



MCS and Protein Phosphorescence

Introduction

Tryptophan phosphorescence within protein molecules is gaining attention as a probe of protein dynamics and structure. The tryptophan phosphorescence lifetime, τ , varies with the protein molecule's local environment and conformation. For example, τ decreases as the solvent viscosity rises. The lifetime also decreases as small molecules diffuse into the protein and quench tryptophan.

Dr. Bruce Kerwin and colleagues at Amgen (Thousand Oaks, CA) and the Istituto di Biofisica (Pisa, Italy) have examined the quenching of tryptophan emission in *N*-acetyl tryptophanamide (NATA, Fig. 1), human serum albumin (HSA, Fig. 2), and recombinant HSA (rHSA) using our FluoroCube lifetime spectrofluorometer.¹

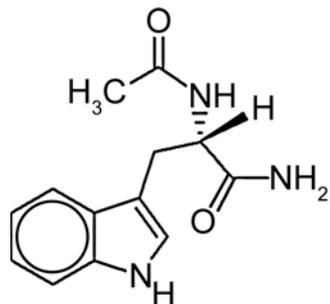


Fig. 1. Molecular structure of NATA.

Experimental method

Both NATA solution (5 μ M, pH = 8) and HSA solutions (1–20 μ M) also in-

¹ D.D. Banks and B.A. Kerwin, "A deoxygenation system for measuring protein phosphorescence", *Anal. Biochem.* **324**(2004), 106–114; G.B. Strambini, *et al.*, "The Triplet-state Lifetime of Indole Derivatives in Aqueous Solution", *Photochem. Photobiol.* **80**(2004), 462–470.

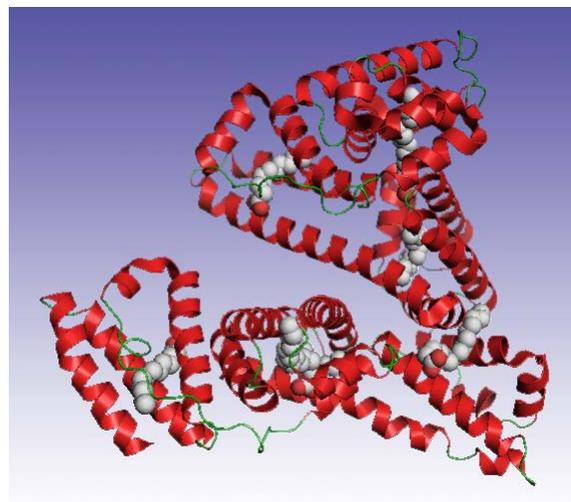


Fig. 2. Molecular structure of human serum albumin.

cluded 1 mM Tris; NATA was held at 4°C. A special deoxygenation apparatus was built to reduce O₂ levels in high-purity N₂ gas to 1 ppb. Deoxygenation of the NATA, HSA, and rHSA solutions in a special cuvette was accomplished by alternately N₂-purging and vacuuming on a gently swirling solution. Other experiments showed that at least seven cycles of deoxygenation were required for optimum effect.



Fig. 3. FluoroCube lifetime spectrofluorometer with 5000XeF xenon flash lamp.

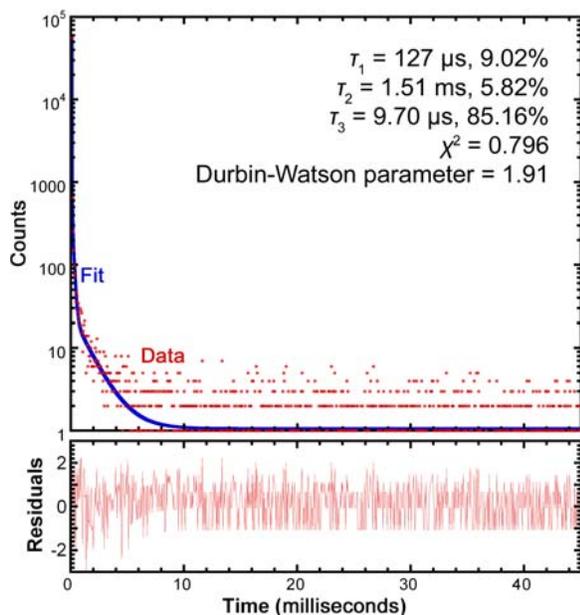


Fig. 4. Oxygenated NATA lifetime decay, tri-exponential fit. Upper plot is the data and fit; lower plot is the residuals.

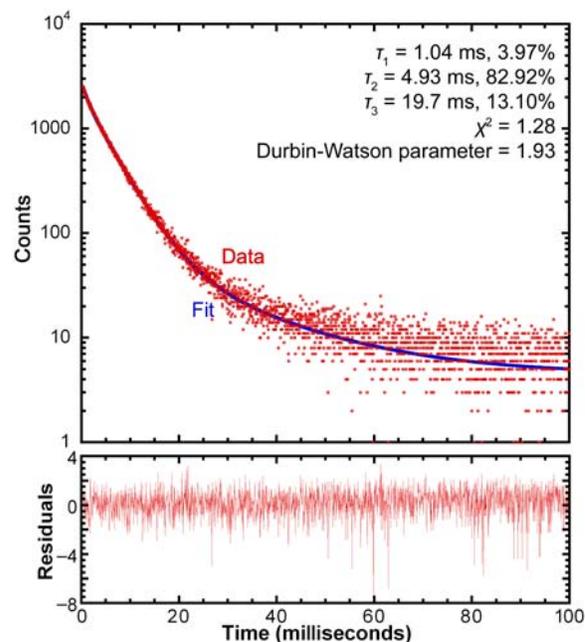


Fig. 6. Deoxygenated HSA lifetime decay, tri-exponential fit. Upper plot is the data and fit; lower plot is the residuals.

