Do Osmolytes Impact the Structure and Dynamics of Myoglobin?

Dorota Kossowska[12], Kyungwon Kwak[12*] and Minhaeng Cho[12*]

[1]Center for Molecular Spectroscopy and Dynamics, Institute for Basic Science (IBS), Korea University, Seoul 136-701, Republic of Korea

[2]Department of Chemistry, Korea University, Seoul 136-713, Korea

[*]kkwak@korea.ac.kr, mcho@korea.ac.kr

Abstract

Osmolytes are small organic compounds that can affect the stability of proteins in living cells. The mechanism of osmolytes' protective effects on protein structure and dynamics has still not been fully explained. However, two possible mechanism have been suggested and discussed - a direct interaction of osmolytes with proteins (water replacement hypothesis) and an indirect interaction (vitrification hypothesis). In order to investigate the effects of osmolytes on protein structure in an aqueous environment, we studied myoglobin-osmolyte systems using FTIR, UV-vis, CD, and femtosecond IR pump-probe spectroscopy. Interestingly, noticeable changes are observed in both the lifetime of the CO stretch of CO-bound myoglobin and the spectra of UV-vis, CD, and FTIR upon addition of the osmolytes. In addition, the temperature-dependent CD studies reveal that the protein's thermal stability depends on molecular structure, size of osmolytes and hydrogen-bonding ability. We believe that the present experimental results provide important clues about the complicated and intricate mechanism of osmolyte effects on protein structure and dynamics in a crowded cellular environment.

UV-VIS spectroscopy

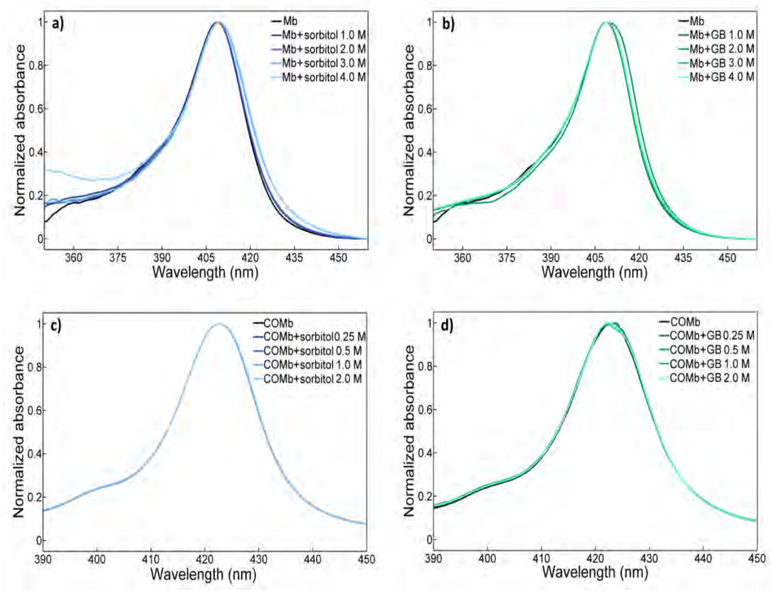

Figure 1. *Concentration dependent UV-vis spectra of (a) myoglobin in the sorbitol solutions, (b) myoglobin in the glycine betaine solutions, (c) carbonmonoxy-myoglobin in the sorbitol solutions, and (d) carbonmonoxy-myoglobin in the glycine betaine solutions.*

