Probing the Long Range Chain Dynamics of a Polypetide in Aqueous Solution

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ABSTRACT

Analytical tools capable of describing the dynamics of a polymer chain in solution provide important information on the folding dynamics of complex macromolecules such as proteins. Over the years, several studies have demonstrated the numerous experimental advantages of fluorescence for probing long range polymer chain dynamics (LRPCD). Poly(*L*-lysine) (PLL), which can exist as a random-coil, α-helix, or β-sheet, was chosen to study the LRPCD of polypeptides in aqueous solution. For such studies, a water-soluble luminophore and quencher are needed. Ideally, both luminophore and quencher should be randomly attached to a polymer to apply the fluorescence blob model (FBM) to study the chain dynamics. This study illustrates how ruthenium (II) bisbypridine 5-amino-1,10-phenanthroline chloride ($RuNH_2$) can be used to probe the dynamics of a model polypeptide. The amine of $RuNH_2$ can be chemically modified into an isothiocyanate to enable its covalent attachment onto PLL. The synthesis, characterization, and modification of $RuNH_2$ will be presented. 3,5-dinitrophenyl isocyanate is used as a quencher. The polypeptide randomly labeled with quencher and luminophore will be studied by time-resolved fluorescence. Analysis of the fluorescence decays with the FBM will provide information on the dynamics of a polypeptide chain in aqueous solution.

INTRODUCTION

Long range polymer chain dynamics (LRPCD) can typically be probed by attaching a luminophore (D) and quencher (Q) at the ends of a polymer chain and measuring the rate constant k_{cy} of their encounter by end-to-end cyclization (EEC).[1]

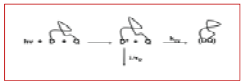
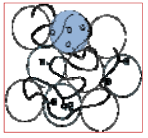


Figure 1: Illustration of a EEC process

Long polymer chains can be randomly labeled with the luminophores and quenchers to bring them close together. However, the data analysis of the rate constants of encounters between luminophores and quenchers becomes complicated. This complication can be overcome by using a fluorescence blob model (FBM).[2]

The FBM arbitrarily divides the coil of a randomly labeled polymer chain into blobs, where a blob is the volume of the polymer coil being probed by the excited chromophore during its lifetime.[2]



N_{blob} = number of monomer units per blob

k_{blob} = rate constant for diffusional encounter between luminophore (D) and quencher (Q)

Figure 2: Schematic representation of the Blob Model approach.

PURPOSE

The goals of this project include:

1) Synthesis, characterization, and modification of a water-soluble ruthenium luminophore ($RuNH_2$).
2) Labeling of PLL with the synthesized luminophore and its appropriate water-soluble quencher.
3) Acquisition of time-resolved fluorescence decays of the labeled PLL under different pH conditions in aqueous solution.
4) Analysis of the fluorescence decays using FBM to gain information about the LRPCD of a polypeptide as PLL undergoes different conformational changes.

The water-soluble luminophore and quencher chosen for this project are, ruthenium bisbipyridine 5–aminophenanthroline chloride ($RuNH_2$) and 3,5-dinitrophenyl isocyanate, respectively.

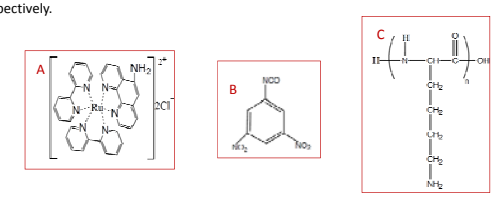


Figure 3: Structures of the A) luminophore, B) quencher, and C) polypeptide used in the project

$RuNH_2$ was synthesized by coupling 5–amino–1,10–phenanthroline to *cis*–bis(bipyridyl) ruthenium (II) dichloride. Since the amine substituent on the phenanthroline ring cannot be directly attached to PLL, it is converted into an isothiocyanate using thiophosgene.

PLL is labeled with the luminophore and quencher by using the procedure described by Ryan et al.[3] The structure of the labeled PLL is shown in Figure 4.

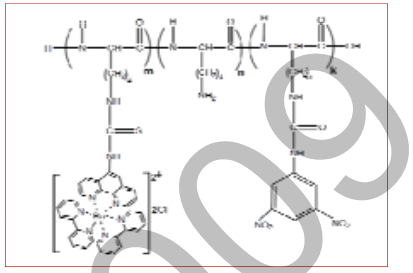


Figure 4: Structure of the labeled PLL

RESULTS

To date, $RuNH_2$ has been successfully synthesized, characterized, and converted to RuNCS. $RuNH_2$ has been synthesized according to the method described by Ellis et al.,[4] and modified by Quinn.[5] The structure of the product was confirmed by both ^1H NMR and MS.

