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Conformation of poly-L-glutamate is independent of ionic strength

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Abstract

CD and UV resonance Raman measurements surprisingly find that the charge screening of even 2 M concentrations of NaCl and KCl do not alter the unfolded PPII and 2.5_1-helix conformations of poly-L-glutamate. These salts appear to be excluded from the region between the side chain charges and the peptide backbone. Furthermore, no direct ion pairing occurs between these salts and the side chain carboxylates.

Keywords

poly-L-glutamate; PPII; 2.5_1-helix; salt exclusion; UV resonance Raman

1. Introduction

The conformations of peptides and proteins depend upon their solution compositions, especially upon the presence of species that specifically interact with the peptides or proteins, or the water solvent.[1–3] In the work here we investigate the dependence of peptide conformation on the presence of salts. Previous studies appear to have demonstrated that ions can interact with peptides and proteins by binding, for example, to form ion pairs.[4–6] Alternatively the impact of ions can be less specific, as when they passively screen sidechain electrostatic interactions.[7] Another mechanism that can impact conformation occurs in the classical Hofmeister series of protein salting in or salting out, that is explained by the impact of ions on the water solvent properties that control aqueous solution protein/peptide hydration.[8]

In this work, we used circular dichroism (CD) and UV resonance Raman spectroscopy (UVRR) to investigate the impact of Na^+ and K^+ on the conformation of poly-L-glutamate (PGA). Surprisingly, we observe a lack of pertubation by high concentrations of Na^+ and K^+ on the conformation of poly-L-glutamate. We find that PGA is not converted to the α-helix conformation at high NaCl and KCl concentrations, and further that the equilibrium between PPII and 2.5_1-helix conformations of PGA is not altered by the presence of 2 M salts.

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Supporting Information
CD spectra at various temperatures: Figures S1 and S2.

2. Results and Discussion

2.1 CD results

The CD spectrum of PGA in pure water at 10 °C (black, Fig. 1) shows a positive band at ~217 nm and a strong negative band at ~197 nm, characteristic of PPII-like conformations.[9, 10] The 0.2 M NaCl or KCl solution spectra are essentially identical to that in pure water. In 1.0 M NaCl or KCl, the positive band slightly decreases in amplitude while the trough becomes less negative, indicating only a slight destabilization of the PPII-like conformation.[10] The PPII-like features slightly further decrease in 2.0 M salt solutions; the NaCl and KCl CD spectra remain identical. These results are consistent with Lizuka et al [11] and Wada's [12] experimental investigations.

We calculated the salt induced fractional α-helical concentration, f_α, by using a two-state model (eq 1) by utilizing the reported θ_{223} value for the pure α-helix of PGA ($[\theta]_\alpha = -35400$ deg.cm^2.dmol^{-1} [13]) and our measured value for PGA in pure water where unfolded conformations dominate [14] ($[\theta]_{unfold} = 3250$ deg.cm^2.dmol^{-1}). The calculated α-helical concentration increases in 1 M and 2 M salts are 0.02 ± 0.01 and 0.04 ± 0.01, respectively. This clearly indicates the negligible α-helical concentration change.

$$f_\alpha = \frac{[\theta]_{223} - [\theta]_{unfold}}{[\theta]_\alpha - [\theta]_{unfold}} \quad \text{eqn1}$$

The CD spectra measured at 30 °C and 50 °C (See Fig. S1–2) also indicate that similar high NaCl or KCl concentration do not significantly alter the PGA conformational equilibrium.

2.2 UVRR results

The UVRR of PGA in pure water (Fig. 2a) show an AmI band at ~1667 cm^{-1} (mainly CO s), an AmII band at ~1568 cm^{-1} (mainly out of phase combination of CN s and NH b), and (C)C_α-H bending bands at ~1395 cm^{-1}. The AmIII region which mainly involves an in phase combination of CN s and NH b occurs between ~1200 cm^{-1} and ~1340 cm^{-1}. The AmIII region contains an $AmIII_2$ band doublet at ~1298 cm^{-1} and ~1317 cm^{-1}, and an $AmIII_3$ band doublet at ~1247 cm^{-1} and ~1269 cm^{-1}. The $AmIII_3$ band at ~1247 cm^{-1} derives from a PPII-like conformation, while the $AmIII_3$ band at ~1269 cm^{-1} derives from a 2.5_1-helix conformation. [14]

