Structure of a model salt bridge in solution investigated with 2D-IR spectroscopy
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Citation for published version (APA):
Structure of a model salt bridge in solution investigated with 2D-IR spectroscopy

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Abstract

Salt bridges are known to be important for the stability of protein conformation, but up to now it has been difficult to study their geometry in solution. Here we characterize the spatial structure of a model salt bridge between guanidinium (Gdm$^+$) and acetate (Ac$^-$) using two-dimensional vibrational (2D-IR) spectroscopy. We find that as a result of salt bridging the infrared response of Gdm$^+$ and Ac$^-$ change significantly, and in the 2D-IR spectrum, salt bridging of the molecules appears as cross peaks. From the 2D-IR spectrum we determine the relative orientation of the transition-dipole moments of the vibrational modes involved in the salt bridge, as well as the coupling between them. In this manner we reconstruct the geometry of the solvated salt bridge.

The stabilization of molecular conformations by the attractive interaction between oppositely charged ions (salt bridges) is of great relevance in many areas of science. In particular, biological systems such as proteins, often contain salt bridges between ionic side chains that determine their structure and function. It is therefore fundamental to characterize the solvated structure of salt-bridged ion pairs, but this is unfortunately not possible with conventional methods like NMR. In this communication we report the study of a biologically-relevant ion pair, that formed by guanidinium (Gdm$^+$) and acetate (Ac$^-$), using two-dimensional infrared (2D-IR) spectroscopy. This ion pair is a model for salt bridges between an arginine and a carboxylate side group (from glutamate or aspartate), which occur commonly in proteins. The molecular structure of this ion pair is shown in Fig. 1A. Isolated Gdm$^+$ has D$_3$ symmetry and a degenerate mode at 1600 cm$^{-1}$ due to a combined CN$_3$ antisymmetric stretch and NH$_2$ scissors motion. This degeneracy is also observed in aqueous solution, but it is broken in viscous solvents.

Interestingly, when an equimolar amount of Ac$^-$ ions is added to the solution (guanidine acetate salt, >98% purity), this splitting becomes larger. It is known that Gdm$^+$ and Ac$^-$ have a strong binding affinity in DMSO, forming more than 98% dimers at the concentration used in our experiments. This suggests that the larger splitting between the Gdm$^+$ modes is due to an interaction with the Ac$^-$ ion. Moreover, Ac$^-$ (tetrabutylammonium acetate, >97% purity) has an absorption band at 1580 cm$^{-1}$ in DMSO (shown in Fig. 1B) due to the COO$^-$ antisymmetric stretch mode. This mode red-shifts after dimerization with Gdm$^+$. The change in the infrared response of both the Gdm$^+$ and the Ac$^-$ ions upon dimerization strongly suggests that there is a coupling between the vibrational modes of these two molecules.

The 2D-IR spectrum of Gdm$^+\cdots$Ac$^-$ confirms unambiguously that the Gdm$_{HF}^+$ and Gdm$_{LF}^+$ modes are both coupled to the COO$^-$ stretch mode of Ac$^-$.

FIG. 1. (A) Molecular structure of the Gdm$^+\cdots$Ac$^-$ dimer obtained using ab initio methods. The corresponding transition-dipole moments of the COO$^-$ stretch mode (1) and of the CN$_3$D$_6^+$ low and high frequency modes (2 and 3, respectively) are indicated by arrows. (B) Infrared absorption spectrum of Gdm$^+\cdots$Ac$^-$, Gdm$^+$ and Ac$^-$ in DMSO (400 mM, solvent subtracted). Shifts of the COO$^-$ and high-frequency CN$_3$D$_6^+$ bands are indicated by arrows.
mode has a smaller magnitude than these cross peaks because of its smaller absorption cross section, see Fig. 1A. Fig. 3B shows a cross section along the probe axis for $\nu_{\text{probe}} = \nu_{\text{COO}^-}$ and for parallel polarization of pump and probe pulses. The negative part at 1550 cm$^{-1}$ is due to the bleaching and $\nu = 1 \rightarrow 0$ stimulated emission of the COO$^-$ stretch mode on the diagonal. The positive region centered at 1580 cm$^{-1}$ is the low-probe-frequency tail of the diagonal induced absorption of the Gdm$^+_\text{HF}$ mode. The negative region at 1610 cm$^{-1}$ is the negative part of the cross peak between the COO$^-$ stretch mode and the Gdm$^+_\text{HF}$ mode.

