Is Your Protein Misbehaving?

Stabilize Your Protein Sample with Choline-*O*-Sulfate!

Certain small organic solutes, also known as "osmolytes" or "chemical chaperones," are naturally occurring molecules that accumulate in the intracellular environment of microbial, plant, and animal cells. These compounds act to increase the thermodynamic stability of folded proteins without perturbing other cellular processes.[1] One such "osmolyte," choline-*O*-sulfate (COS), is synthesized by a variety of plants, lichens, algae, fungi and bacteria. It serves as an osmoprotective compound in halophytic plants subjected to salt stress and has proved to be osmoprotective for several gram-negative bacterial species and the fungus *Penicillium fellutanum*.[2]

Dr. Wakamatsu from Gunma University (Gunma, Japan) has borrowed lessons learned from Mother Nature by pioneering the use of COS and COS-d_{13} for use in protein NMR studies. As presented here, his results show **significant improvement in stabilizing soluble forms of protein in solution** with a concurrent increase in sensitivity in multidimensional NMR data sets acquired at near human physiological temperatures.

Advantages of Choline-*O*-Sulfate:

- Prevention of thermal denaturation of membrane proteins, including GPCRs
- Prevention of precipitation of protein and protein/peptide complexes
- Facilitation of NMR measurements, especially at elevated temperatures
- Improvement of protein recovery during purification

CIL is proud to offer unlabeled COS for the worldwide protein NMR community. Once you are satisfied with the results using unlabeled COS, please ask your local sales representative for a quote for uniformly deuterated COS (COS-d_{13}).

Catalog No.	Description	Size	Price
ULM-8703	Choline-*O*-Sulfate	1 g	Inquire
ULM-8703	Choline-*O*-Sulfate	10 g	Inquire

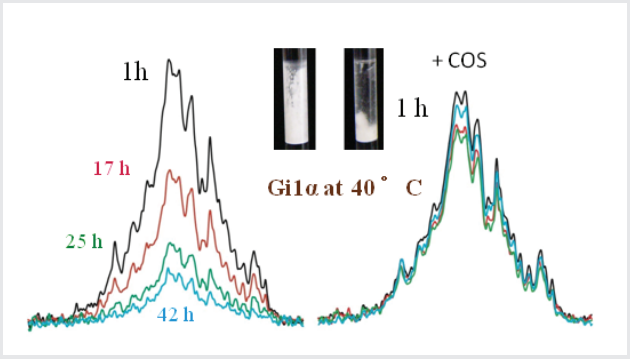

Figure 1. 1D HSQC spectra of uniformly ^{15}N labeled Gi1α in the absence (left) or presence of 1 *M* COS (right) were recorded on a Bruker ARX-400 spectrometer at 40°C at 1 h (black), 17 h (red), 25 h (green), and 42 h (cyan) after temperature control. The photographs demonstrate much less precipitate in the presence of COS at 43 h.

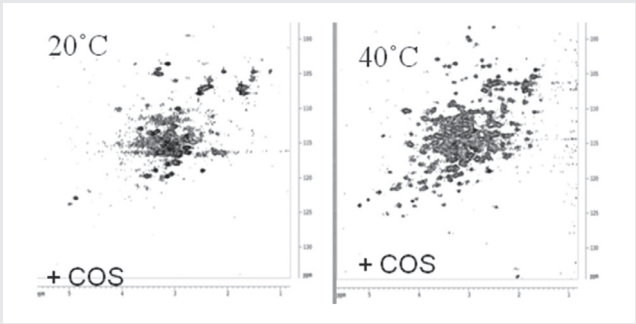

Figure 2. HSQC spectra of uniformly ^{15}N-labeled Gi1α in the presence of 1 *M* COS at 20 °C (left) and 40 °C (right). Spectra were recorded on a Bruker ARX-400 spectrometer.

1. Kumar, R. **2009**. Role of natural occurring osmolytes in protein folding and stability. *Archive of Biochemistry and Biophysics,* (491) 1-6.

2. Nau-Wagner, G.; Boch, J.; LeGood, A.; Bremer, E. **1999**. High-affinity transport of choline-*o*-sulfate and its use as a compatible solute in bacillus subtilis. *Applied and Environmental Microbiology, 65*(2) 560-568.

Please see figures 3-7 on reverse side.

