Utilization of IR probes for studying the chosen biologically important systems

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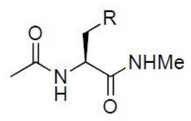
β-Isocyanoalanine as an IR probe: comparison of vibrational dynamics between isonitrile and nitrile-derivatized IR probes

Abstract

*A vibrational probe based on isonitrile (NC)-derivatized alanine **1** (Ac-Ala(NC)-NHMe) was synthesized and its structure and vibrational dynamics of its NC stretch mode were examined utilizing FTIR and femtosecond IR pump−probe spectroscopy. It has been found that the probe is characterized by very high sensivity to the hydrogen bonding environment and in comparison to nitrile (CN) the NC stretch mode possesses larger dipole strength when attached to the aliphatic group. Its vibrational lifetime is several times longer than that of azido (N_3) stretch mode in azido-derivatized IR probes and it was determined to be 5.52 ps and 5.53 ps in D2O and DMF, respectively. Furthermore, to understand the phenomenon of the vibrational solvatochromism of the new IR probe quantum chemistry calculations of methyl isocyanide in water clusters were carried out and the distributed site model for vibrational solvatochromism was applied.*

FTIR spectroscopy



0. R = H
1. R = NC
2. R = CN
3. R = N_3

Figure 1. *Structures of compounds **0**−**3** used for IR study.*

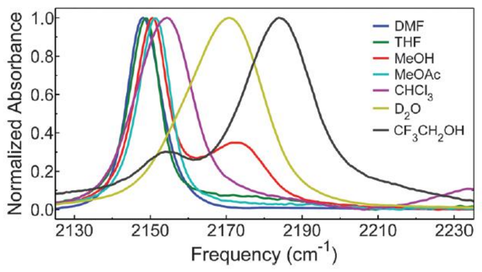


Figure 2. *Isonitrile (NC) stretch FTIR spectra of **1** in various solvents.*

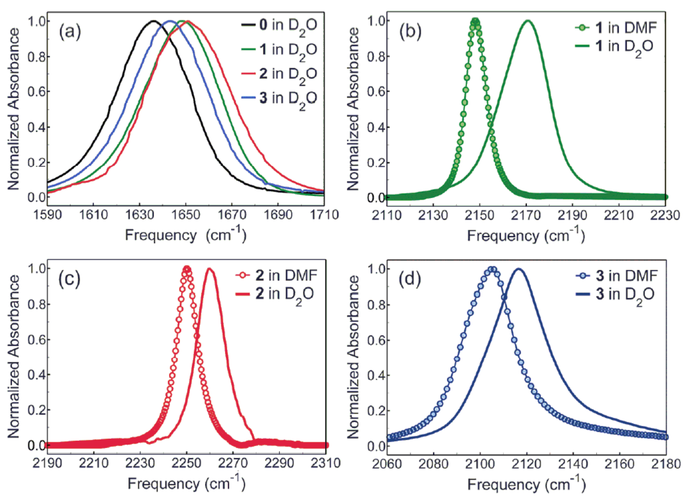


Figure 3. *(a) Amide I' FTIR spectra of **0**–**3** in D_2O. (b–d) Isonitrile (NC), nitrile (CN), and azido (N_3) stretching FTIR spectra of **1**–**3** in DMF and D_2O.*

Polarization controled IR pump–probe spectroscopy

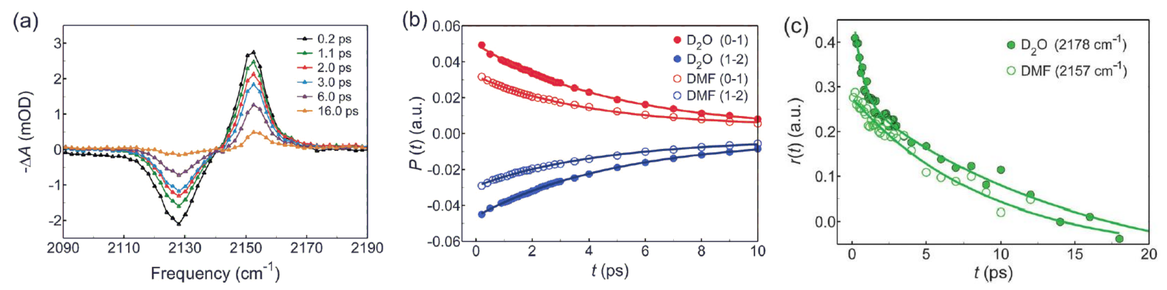


Figure 4. *Polarization-controlled IR pump–probe data of **1**. (a) Isotropic IR pump–probe spectra of **1** in DMF at the delay time t. (b)Vibrational population decays of **1** in D_2O and DMF. (c) Anisotropy decays of **1** in D_2O and DMF.*

