Rapid Electrophoretic Screening of Homocysteine Thiolactone-Induced Protein Aggregation Using Cationic Polyelectrolyte-Coated Capillaries

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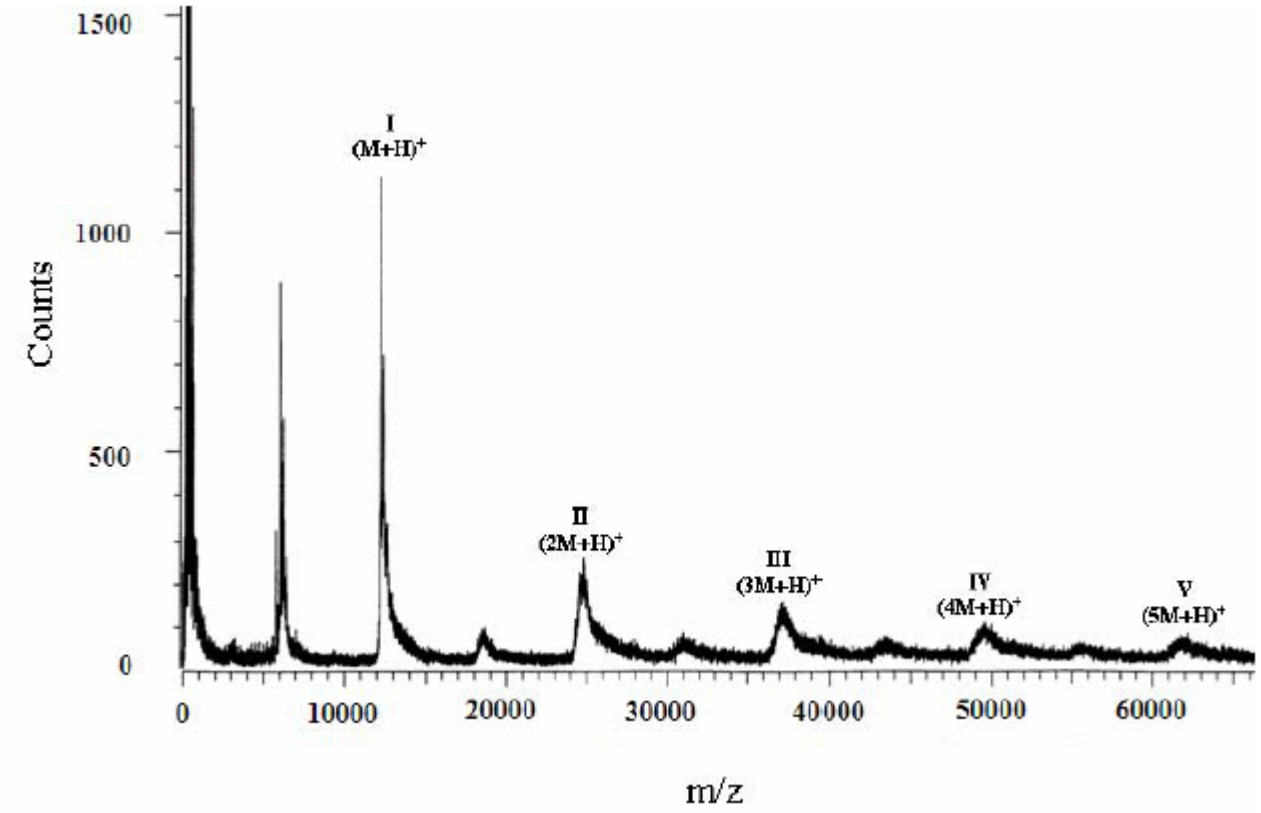


Figure S1. MALDI-TOF mass spectrum for the protein reaction mixture (10 mg/mL bovine cytochrome c + 2.5 mM homocysteine thiolactone) acquired after 24 hrs. The molecular ion $(M+H)^+$ for monomeric bovine cyt c was detected at approximately 12.3 kDal, which is consistent with the literature. The roman numerals indicate molecular ion species corresponding to monomeric (I), dimeric (II), trimeric (III), tetrameric (IV), and pentameric (V) bovine cytochrome c. The peak preceding the monomer molecular ion is due to the doubly protonated monomeric species. The minor peaks preceding the aggregate molecular ion peaks are due to various trace higher order aggregate species.

