Notes & Tips

Guanidinium chloride-induced spectral perturbations of 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid confound interpretation of data on molten globule states

M.N. Zakharov\textsuperscript{a,1}, J. Ulloor\textsuperscript{a,1}, S. Bhasin\textsuperscript{a}, J.A. Ross\textsuperscript{b,2}, N.S. Narula\textsuperscript{a}, M. Bakhita\textsuperscript{a}, B.K. Pillai\textsuperscript{a,3}, R. Kumar\textsuperscript{c}, D.M. Jameson\textsuperscript{b}, R. Jasuja\textsuperscript{a,4}

\textsuperscript{a}Section of Endocrinology, Diabetes, and Nutrition, Boston University School of Medicine, Boston, MA 02118, USA
\textsuperscript{b}Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawai'i at Manoa, Honolulu, HI 96813, USA
\textsuperscript{c}Department of Basic Sciences, Commonwealth Medical College, Scranton, PA 18510, USA

\textsuperscript{1}These authors contributed equally to this article.
\textsuperscript{2}Present address: Center for Biomedical Engineering, University of Texas Medical Branch, Galveston, TX 77555, USA.
\textsuperscript{3}Present address: Center for Biomedical Engineering, University of Texas Medical Branch, Galveston, TX 77555, USA.
\textsuperscript{4}Abbreviations used: bis-ANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid; GuHCl, guanidinium chloride; UV–Vis, ultraviolet–visible; FWHM, full-width half-maximum.

\textbf{A R T I C L E  I N F O}

Article history:
Received 23 February 2011
Received in revised form 9 April 2011
Accepted 12 April 2011
Available online 20 April 2011

\textbf{A B S T R A C T}

We describe limitations in the use of 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid (bis-ANS) to examine unfolding intermediates associated with guanidinium chloride (GuHCl)-induced protein denaturation. Several studies have used alterations in fluorescence emission of bis-ANS to quantify the population of “molten globule” states. Our findings indicate that the observed changes in bis-ANS spectroscopic properties could originate from the interactions of bis-ANS and GuHCl and the aggregation of the dye at higher GuHCl concentrations. We posit that in the absence of additional complementary structural or spectroscopic measurements, the use of bis-ANS emission alone to monitor protein conformations can be misleading.

© 2011 Elsevier Inc. All rights reserved.
experiments using bis-ANS and GuHCl combinations may have been misinterpreted.

Bis-ANS (Invitrogen, Eugene, OR, USA) was reconstituted in deionized water to a stock concentration of 3.2 mM (extinction coefficient of 16,790 cm⁻¹ mmol⁻¹ at 385 nm [6]). GuHCl (99% pure) was obtained from Sigma–Aldrich and reconstituted to 8 M stock using deionized water. A matrix of 12 × 8 1.5-mL Plastibrand PMMA (polymethyl methacrylate) cuvettes (Perfector Scientific, cat. no. 9008) was prepared from respective stocks covering a GuHCl concentration range from 0 to 5 M and bis-ANS from 0 to 50 μM in 100 mM Tris buffer (pH 7.4, Fisher Scientific, cat. no. BP152-1, impurities Cu, Pb < 1 ppm, reconstituted with deionized water, and filtered through 0.2-μm membrane) containing 200 mM NaCl. The absorption spectrum (300–500 nm) of each sample (10 mm pathlength) was measured using a Beckman Coulter DU 800 ultraviolet–visible (UV–Vis) spectrophotometer blanked against a deionized water-filled cuvette.

Steady-state fluorescence spectroscopy was performed using ISS PC1 and ISS K2 instruments (ISS, Champaign, IL, USA). The excitation wavelength was set to 385 nm with 16-nm slits (full-width half-maximum [FWHM]). Emission spectra were measured from 430 to 650 nm using 16-nm slits (FWHM) and 1-nm steps. To correct for the Wood's anomaly, a polarizer oriented at 0° was inserted into the emission path. To account for background scattering from UV clear disposable cuvettes, the signal from a water-filled cuvette was subtracted at every wavelength. Inner filter effects were negligible for concentrations up to 50 μM bis-ANS (no GuHCl). The fluorescence of GuHCl and Tris alone was negligible. Manufacturer-supplied corrections for grating efficiency and PMT (photomultiplier tube) response were applied to obtain corrected emission spectra.

Time-resolved measurements were obtained using an ISS K2 fluorometer. Excitation was accomplished using a 370-nm LED (light-emitting diode). Emission of bis-ANS was observed through a 435-nm longpass filter (Melles Griot, cat. no. 03FCG461). In the multifrequency phase and modulation technique, the intensity of the exciting light is modulated, the phase shift and relative modulation of the emitted light (with respect to the excitation) are determined, and lifetimes are then calculated according to well-known equations [7,8]. The measured phase and modulation values were analyzed as a sum of exponentials by using a nonlinear least squares procedure implemented using ISS Vinci software. The goodness of the fit to a particular model was judged by the value of the reduced chi-square [9].

We determined the effect of increasing GuHCl concentrations on the integrated bis-ANS emission intensity (Fig. 1A) at varying concentrations of bis-ANS. The fluorescence intensity of bis-ANS emission was markedly affected by the GuHCl concentration (Fig. 1A). This effect is independent of excitation wavelength (295 or 370 nm) (data not shown). For bis-ANS concentrations higher than 5 μM, the integrated fluorescence intensity increased 5 to 10-fold as GuHCl concentrations were increased up to 1.5 M. Interestingly, as the GuHCl concentration was increased further from 1.5 to 5 M, the integrated fluorescence intensity returned back to the levels observed in the absence of GuHCl. The observed spectral changes are quite similar to those reported previously during equilibrium unfolding in multiple published studies (Fig. 4 in Ref. [3], Fig. 9 in Ref. [4], and Fig. 9 in Ref. [5]). Our findings call into question the inferences of partially unfolded protein states drawn from1.5 to 5 M, the integrated fluorescence intensity returned back to the levels observed in the absence of GuHCl. The observed spectral changes are quite similar to those reported previously during equilibrium unfolding in multiple published studies (Fig. 4 in Ref. [3], Fig. 9 in Ref. [4], and Fig. 9 in Ref. [5]). Our findings call into question the inferences of partially unfolded protein states drawn from...
protein folding intermediates in published studies that used bis-ANS as a sole conformational probe and GuHCl as a denaturing agent; these inferences should be reexamined and verified using other methodological approaches. Our findings indicate that the observed changes in bis-ANS spectroscopic properties in the presence of increasing concentrations GuHCl, on which these inferences were based in these previous publications, could partly or wholly be related to interactions of bis-ANS and GuHCl and the aggregation of the dye at higher GuHCl concentrations.

Acknowledgements

The work was supported by Evans Foundation grant 01346002591, NIH grant U01 AG014369-S, ACS grant IRG-72-001-33-IRG, NIH grant R01 AG037193.

Appendix A. Supplementary data


References