Table 1. *Spectral properties of **1**–**3**.*

	1		**2**		**3**	
	D_2O	DMF	D_2O	DMF	D_2O	DMF
ω_{center} (cm^{-1})	2169.53	2151.63	2260.37	2250.21	2116.05	2103.59
FWHM (cm^{-1})	24.14	10.58	14.33	10.98	28.95	26.36
ε ($cm^{-1}M^{-1}$)	142	390	14	27	349	333
D* (Debye^2)	1.82	2.21	0.06	0.15	5.49	4.80
T_1 (ps)	5.52±0.18	5.53±0.17	-	-	1.14±0.04 (0.10)** 0.13±0.01 (0.24)**	1.34±0.08 (0.10)** 0.065±0.004 (0.15)**
τ_{or} (ps)	10.33±2.24* 0.52 ± 0.20	7.28±0.94	-	-	4.50±0.95	3.82±1.98

Computational data

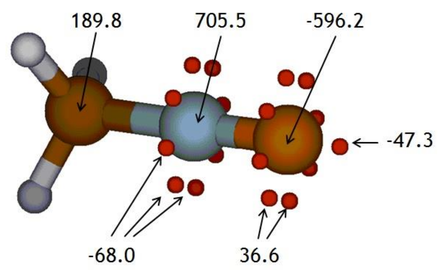


Figure 5. *Positions of the distributed interaction sites for MeCN. The unit of solvatochromic coefficients shown is [cm^{-1}/V].*

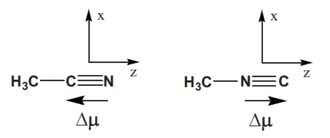


Figure 6. *Directions of the solvatochromic dipole moment in MeCN and MeNC molecules.*

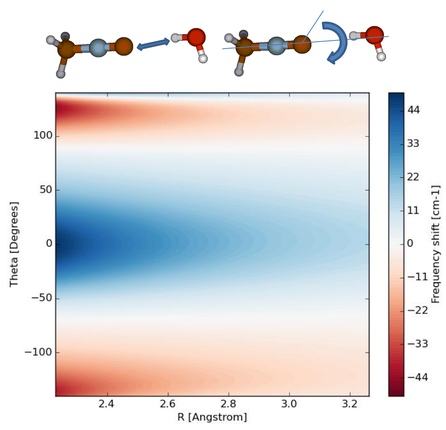


Figure 7. *Scan of the frequency shift depending on the MeNC – water hydrogen bond lenght and the angle θ.*

Osmolytes impact on the structure and dynamics of horse heart myoglobin

Abstract

Osmolytes are the organic compounds which can affect the proteins stability and they contribute to proteins folding. The certain mechanism of osmolytes protective effect is not fully explained. In general two possible ways are considered - the direct interaction of osmolytes with protein (water replacement hypothesis) and the indirect way of interaction (vitrification hypothesis). In order to know more about the effect of the osmolytes on protein-water environment myoglobin-osmolytes systems were studied using FTIR, UV-VIS, CD and pump-probe spectroscopy. Interestingly, a change in the lifetime of CO myoglobin, and a change in the UV-VIS and CD spectra upon addition of the osmolytes (glycine betaine and sorbitol) were observed. In addition, the temperature-dependent CD studies revealed the different behaviour of protein unfolding process in case of GB and sorbitol.

UV-VIS spectroscopy

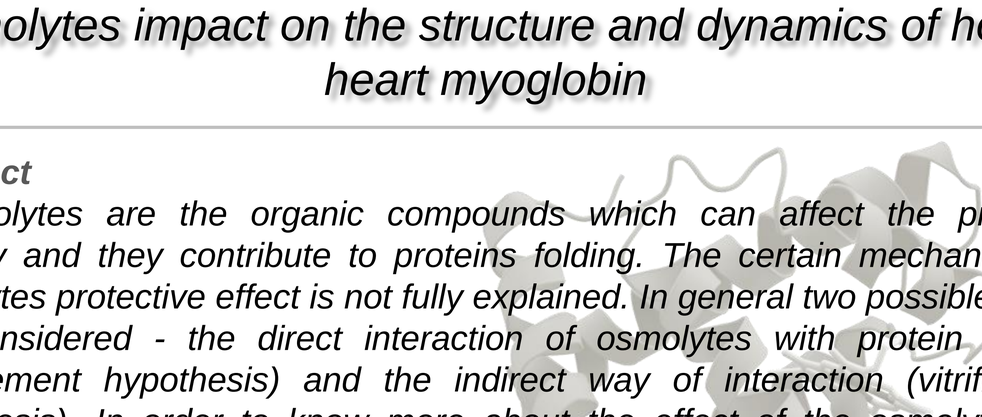


Figure 8. *Concentration-dependent UV-VIS spectra of (a) metmyoglobin in sorbitol solutions, (b) metmyoglobin in glycine betaine solutions, (c) COmyoglobin in sorbitol solutions and (d) COmyoglobin in glycine betaine solutions .*

References

[1] Kim H. and Cho M. *Chem. Rev.* **2013**. *113*, pp 5817–5847,

[2] Maj M, Ahn C, Kossowska D, Park K, Kwak K, Han H, Cho M. *Phys. Chem. Chem. Phys.* **2015**. *17*, 11770-11778,

[3] K. L. Koster et al. *Biochim. Biophys. Acta* **1994.** *1193*, 143-150.