Circular dichroism spectroscopy

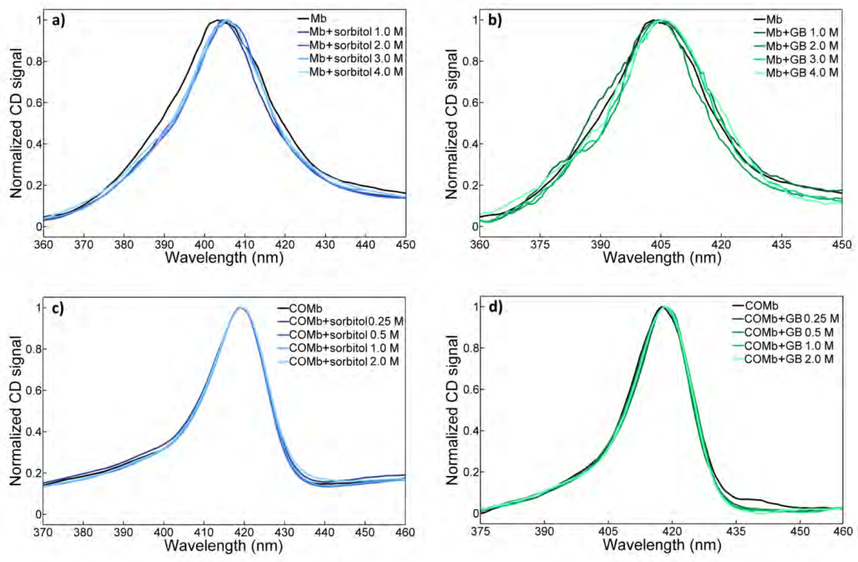

Figure 2. *Concentration-dependent CD spectra (Soret band) of (a) metmyoglobin in sorbitol solutions, (b) metmyoglobin in glycine betaine solutions, (c) carbonmonoxy-myoglobin in sorbitol solutions and (d) carbonmonoxy-myoglobin in glycine betaine solutions.*

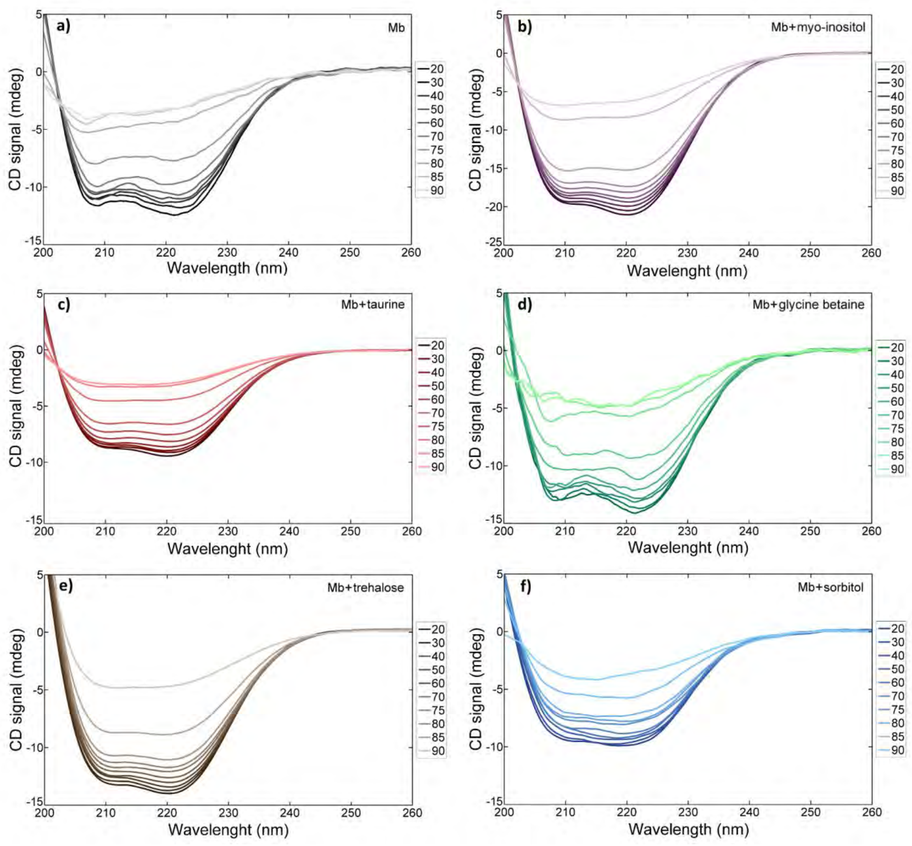

Figure 3. *Temperature-dependent CD spectra of metmyoglobin backbone in (a) 7.0 pH phosphate buffer, (b) 0.9 M myo-inositol, (c) 0.9 M taurine, (d) 2.0 M glycine betaine, (e) 1.5 M trehalose and (f) 2.0 M sorbitol.*

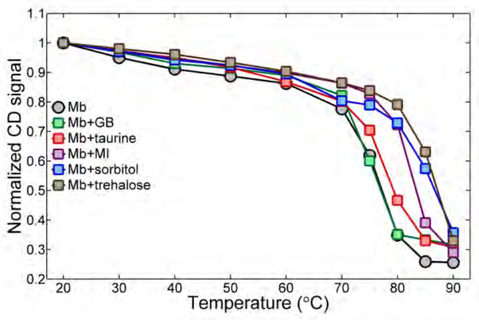

Figure 4. *Temperature-dependent CD signal of metmyoglobin backbone in different solutions: 7.0 pH phosphate buffer, 2.0 M sorbitol, 2.0 M glycine betaine, 1.5 M trehalose, 0.9 M taurine and 0.9 M myo-inositol. The data was normalized by dividing by the minimum CD signal value. Data shown is taken at the 222 nm wavelength, which is the minimum of the backbone signal..*