In 2 M NaCl and KCl, the C_α-H intensity slightly decreases compared to pure water, indicating a small α-helix content increase.[15] Also, the $AmIII_3$ region intensity slightly decreases.[1, 16] Surprisingly, the relative intensity of PPII to 2.5_1-helix bands does not change, even though the 2.5_1-helix conformation is stabilized by electrostatic repulsion between side chains.[14, 17] The intensity in the AmI region increases dramatically because of a surprising increase in the Raman cross section of the underlying ~1660 cm^{-1} water O-H bending band due to the presence of a $Cl^- \rightarrow$ water charge transfer band.[18–20]

To calculate the magnitude of the salt induced α-helical conformation concentration increase, we subtracted the UVRR of PGA in pure water (where it exists in a PPII-like and 2.5_1-helix conformation equilibrium [14]) from that of PGA in 2 M NaCl and KCl such that the C_α-H b intensity was minimized in the UVRR difference spectrum. The resulting PGA pure water UVRR intensities subtracted are directly proportional to the concentration of the PPII-like and 2.5_1-helix conformations at each temperature. From the difference spectra we can calculate a fractional α-helical concentration increase of 0.08 ± 0.03 and 0.07 ± 0.03 for 2 M NaCl and KCl, respectively. Thus, neither NaCl nor KCl induces much PGA α-helix formation.

NaCl and KCl occur in the middle of Hofmeister series, indicating that NaCl and KCl should have intermediate effects on the dehydration of PGA.[8] Collins et al.'s model of matching water affinities [4–6], that predicts that preferential ion pair formation occurs between oppositely charged ions of similar charge densities, predicts that the penultimate carboxylate would preferentially ion pair with Na^+ compared to K^+.[5] This is supported by recent experimental and theoretical studies showing that $-COO^-$ groups preferentially pair with Na^+. [21–25]

We measured the UVRR of Na^+ (K^+) acetate in the presence of 4 M NaCl (KCl). Previous studies indicate that ion binding to the $-COO^-$ groups significantly shifts the carboxylate symmetric stretching band, $\nu_{s\,(COO^-)}$ and the C-C stretching band, $\nu_{(C\text{-}C)}$ to higher frequencies.[26, 27] The $\nu_{s\,(COO^-)}$ and $\nu_{(C\text{-}C)}$ band frequencies do not change (Fig. 3) in 4 M Na^+ and 4 M K^+, indicating that neither Na^+ nor K^+ directly binds to the COO^- groups.

Electrostatic repulsion between glu side chains is responsible for the formation of the 2.5_1-helix conformation because it minimizes the repulsion between its splayed side chains.[14, 17] Surprisingly we observe a lack of a NaCl or KCl dependence of the PPII and 2.5_1-helix PGA conformational equilibrium. We expected that high salt concentrations would decrease the electrostatic repulsion between glu side chains. We can estimate the electrostatic repulsion decrease induced by salt screening from eq 2:

$$\psi(l)=\psi_0 \cdot \frac{\varepsilon_w}{\varepsilon_r} \cdot e^{-l\cdot\kappa} \qquad \text{eqn2}$$

where $\psi(l)$ is the electrostatic potential at distance l in presence of salt screening; ψ_0 is the electrostatic potential with no screening; ε_w is the dielectric constant of pure water (ε_w=83 [28]); ε_r is the dielectric constant of salt solutions (ε_r in 0.2 M, 1.0 M and 2.0 M NaCl and KCl solutions are 80, 70 and 60, respectively.[28, 29]); κ^{-1} is the Debye length which is defined by eq 3 [7]:

$$\kappa^{-1}=\sqrt{\frac{\varepsilon_r\varepsilon_0 k_B T}{2N_A e^2 I}} \qquad \text{eqn 3}$$

where ε_0 is the permittivity of free space (ε_0= 8.854× 10^{-12} $C^2\cdot N^{-1}\cdot m^{-2}$); k_B is the Boltzmann constant (k_B=1.380 ×10^{-23} $N\cdot m\cdot K^{-1}$); T is the absolute temperature (T=283.15 K); N_A is the the Avogadro number (N_A= 6.022×10^{23} mol^{-1}); e is the elementary charge (e= 1.602×10^{-19} C); I is the ionic strength of the salt solutions. The Debye lengths, κ^{-1} in 0.2 M, 1.0 M and 2.0 M NaCl and KCl solutions are ~6.8 Å, 2.8 Å and 1.9 Å, while the distances between neighboring glu sidechain charges are 8.3 Å and 8.4 Å for the PPII and 2.5_1-helix conformations, respectively.[14] We estimate that the glu sidechain electrostatic repulsion should decrease by more than 50-fold at 2 M salt relative to that in pure water.

