black); the geometry of the latter is visualized in Figure 1b. The line spectrum of the calculation of the latter is given on the right. The colors indicate on what chemical entity the normal mode vibration is localized: vancomycin (black), Ala-γGlu-Lys-D-Ala-D-Ala (red), and both together (orange); these were automatically assigned by the algorithm described in Aerts et al.[7] [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 The results of an the automated normal mode analysis on the calculation of vancomycin bound to Ala-γGlu-Lys-D-Ala-D-Ala (labeled *pentapeptide*) in Figure 3: the number of normal modes assigned to vibrations localized on either vancomycin, Ala-γGlu-Lys-D-Ala-D-Ala, or both (first column), the contribution of each of the three categories to the total Raman intensity (second column; between brackets the average intensity carried by one normal mode with respect to the total intensity of all the normal modes) and to the total Raman optical activity (ROA) intensity (third column; between brackets the average intensity carried by one normal mode with respect to the total intensity of all the normal modes)

	No. of normal modes	Raman intensity	ROA intensity
Vancomycin	257 (61%)	70% (0.27%)	70% (0.27%)
Pentapeptide	79 (19%)	12% (0.15%)	12% (0.15%)
Complex	84 (20%)	18% (0.21%)	18% (0.22%)

pentapeptide alone, or the complex -the automated assignment as described by Aerts et al. was used.[7] A visual picture of the assignment can be consulted in Figure 3 and the quantitative analysis thereof can be found in Table 1. When inspecting these it becomes immediately apparent that vancomycin plays the largest role in both the Raman and ROA intensities. Not only are most of the normal modes attributable to vancomycin (61% for vancomycin, 20% for the complex, 20% for the pentapeptide), also the Raman and ROA intensity that is being carried by each normal mode vibrations on average of vancomycin is the highest (0.27% for vancomycin, 0.22% for the complex, 0.15% for the pentapeptide). Altogether, these computations support the experimental ROA observations and show why the inclusion of 201 ($3N - 6$; $N = 69$) additional normal modes the pentapeptide in the spectra is accompanied by minor spectral differences only. The small differences observed are then attributable to the change in nature of the vibrational modes with respect to the nature of those of the isolated molecules, manifested in frequency shifts and Raman/ROA intensity changes. In the case of the appearing band at ~1100 cm^{-1} for the complex it can be seen that the vibrational mode at 1130 cm^{-1}, originating from vancomycin, couples with vibrations from the pentapeptide, and happens to display a strong positive ROA intensity (see orange line transition at 1130 cm^{-1} in Figure 3). Finally, it should be noted that no direct comparative analysis between the experimental and calculated spectra has been conducted. At first instance, one can find certain discrepancies. However, from previous work we have found that a certain mismatch is inevitable and should, therefore, be tolerated.[7] Moreover, in the same work it was found that the estimation of the contributions of the different

molecular parts in the system to the Raman and ROA intensities is independent of the exact conformation or spectral (mis)match, justifying the usage of the current calculated spectra and normal mode analysis.

The final question that remains is: What information regarding the complex can be deduced from its ROA spectrum? We now know that the ROA spectrum of the complex is dominated by vancomycin, and therefore, its conformation within the complex is selectively targeted. It is possible to investigate the conformational behavior of the tricyclic part of vancomycin (see red part of the molecular structure of vancomycin in Figure 1a) in the sense that there is an ROA spectral response if the conformation is to change.[7] As there is no ROA spectral response, we can conclude that, based on ROA, no conformational change happens during the binding of the pentapeptide in the molecular part of vancomycin that ROA is sensitive to. In conclusion, we demonstrate that ROA can be a valuable tool when studying (drug-target) complexed systems, either complementing or confirming the findings in other studies.

4 | CONCLUSION AND FUTURE PERSPECTIVE

The antibiotic vancomycin bound to its biological target, the Lipid II cell wall precursor (here Ala-γGlu-Lys-D-Ala-D-Ala), has been investigated by means of Raman and ROA spectroscopy. Where minor spectral differences were observed between the Raman spectra of vancomycin and the complex, none were found between the corresponding ROA spectra. The reason for this is twofold: (1) vancomycin swamps the ROA intensities of the pentapeptide, making the latter ROA-invisible, and (2) the conformation of vancomycin does not alter upon binding its biological target, or at least in the molecular parts of vancomycin that can be examined by ROA. Thus, we end up with a clearer picture of the ROA spectral response of vancomycin and the general applicability of ROA in a pharmaceutical context.

Irrespective of the specific conclusions drawn based on ROA regarding the complex itself, the measurements expounded herein usher in a fresh chapter for the ROA technique. Namely, it is possible to measure systems that interact with each other and to draw useful structural conclusions. Of course, what exact structural information can be extracted is highly dependent on the system. Here, ROA is capable of examining a specific part of vancomycin, without interference from the biological target that is present in the sample. As such, ROA can play an important role in conformational characterization of interacting systems by complementing or confirming the results obtained using other methods. Hence, with this work, we hope to promote future investigations of interacting systems by means of ROA.

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