FTIR and polarization controled IR pump–probe spectroscopy

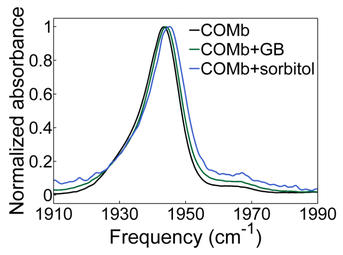


Figure 9. *FTIR spectra of COmyoglobin in various osmolytes solutions.*

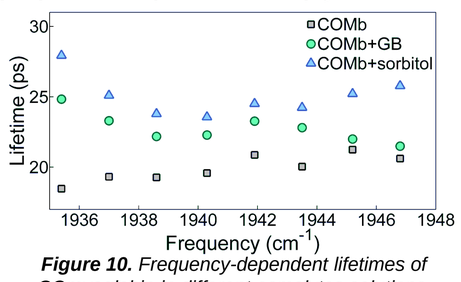


Figure 10. *Frequency-dependent lifetimes of COmyoglobin in different osmolytes solutions.*

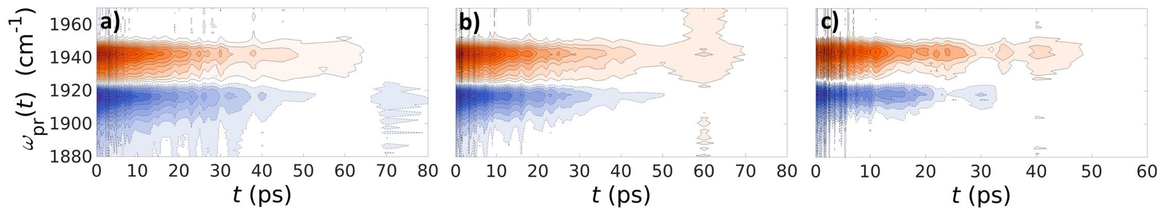


Figure 11. *IR pump-probe spectra of COmyoglobin in (a) buffer, (b) 2.0 M glycine betaine and (c) 2.0 M sorbitol solutions.*

Circular dichroism spectroscopy

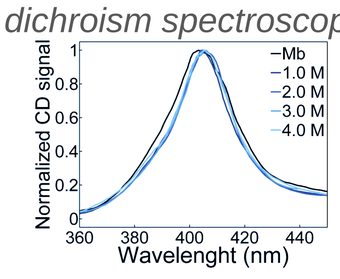


Figure 12. *Concentration-dependent CD spectra of metmyoglobin in sorbitol solutions (Soret band).*

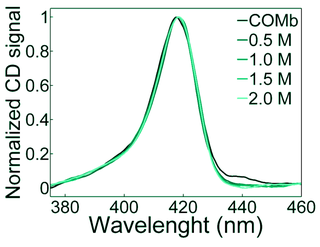


Figure 13. *Concentration-dependent CD spectra of COmyoglobin in glycine betaine solutions (Soret band).*

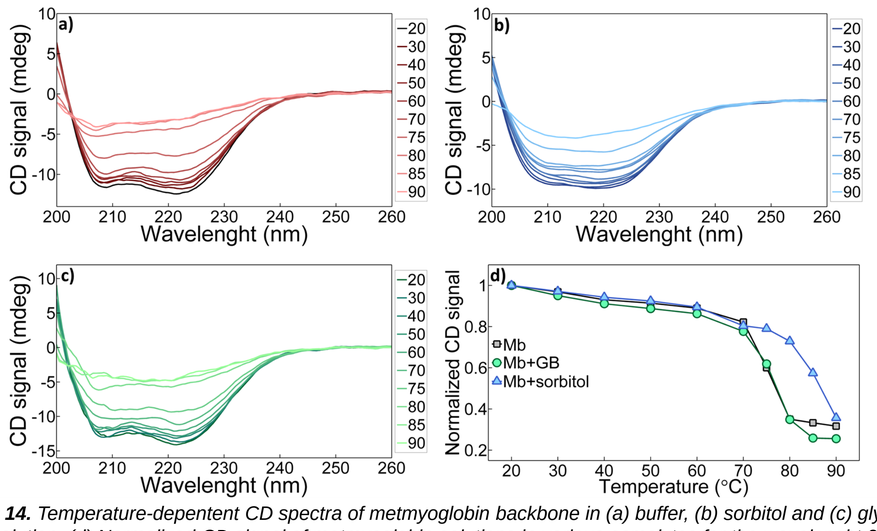


Figure 14. *Temperature-depentent CD spectra of metmyoglobin backbone in (a) buffer, (b) sorbitol and (c) glycine betaine solution. (d) Normalized CD signal of metmyoglobin solutions in various osmolytes for the wavelenght 222 nm.*