Mass spectrometry experiments were performed using a Bruker ProFLEX III™ matrix assisted laser desorption ionization time of flight- mass spectrometer (MALDI TOF-MS) in linear mode with a sinapinic acid (SA) matrix. The matrix was prepared by dissolving SA in a 2:3 acetonitrile/0.1% TFA mixture to obtain a 10 mg/mL SA solution. Protein reaction mixture samples and SA were mixed to obtain a 1:1 protein/matrix solution. The resultant solution was deposited drop-wise onto a MALDI

target and allowed to dry and crystallize. Mass spectra were acquired using 100 shots with a 0 ns delay. Relevant instrumental parameters: linear mode, 100 shots, 0 ns delay, and sinapinic acid matrix.

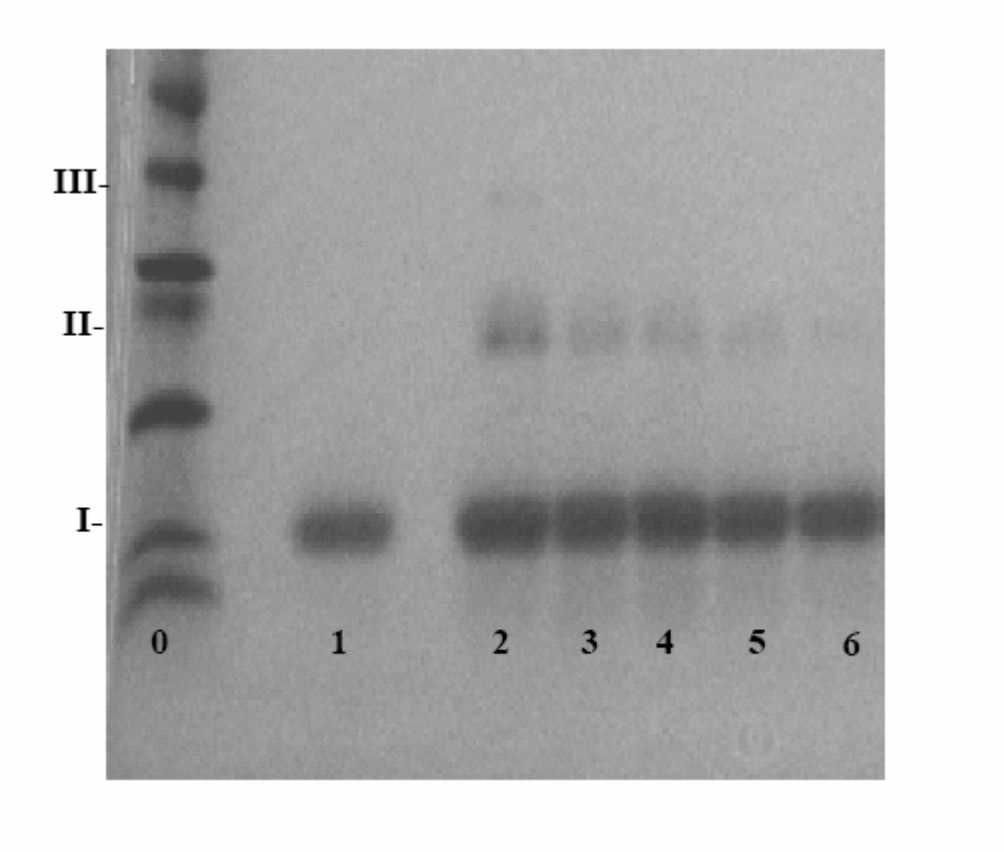
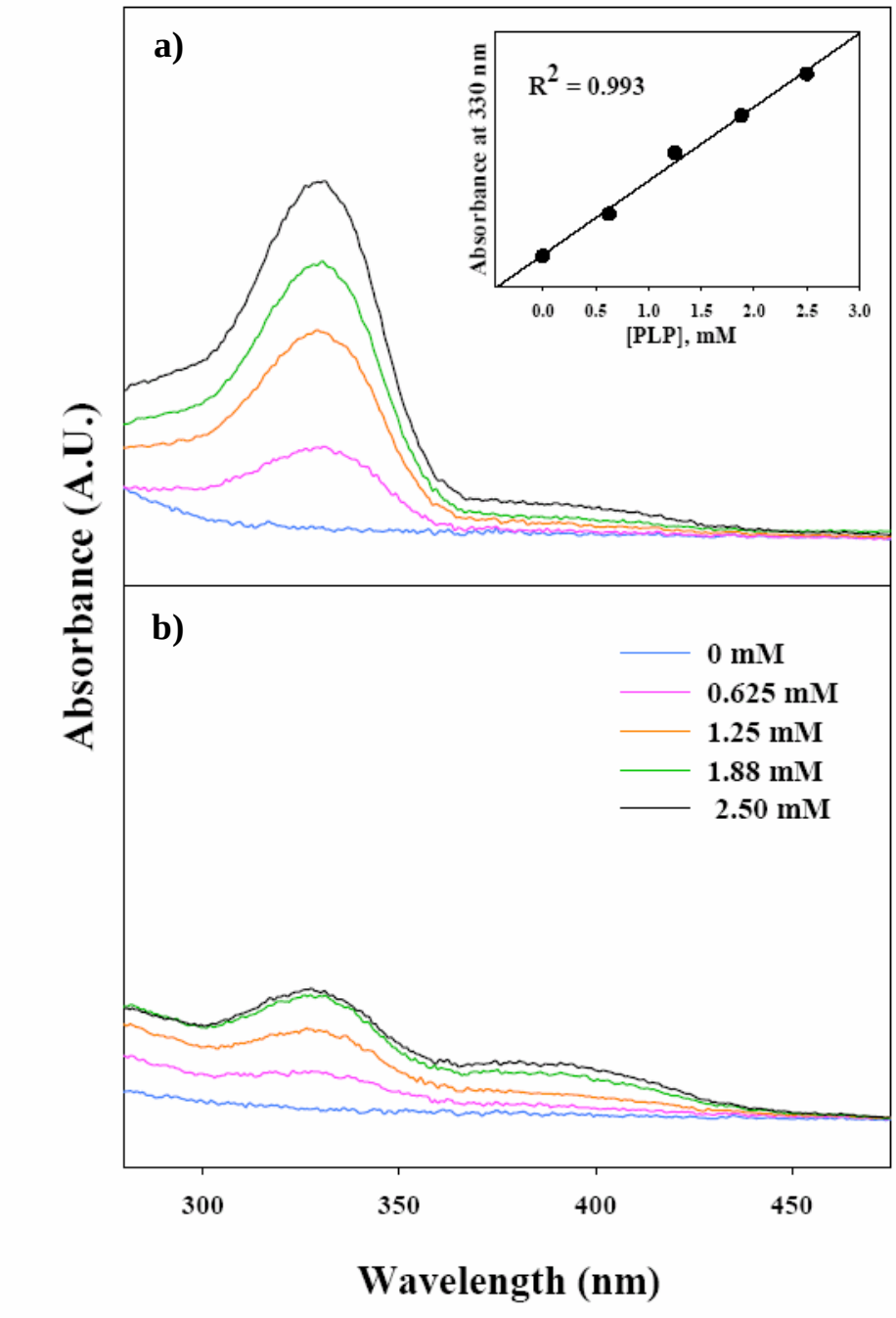


