Fluorescent and affinity-based tools to detect cysteine sulfenic acid formation in proteins

Leslie B. Poole, Chananat Klomsiri, Sarah A. Knaggs, Cristina M. Furdui, Kimberly J. Nelson, Michael J. Thomas, Jacquelyn S. Fetrow, Larry W. Daniel, and S. Bruce King

CONTENTS OF SUPPORTING INFORMATION

I. 2D gel analyses of DCP-FL1 modified AhpC

To determine the sensitivity of visualization for DCP-FL1 modified proteins, various amounts of labeled C165S AhpC were loaded onto 11 cm IPG strips and focused in a Protean IEF cell, then incubated in buffer containing dithiothreitol followed by one containing iodacetamide (as recommended in the Bio-Rad manual), trimmed and transferred to the tops of Criterion gels for separation in the second dimension. As shown in Fig. S1, 0.5 pmol was readily detected in a two dimensional gel spot with good linearity up to at least 5 pmol.

II. MS-MS analysis of the labeled peptide from DCP-FL1 modified AhpC

To confirm the site of AhpC modification with DCP-FL1, AspN digestion of the labeled AhpC was carried out to generate an 11-residue peptide containing the expected cysteine site susceptible to oxidation. The peptide mixture was analyzed using liquid chromatography and a Q-TOF MS. As shown in Fig. S2, MS-MS analysis of the parent peptide gives a high quality spectrum that accurately sequences the labeled peptide and confirms the presence of the covalent adduct on Cys46 of this

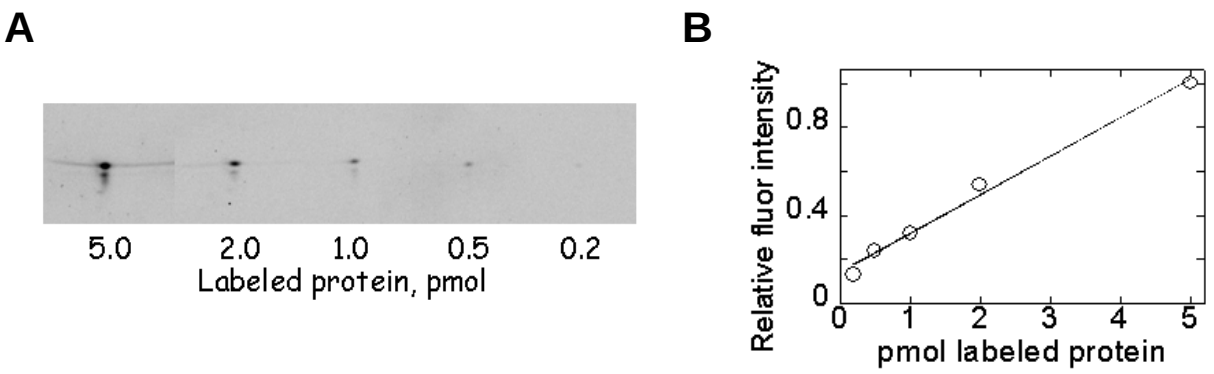


Figure S1. Visualization of DCP-FL1 labeled AhpC protein after 2D gel electrophoresis. Varying amounts of AhpC C165S labeled with DCP-FL1 were focused on pH 3-10NL IPG strips, then trimmed and loaded, 3 strips per gel, onto Criterion 8-16% gradient SDS-polyacylamide gels for resolution in the second dimension (panel A). Fluorescence intensity was determined for each protein amount directly from the images using MATLAB software and plotted *versus* amount loaded (panel B).

AhpC peptide. Furthermore, a characteristic ion is detected of a mass that suggests fragmentation during collision-induced decay between the sulfur and β-carbon of the adducted cysteinyl residue (Table 1 in the main text). For appropriately equipped mass spectrometers, this specific ion can be used to trigger MS-MS analysis as was carried out for the DCP-FL1-modified peptide shown in Fig. S2.