References

[1] D. Kossowska, K. Kwak, and M. Cho, *Molecules* **2018**, *23*, 3189,

[2] J. S. Clegg et al., *Cryobiology* **1982**, *19*, 306-16,

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

FTIR and polarization controled IR pump–probe spectroscopy

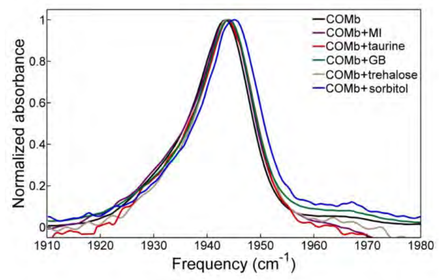

Figure 5. *FTIR spectra of carbonmonoxy-myoglobin in various osmolytes solutions.*

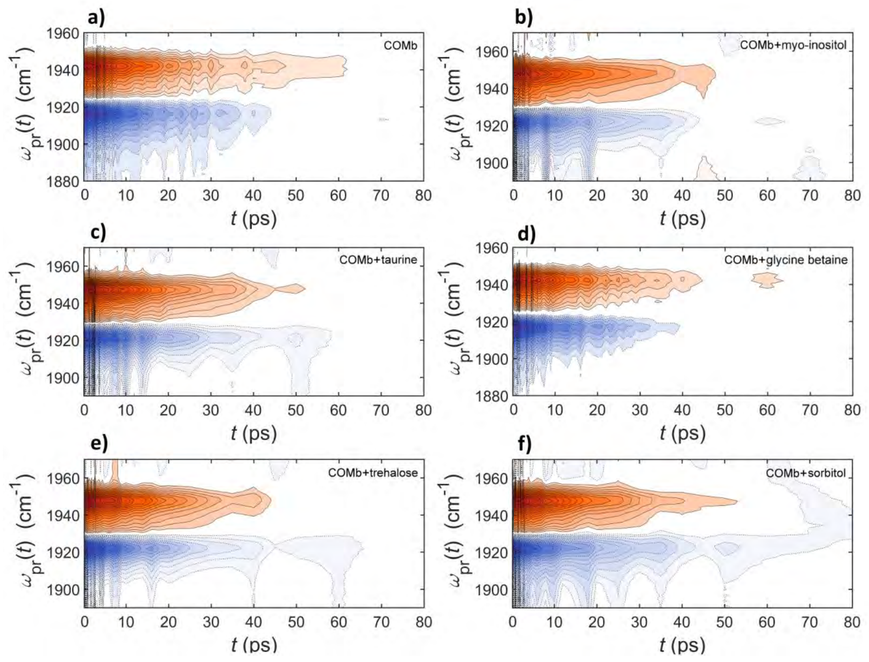

Figure 6. *Isotropic pump-probe spectra of carbonmonoxy-myoglobin in the following solutions: (a) phosphate buffer in D2O, (b) 0.9 M myo-inositol, (c) 0.9 M taurine, (d) 3.0 M glycine betaine, (e) 1.5 M trehalose and (f) 3.0 M sorbitol..*

Table 1. *Spectral properties of COMb in different osmolytes solutions. "MI" stands for myo-inositol, and "GB" for glycine betaine.*

		COMb	COMb + MI 0.9 M	COMb + Taurine 0.9 M	COMb + GB 3 M	COMb + Trehalose 1.5 M	COMb + Sorbitol 3 M
ω_{center} (cm^{-1})	A_1	1943.0	1943.8	1943.8	1943.9	1944.0	1944.6
	A_3	1930.5	1931.3	1933.2	1935.6	1935.2	1933.9
	A_0	1967.6	1963.2	1967.3	1967.2	1966.9	1965.1
T_1 (ps)		19.1 ± 0.5	18.9 ± 0.9	19.7 ± 0.8	20.1 ± 0.6	20.5 ± 0.5	20.4 ± 0.6

Summary

- The addition of the osmolytes does not cause any strong perturbation to the inner active site of the protein.
- Sorbitol induces the largest frequency shift in FTIR.
- myoglobin unfolding temperature increases in the following order: Mb and Mb + GB (75 °C) < Mb + taurine (79 °C) < Mb + MI (83 °C) < Mb + sorbitol and Mb + trehalose (90 °C).
- Polyols like trehalose and sorbitol having multiple H-bonding sites are found to be protein-protectants.
- There is no direct evidence for the direct interaction of the osmolytes with protein.