We naively expected that this change in electrostatic interactions should alter the equilibrium between the PPII and 2.5_1-helix conformations. The fact that this does not occur suggests that the ions are excluded from the region between side chains. This would also explain why the 2.5 M NaCl does not alter the PPII and 2.5_1-helix conformational equilibrium in polylys.[30] It should be noted that our experimental results contradict the recent molecular dynamics simulation studies that indicate that Na^+ controls peptide conformations by binding to carboxylate side chain. [31, 32]

3. Conclusion

We used circular dichroism and UV resonance Raman spectroscopy, to study the impact of screening of NaCl and KCl on the conformational equilibria of poly-L-glutamate. In contradiction to expectations, we observe a lack of impact of high concentrations of NaCl and KCl on the conformation of poly-L-glutamate. These salts appear to be excluded from the region between the side chain charges and the peptide backbone. Furthermore, we see no evidence of formation of ion pairs between Na^+ and K^+ salts and the side chain carboxylates.

4. Experimental Methods

Samples

PGA (MW_{vis}=11600, MW_{mALLS}=6649) was purchased from Sigma Chemical. Peptide samples were prepared at 1 mg/ml concentrations by dissolving PGA in pure water or 2 M salt, and adjusted to pH 8.3. Sodium acetate (>99% purity) was purchased from EM Science; potassium acetate (>99% purity) was purchased from Sigma.

CD Spectra

CD spectra were measured for poly-L-glutamate by using a Jasco-715 spectropolarimeter with a 0.02 cm path length cuvette. We collected CD spectra from 250 – 190 nm. We utilized 3-min accumulation times and five accumulations were averaged.

UVRR Spectra

The UVRR apparatus has been described in detail by Bykov et al.[33] Briefly, 204 nm UV light (2 mW average power, 100 μm diameter spot, 25–40 ns pulse width) was obtained by mixing the 3rd harmonic with the 816 nm fundamental of a 1 kHz repetition rate tunable Ti:Sapphire laser system (DM20-527 TU-L-FHG) from Photonics Industries. The sample was circulated in a free surface, temperature-controlled stream. A 165° sampling backscattering geometry was used. The collected light was dispersed by a double monochromator onto a back thinned CCD camera with a Lumogen E coating (Princeton Instruments-Spec 10 System). We utilized 5-min accumulation times, and four accumulations were averaged.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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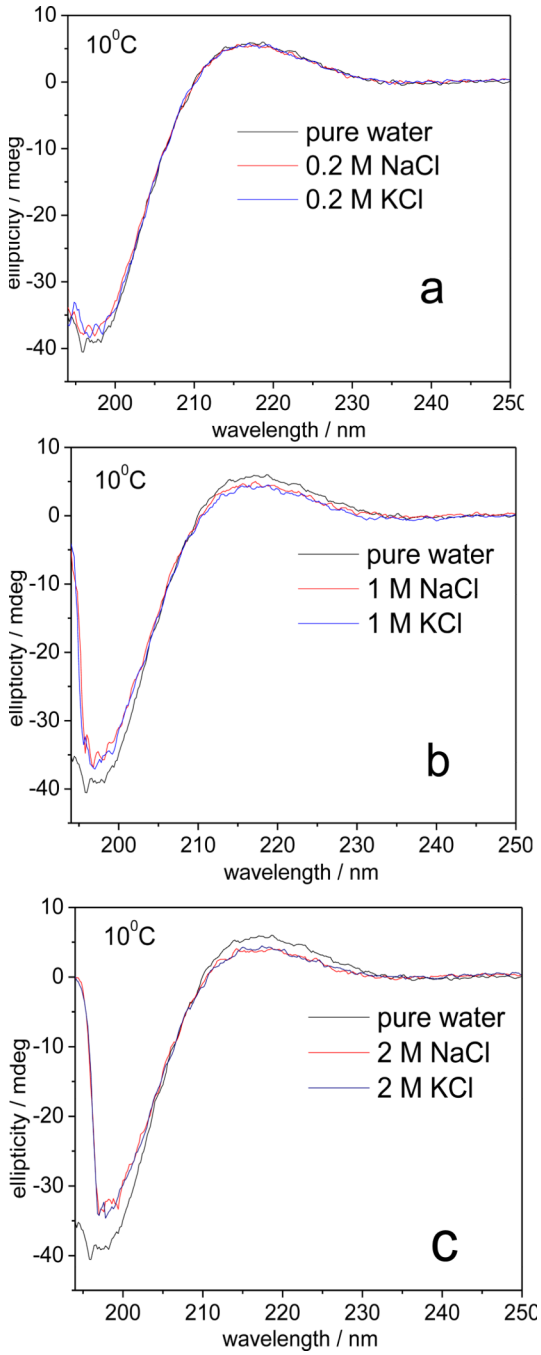

Figure 1.
CD spectra of 1 mg/ml PGA in pure water and in a) 0.2 M, b) 1.0 M and c) 2 M NaCl and KCl at pH 8.3 at 10 °C.