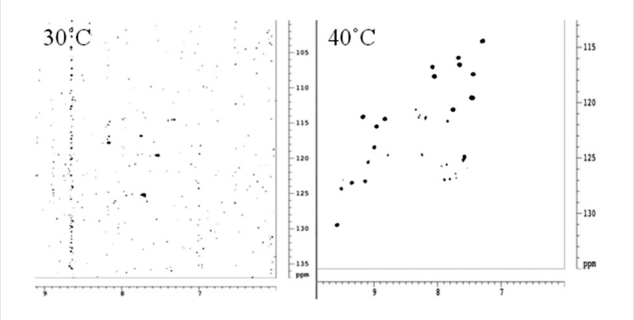

Figure 3. HSQC spectra of Gi1α labeled with [^{15}N] phenylalanine in the presence of 1 M COS-d_{13} at 30°C (left) and 40°C (right). Almost all (18 out of 19) phenylalanine signals are clearly observed at 40°C. Spectra were recorded on a Bruker Avance-700 spectrometer.

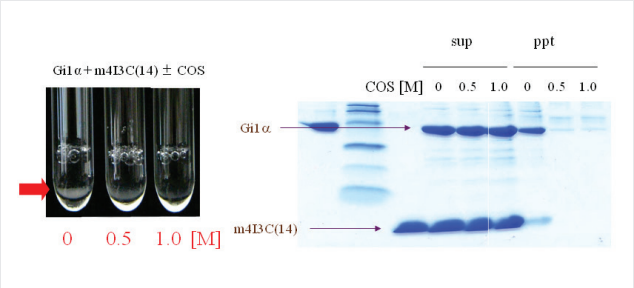

Figure 4. COS prevents co-precipitation of Gi1α and its selective activator, m4I3C(14), on mixing. Gi1α and m4I3C(14) form precipitates on mixing (photograph at bottom, left tube). The composition of the precipitates is confirmed by SDS-PAGE (bottom right panel, lane ppt/0). In the presence of COS, the precipitates are not observed (photograph, SDS-PAGE).

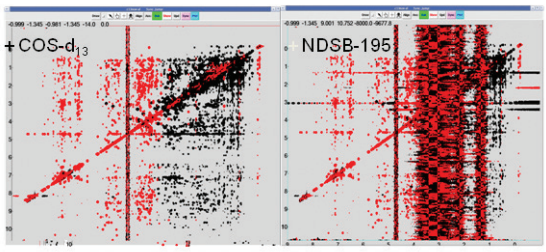

Figure 5. ^{13}C-edited NOESY spectra of uniformly ^{13}C and ^{15}N-labeled acidic fibroblast growth factor (αFGF) in the presence of 0.5 *M* COS-d_{13} (left) or 0.5 *M* NDSB-195 (right) recorded on a Bruker Avance-800 spectrometer. Deuteration of COS is critical in ^{13}C-edited NOESY spectra due to the absence of significant t_1 noise.

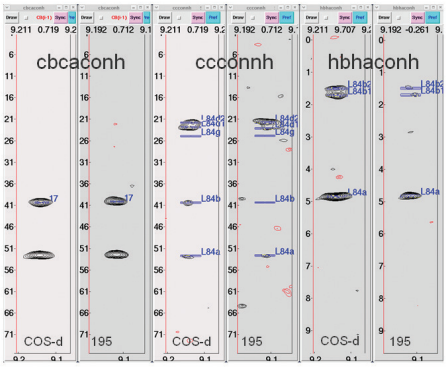

Figure 6. Comparison of spectral strips of αFGF in the presence of COS-d_{13} (left) vs. NDSB-195 (right) for cbcaconh, ccconnh, and hbhaconh measurements. Although the concentration of the protein is the same, COS-d_{13} gives larger cross peaks than does NDSB-195. Recorded on a Bruker Avance-600 spectrometer.

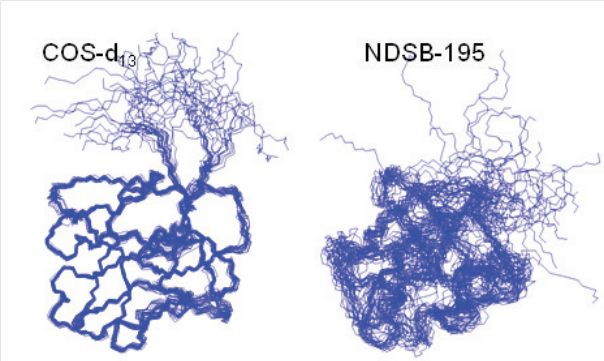

Figure 7. Overlay of 20 αFGF structures calculated by using NOE data in the presence of COS-d_{13} (left) or NDSB-195 (right). Well-defined structures are obtained in the presence of COS-d_{13} but not in the presence of NDSB-195.

All images are courtesy of Dr. Wakamatsu of Gunma University (Gunma, Japan).