Figure S2. Bovine cytochrome *c* and hcy thiolactone (10 mg/mL and 2.5 mM, respectively) were reacted with pyridoxal-5-phosphate (PLP) for 24 h at room temperature. The resultant samples were denatured by heating at 95°C for 5 min in the presence of SDS and separated using SDS-PAGE on 4–20% gels at approximately pH 9. A representative gel is shown above. Unmodified cytochrome *c* migrated as a single band (lane 1). Lanes 2-6 show 1:0, 1:0.25, 1:0.5, 1:0.75, and 1:1 hcy thiolactone:PLP, respectively. Cytochrome *c* monomers, dimers, and trimers are denoted as I, II, and II, respectively as determined by comparison with protein mass standards (lane 0). Note the decrease in aggregate band intensities as the concentration of PLP increases, eventually resulting in the disappearance of the trimeric species as well as an appreciable reduction in the dimeric species.



Figures S3. Spectrophotometric detection of in situ pyridoxal tetrahydrothiazine formation after 12 h in a) control reaction mixtures containing 2.5 mM Hcy thiolactone and PLP in the indicated concentrations (inset: pyridoxal tetrahydrothiazine production monitored at 330 nm), and b) protein reaction mixtures containing 10 mg/mL bovine cyt c, 2.5 mM Hcy thiolactone, and PLP in the indicated concentrations after 12 h. All samples were diluted 10-fold with pH 7.4, 100 mM sodium phosphate, 0.2 mM EDTA buffer prior to analysis. UV-Vis spectra acquired in triplicate at 25°C in a 1 cm quartz cell with a Shimadzu model UV-3101PC spectrophotometer.