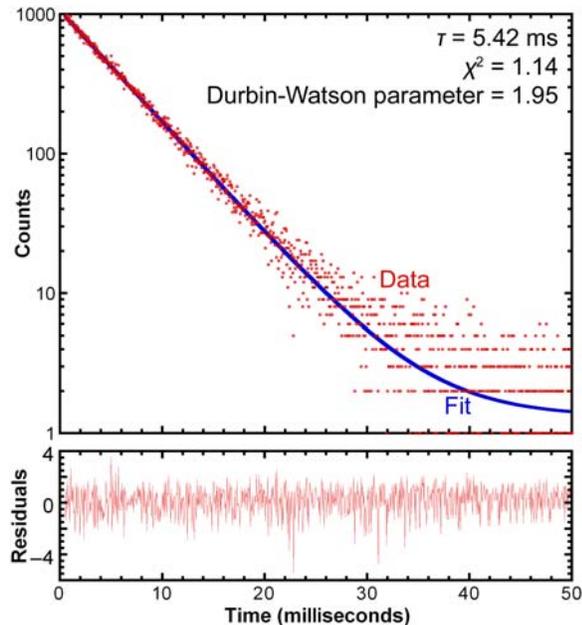


Fig. 5. Deoxygenated NATA lifetime decay, mono-exponential fit. Upper plot is the data and fit; lower plot is the residuals.

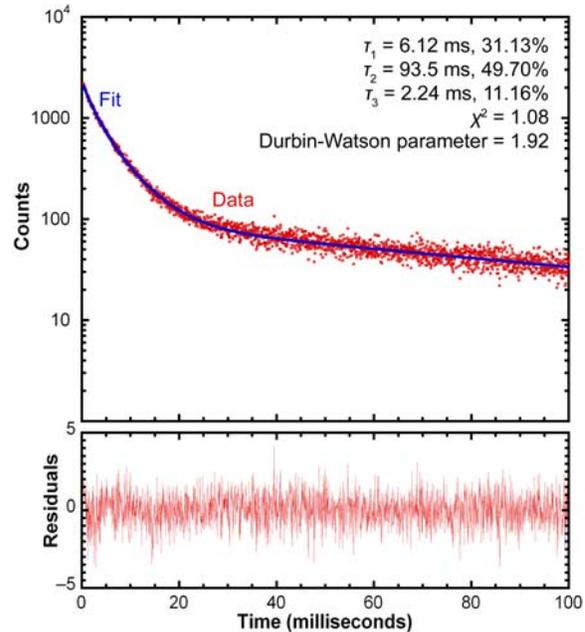


Fig. 7. Deoxygenated rHSA lifetime decay, tri-exponential fit. Upper plot is the data and fit; lower plot is the residuals.



Multichannel scaling (MCS) single-photon-counting spectroscopy was performed using HORIBA Jobin Yvon's FluoroCube fluorescence lifetime system (Fig. 3). The excitation source was our 5000XeF broad-band (250–800 nm) xenon flash lamp (pulse-width ~ 120 ns). The excitation monochromator was tuned to 294 nm with a bandpass of 32 nm. The emission monochromator was set to 448 nm (NATA's room-temperature peak emission wavelength) using a 32 nm bandpass. The detector was a TBX-04 module. The system was controlled with our DataStation Hub and DAS6 software.

To detect luminescence, 2000 flashes from the source were required. For HSA and rHSA, 50 μs /channel and 5000 channels were used, while for NATA, 20 μs /channel and 1000 channels were used.

Results

For the NATA lifetime decay (Figs. 4 and 5), the first 70 μs of the data were ignored, for the excitation source's decay interfered. To boost the excitation, several thousand flashes of the 5000XeF were recorded. Background was recorded after equilibrating the NATA with O_2 and rerunning the experiment.

The first five data points of the HSA and rHSA lifetime decays (Figs. 6 and 7) were discounted, owing to the interference of the excitation prompt. As with the NATA, 2000 flashes of the excitation source were required to create the dataset.

Discussion

Note the obvious difference between the oxygenated NATA (Fig. 4), whose primary lifetime ($\tau_3 = 9.70 \mu\text{s}$)

quenched to over 500 times shorter than the deoxygenated NATA ($\tau = 5.42 \text{ ms}$, Fig. 5). Clearly, as demonstrated previously¹, NATA is highly sensitive to the presence of oxygen.

The difference in phosphorescence between the deoxygenated HSA and rHSA (Fig. 5 vs. Fig. 6) is likewise striking. Banks and Kerwin propose that the difference stems from slight contamination of the protein with copurifying agents (e.g., fatty acids) that quench the luminescence, or possibly from variations in structural flexibility near the tryptophan.

Conclusions

Deoxygenation of proteins is crucial to determination of their intrinsic lifetimes. The HORIBA Jobin Yvon FluoroCube lifetime spectrofluorometer is a sensitive and important tool for investigation of the properties of proteins and changes in these proteins' microenvironments.



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