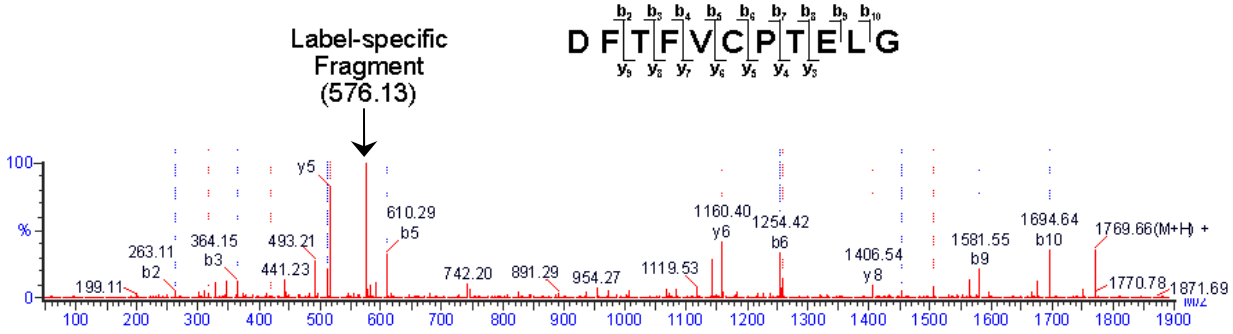


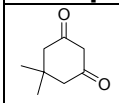
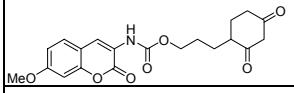
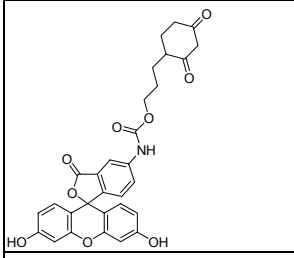
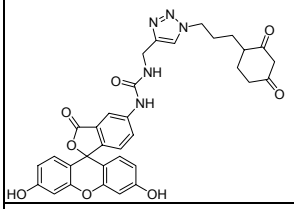
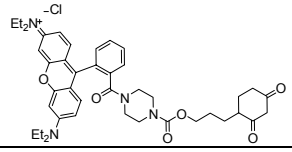
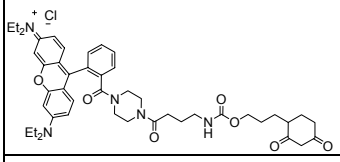
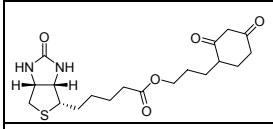
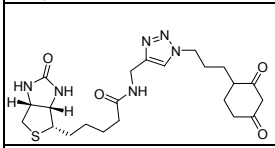
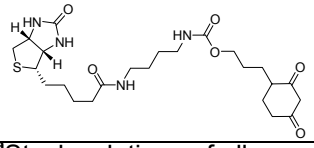
Figure S2. LC-MS/MS spectrum of the DCP-FL1-labeled peptide of C165S AhpC containing an adduct at Cys46. The covalent adduct with DCP-FL1 was prepared and digested with AspN in order to generate an 11-residue peptide (MH+ = 1769.66). Reverse phase chromatography on a C-18 column was used to resolve peptides, with a portion of the eluant injected directly into the Q-TOF mass spectrometer. Based on the detection of the label-specific fragment, the parent ion of *m/z* 885.33 (+2 charge) was selected for fragmentation. Cleavage of the amide bond results in N-terminal fragments designated as "b" and C-terminal fragments designated as "y". The masses of both sets of ions are consistent with DCP-FL1 linked covalently to Cys46 (b6 – b5 = y6 – y5 = 644.14 *m/z*).

III. Log P analyses for the compounds synthesized

The partition coefficient (P) was determined by the shake-flask method (following OCED guidelines) (for a review, see *1*). Briefly, in preparation, the two phases of the solvent system (n-octanol, spectrophotometric grade, and phosphate buffer, pH 7.2) were mutually saturated. Stock solutions of the test substances were prepared in either n-octanol presaturated with phosphate buffer, pH 7.2 or in phosphate buffer, pH 7.2 (using up to 5% DMSO to aid solubility) presaturated with n-octanol, or both. Accurately measured volumes of the two phases (one phase containing the test substance) in the ratios of 1/1, 1/2 and 2/1 in test vials were prepared. These were either vortexed for 1 h or shaken by hand for 5 min (tests indicated that there was no difference in efficiency of partitioning between the two methods) and then the two phases were allowed to separate and equilibrate for 1 h before analysis. A 1 mL aliquot of each of the phases from each test vial for each test condition was taken and analyzed by UV-Vis spectrophotometry for determination of the concentration of test substance in each phase. In most cases, the partition coefficient was determined using two different stock concentrations of the test substance and the three phase volumes.

The distribution of a solute in a biphasic liquid system consisting of octanol and water is the accepted physicochemical property measuring the hydrophobicity of chemicals ((*1*). This property is of critical interest to the pharmaceutical industry using quantitative structure–activity relationships (QSAR) in drug design and toxicology given its influence on drug absorption, distribution, metabolism and excretion. A high log*P* value of a molecule indicates that the molecule dissolves easily in hydrophobic materials and dissolves poorly in water. Not surprisingly, a high log*P* value is associated with better permeability through membranes, as well (*2*). Of our sulfenic acid-reactive compounds tested and listed in Table S1, both rhodamine compounds and the methoxycoumarin conjugate reported earlier exhibited positive logP values, suggesting that these three compounds have the highest likelihood of being membrane permeable.

Table S1. LogP values for various sulfenic acid-reactive reagents.

Compound	Name	LogP (± SD)[a]	LogP (± SD)[b]
	Dimedone	–0.63 ± 0.28	–0.61 ± 0.11
	DCP-MCC[c]	1.25 ± 0.04	1.27 ± 0.05
	DCP-FL1	–2.25 ± 0.21	–0.88 ± 0.15
	DCP-FL2	–1.33 ± 0.06	–1.61 ± 0.22
	DCP-Rho1	ND	1.03 ± 0.09
	DCP-Rho2	ND	0.78 ± 0.10
	DCP-Bio1	ND	–0.91 ± 0.13
	DCP-Bio2	ND	–1.57 ± 0.11
	DCP-Bio3	ND	–1.22 ± 0.08

[a]Stock solutions of all compounds were made in n-octanol (NOTE: there were solubility problems for the fluorescein derivatives) and partitions with phosphate buffer, pH 7.2, were performed (using 3 different phase volumes).
[b]Stock solutions of all compounds were made in phosphate buffer, pH 7.2 (2% DMSO required for solubilization of the coumarin derivative and biotin derivatives) and partitions with n-octanol were performed (using 3 different phase volumes).
[c]Abbreviated name from (*3*).

REFERENCES

1. Berthod, A., and Carda-Broch, S. (2004) Determination of liquid-liquid partition coefficients by separation methods, *J Chromatogr A 1037*, 3-14.
2. Chen, L. L., Yao, J., Yang, J. B., and Yang, J. (2005) Predicting MDCK cell permeation coefficients of organic molecules using membrane-interaction QSAR analysis, *Acta Pharmacol Sin 26*, 1322-1333.
3. Poole, L. B., Zeng, B. B., Knaggs, S. A., Yakubu, M., and King, S. B. (2005) Synthesis of chemical probes to map sulfenic acid modifications on proteins, *Bioconjug Chem 16*, 1624-1628.