In order to obtain structural information from the 2D-IR spectra, we model them with an exciton model, which is a well established way of analyzing 2D-IR data. It has been shown before that, in addition to coupling between the two CN$_3$D$_6^+$ modes, there is also energy transfer between them, but in DMSO this process is slow enough to be neglected at the delay at which we measured the 2D-IR spectra used for the structural analysis (2 ps). Figs. 2F and G show the calculated 2D-IR spectra, which are in very good agreement with the measured ones, specially considering that the difference 2D-IR spectrum between parallel and perpendicular polarizations is reproduced very well (even though it is not included in the fit). The couplings between the three modes involved in the salt bridge (Gdm$^+_\text{HF}$, Gdm$^+_\text{LF}$ and Ac$^-$), and the angles between their transition-dipole moments were parameters of the fit and are listed in Table I. The coupling between the Gdm$^+_\text{HF}$ and Gdm$^+_\text{LF}$ modes is 9 cm$^{-1}$, but a large coupling was expected between these vibrational modes because they share atoms and bonds. Remarkably, the coupling between each of the Gdm$^+_\text{HF}$ and Gdm$^+_\text{LF}$ modes and the COO$^-$ stretch mode is also large, (10 and 7 cm$^{-1}$, respectively) even though these vibrational modes neither share atoms nor bonds. This coupling rather originates from salt bridging between the two molecules, an interaction that has both an electrostatic and hydrogen-bond nature. The large couplings between the COO$^-$ and the Gdm$^+_\text{HF}$ and Gdm$^+_\text{LF}$ modes show that the large frequency shifts observed in the IR spectrum upon salt-bridge formation (Fig. 1A) are mostly due to splitting of the coupled modes rather than to a change...
in the local-mode frequencies. The angles between the transition-dipole moments of the salt-bridged vibrational modes are listed in Table I and these values are in agreement with a planar geometry. The planarity of a salt bridge is often taken as a metric of its quality in X-ray studies, [9] and our results seem to suggest that in DMSO solution, the geometry of an isolated salt bridge, in which steric constraints are absent, is indeed planar. We have performed complementary ab initio calculations on the Gdm$^+$···Ac$^-$ dimer using Gaussian03 [10] at MP2/6-311+G(d) level of theory. The calculation predicts an approximately planar geometry for the salt bridge, in which all three transition-dipole moments lie almost in the same plane, as shown in Fig. 1A. The angle between the transition-dipole vectors of the two Gdm$^+$ modes is smaller than for isolated guanidinium [5], most likely as a result of salt bridging with the Ac$^-$ ion. We find in the calculation that the Gdm$^+$HF mode is antisymmetric with respect to the symmetry axis through the C-C bond of Ac$^-$, which explains the large coupling with the also antisymmetric COO$^-$ stretch mode. [11] The Gdm$^+$LF mode is symmetric with respect to this symmetry axis, so it is remarkable that it also couples strongly to the COO$^-$ stretch mode, despite their different symmetry.

In conclusion, we were able to detect the existence of a salt bridge between Gdm$^+$ and Ac$^-$ in solution using 2D-IR spectroscopy. We characterize the coupling between two CN$_3$D$_6^+$ modes of Gdm$^+$ and the COO$^-$ stretch mode of Ac$^-$. We find that the COO$^-$ stretch mode couples more strongly to the high-frequency CN$_3$D$_6^+$ mode than to the low-frequency one, most likely because of their similar symmetry. We determine the geometry of the salt bridge and find that it is in good agreement with a salt-bridge geometry in which the Gdm$^+$ and COO$^-$ moieties are coplanar.

TABLE I. Coupling $\beta$ and angle $\theta$ between the transition-dipole moments of the COO$^-$ stretch of Ac$^-$ and the two CN$_3$D$_6^+$ modes of Gdm$^+$ as obtained from the fit ($\chi^2 = 43$).

<table>
<thead>
<tr>
<th>Modes</th>
<th>$\beta$ (cm$^{-1}$)</th>
<th>$\theta$</th>
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<tbody>
<tr>
<td>Gdm$^+$HF-Gdm$^+$HF</td>
<td>9</td>
<td>80$^\circ$</td>
</tr>
<tr>
<td>Gdm$^+$HF-Ac$^-$</td>
<td>10</td>
<td>-3$^\circ$</td>
</tr>
<tr>
<td>Gdm$^+$LF-Ac$^-$</td>
<td>7</td>
<td>80$^\circ$</td>
</tr>
</tbody>
</table>

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