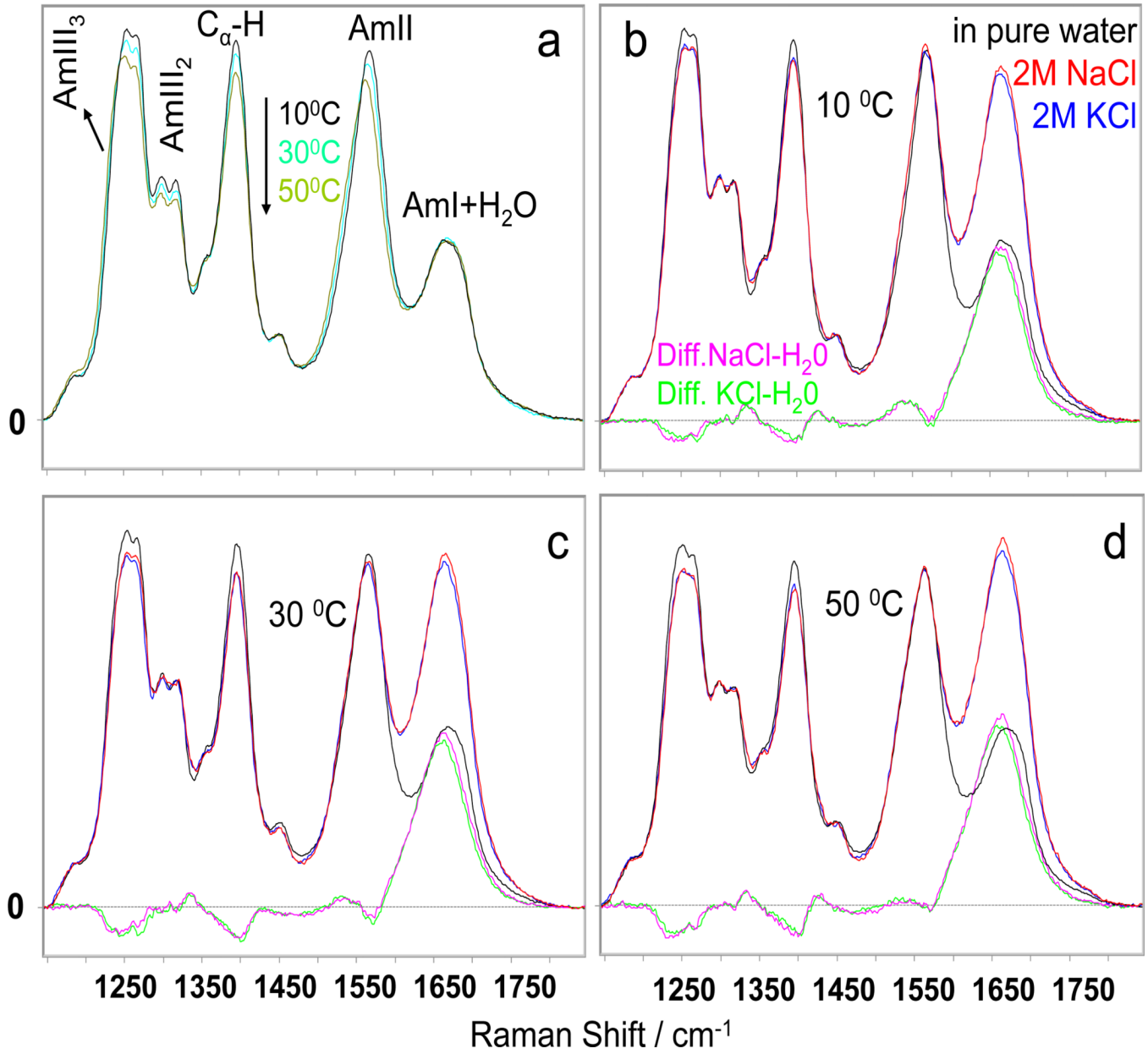

Figure 2.