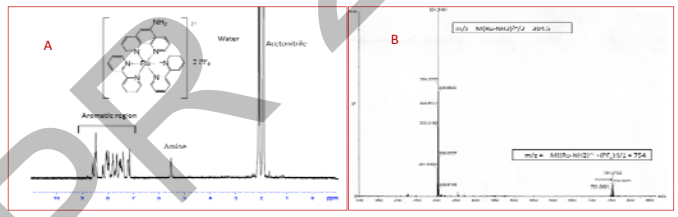


Figure 5: ^1H NMR (A) and mass spectrum (B) of $RuNH_2$

$RuNH_2$ was converted to RuNCS by using the procedure of Ryan et al.[3] The structure of the product was confirmed by both ^1H NMR and MS.

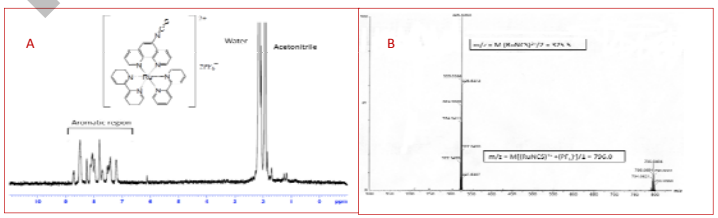


Figure 6: ^1H NMR (A) and mass spectrum (B) of RuNCS

UV-visible absorption and emission spectra of $RuNH_2$ in 0.1 M Na_2CO_3 aqueous solution at pH 9.6 were acquired. The absorption peak at 454 nm and emission peak at 610 nm match well the reported values.[3]

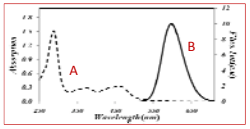
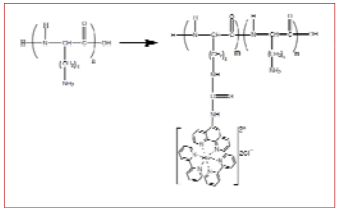


Figure 7: Absorption (A) and emission (B) spectra of $RuNH_2$ in 0.1 M Na_2CO_3 solution

The procedure described by Ryan et al.[3] was used to label PLL with RuNCS, and is described in Scheme 1.



Scheme 1: Labeling of PLL with RuNCS

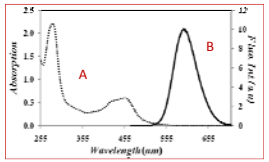


Figure 8: Absorption (A) and emission (B) spectra of labeled PLL in 0.1 M Na2CO3 solution

Figure 8 shows the absorption and emission spectra of the labeled PLL in 0.1 M Na_2CO_3 aqueous solution at pH 9.6. Both absorption and emission spectra of the labeled PLL resemble closely those of $RuNH_2$ shown in Figure 7.

The luminescence decay of a degassed $RuNH_2$ solution in 0.1 M Na_2CO_3 at pH 9.6 was obtained by time-resolved fluorescence as shown in Figure 9A. The decay was slightly biexponential and an average lifetime of 667 ns was obtained which compares well with the reported value of 639 ns.[3] Four exponential decays were needed to fit the decay of the labeled PLL shown in Figure 9B. The decay of ruthenium labeled PLL (RuPLL) had an average lifetime of 297 ns.

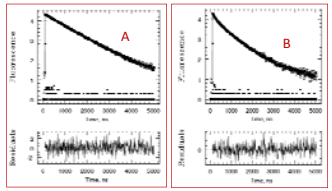


Figure 9: Time-resolved fluorescence decays of A)$RuNH_2$ B) labeled PLL in 0.1 M Na_2CO_3 solution

The shorter <τ> might be the result of the high luminophore content of PLL which was found to equal 14 mol%. The effect that the luminophore content of RuPLL has on fluorescence decay of RuPLL will be investigated in the future.

FUTURE WORK

Next, RuPLL will be randomly labeled with different amounts of 3,5-dinitrophenyl isocyanate which is expected to act as a quencher of RuNCS based on an earlier study.[5] Luminophore and quencher content of the labeled PLL will then be determined and time-resolved fluorescence decays will be acquired using the SPC technique. These decays will be fitted with the fluorescence blob model to get the parameters N_{blob} and k_{blob} that provide useful information about LRPCD.

ACKNOWLEDGEMENTS

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