a) Temperature dependence of 204 nm excited UVRS of 1 mg/ml PGA in pure water. UVRS of PGA in pure water and in the presence of 2 M NaCl and KCl and their difference spectra: b) at 10 °C; c) at 30 °C; d) at 50 °C; All spectra were normalized to the 1450 cm^{-1} band which shows little intensity variation. [14]

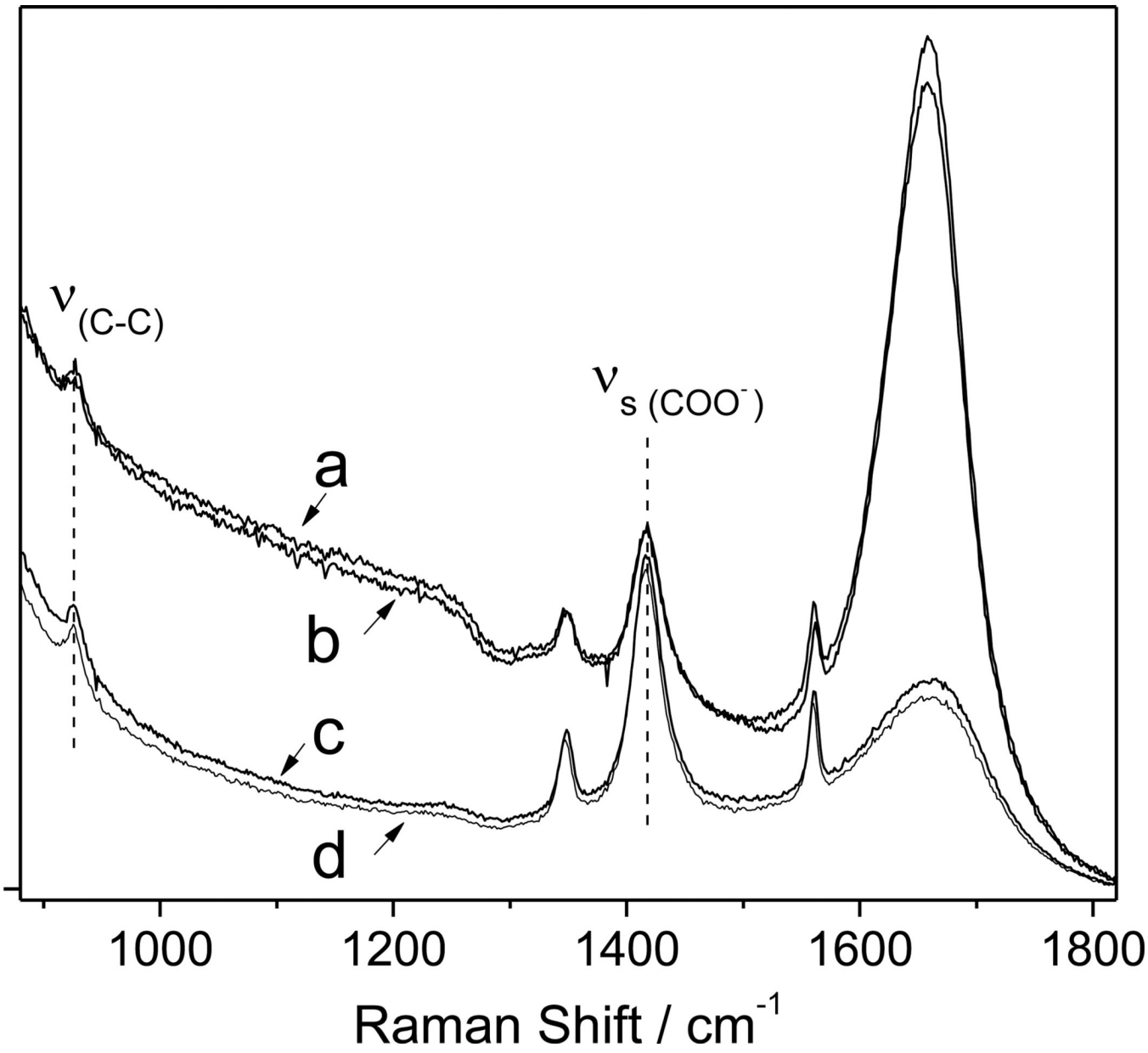

Figure 3.
204 nm excited UVRS of sodium acetate (NaAc) and potassium acetate (KAc) and in the presence of 4 M NaCl and 4 M KCl at 10 °C: a) 0.02 M NaAc + 4 M NaCl; b) 0.02 M KAc + 4 M KCl; c) 0.02 M KAc; d) 0.02 M NaAc.