Compression of Nano□sized Amylopectin Fragments Probed by Pyrene Excimer Fluorescence (PEF) in DMSO

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INTRODUCTION

Starch is biodegradable, inexpensive, and non-toxic. It is the second most abundant polysaccharide produced on earth and it can be easily extracted from plants. Starch is composed of linear amylose and branched amylopectin. The contribution of each component to the composition of starch depends on the botanical source of starch. For example, maize corn starch contains only amylopectin while amylomaize contains 30 wt% of amylopectin. Unlikely amylose, amylopectin can shrink upon drying.[1] In terms of industrial applications, shrinkage of amylopectin upon drying is a drawback if amylopectin is applied as a coating binder as it can reduce the gloss of paper by causing a rougher paper surface upon drying. A better understanding of the mechanism undergone by amylopectin as it shrinks upon drying requires the characterization of the internal molecular architecture of amylopectin in solution.

We have proposed the Solution-Cluster model (SCM) to describe the interior of amylopectin in solution. The SCM is inspired from the Cluster model that describes the structure of amylopectin in the solid state and which has been widely accepted.[2] According to the Cluster model, amylopectin helices pack hexagonally in crystalline lamellae. The crystalline lamellae are separated by amorphous domains which are the loci for the branching points of amylopectin. The inter-helix distance ($d_{h\text{-}h}$) in a lamella equals 10 Å. In solution, the helices are solvated and the inter-helix distance can be probed in solution by labeling amylopectin with a dye whose fluorescence signal responds to the local dye concentration.

This presentation will describe how pyrene excimer fluorescence (PEF) can be applied to study the shrinkage of amylopectin. PEF has been widely applied to study the dynamics of polymers. A pyrene excimer can be formed when a ground-state pyrene collides with an excited pyrene within its lifetime. Excited pyrene monomers that do not form excimer decay with their natural lifetime τ_M while a pyrene excimer decays with a lifetime, τ_E. The fraction of pyrene molecules that emit as monomer or excimer can be inferred from the fluorescence intensity ratio of the excimer (I_E) over that of the monomer (I_M), namely the I_E/I_M ratio as well as from the analysis of the time-resolved fluorescence decays. PEF depends on the viscosity of the local environment of pyrene as well as the local pyrene concentration.

Amylopectin extracted from maize and three different research-grade nano-sized amylopectin fragments (NAFs) obtained from EcoSynthetix were labelled with 1-pyrenebutyric acid. The fluorescence signal of the pyrene excimer was monitored as a function of the concentration of starch that was added to the solution. PEF was found to increase with increasing starch concentration indicating that amylopectin shrank at concentrations above its overlap concentration (C*). The shrinkage resulted in an increase in the local concentration of pyrene due to a decrease in the inter-helical distance.

EXPERIMENTAL

*Materials***:** NSFs were obtained from EcoSynthetix, purified by dialysis against water, and lyophilized. All other chemicals were purchased from Sigma Aldrich and used as is.

Size of Nano-Sized Amylopectin Fragments: The number-average hydrodynamic diameter (D_h) of NAFs was determined by Dynamic Light Scattering (DLS) using 0.1 wt% NAF dispersions in DMSO at 25 °C. A NAF with a D_h of 56 nm was represented as NAF(56). Amylopectin, NAF(8), NAF(17), and NAF(56) were used in this study.

Preparation of Pyrene Labeled Nano-Sized Amylopectin Fragments (Py-NAF): The synthesis, purification, and determination of the pyrene content of Py-NAFs followed a procedure that was developed for amylose and which has been described earlier.[3] Py(*x*)-NAF describes a NAF where a molar percentage *x* of the anhydroglucose units were labeled with 1-pyrenebutyric acid.

Preparation of Py(0.004)-NAF(56) Dispersions: Py(0.004)-NAF dispersions were prepared with a NAF labeled with a low pyrene content to minimize PEF and measure the lifetime of isolated pyrene labels. Up to a Py(0.004)-NAF(56) concentration of 13 wt%, very little PEF could be detected and the Py(0.004)-NAF(56) dispersions were prepared by dissolving the proper mass of Py(0.004)-NAF(56) in DMSO. More significant PEF was observed at Py(0.004)-NAF(56) concentrations above 13 wt%. For this reason, unlabeled NAF(56) was mixed with Py(0.004)-NAF(56) to increase the starch concentration without inducing PEF. For dispersions with a NAF concentration higher than 13 wt%, a stock solution was prepared by dispersing 4 wt% of Py(0.004)-NAF(56) in DMSO. Unlabeled NAF(56) (0.45-0.94 g) was added to 1.1-1.65 mL of the stock solution. The mixture was further diluted with DMSO to a final volume of 3.3 mL. At this stage, the mixture had a final concentration of less than 33 wt%. It contained enough solvent to disperse the solids. After a homogenous dispersion was obtained by stirring, the DMSO was evaporated by flowing N_2 over the dispersions. The dispersions with a concentration lower than 40 wt% were fluid enough to be transferred to a fluorescence cell to acquire their fluorescence spectra and decays. However, the dispersions with a concentration greater than 40 wt% could not be pipetted into a fluorescence cell due to their high viscosity. Those samples were simply smeared against the fluorescence cell wall as a paste. Any bubble generated from this process was removed by sonication at room temperature. The dispersions were analyzed by steady-state (SSF) and time-resolved (TRF) fluorescence without degassing.

Preparation of Mixtures of Py-NAF and Unlabeled NAF(56): Dispersions of naked NAF(56) with a concentration smaller than 20 wt% were prepared by adding the required amount of unlabelled NAF(56) (0-30 wt %) to a Py-NAF dispersion in DMSO with a pyrene concentration equal to 24 μM. The dispersions were stirred for two days at room temperature to obtain a homogeneous dispersion. For dispersions with a NAF(56) content greater than 30 wt%, the proper mass of NAF(56) was added to a dilute Py-NAF dispersion in DMSO. The DMSO was then evaporated so that the final dispersion would contain about 24 μM pyrene from the Py-NAF sample and a large excess of NAF(56) sample that would result in the desired starch concentration (> 30 wt%). In each case, the concentration of Py-NAF was sufficiently dilute to prevent intermolecular PEF. The transfer of the samples to a fluorescence cell was done in the same manner as described in the previous section, with a pipette or a spatula for dispersions with a starch concentration lower or higher than 40 wt%, respectively.

RESULTS AND DISCUSSION

The lifetime of the pyrene monomer (τ_M) obtained from the analysis of the TRF decays of Py(0.004)-NAF(56) and the wavelength of the excimer fluorescence maximum obtained from the SSF spectra of Py(5)-NAF(56) were monitored as a function of the unlabeled NAF(56) concentration. Due to their paste like nature, oxygen, which is a strong quencher of pyrene fluorescence, was not removed from the Py(0.004)-NAF(56) samples. Fortunately, the concentration of oxygen is low in polar solvents like DMSO so that a relatively long fluorescence τ_M value was obtained. Increasing the NAF(56) concentration in the Py(0.004)-NAF(56) dispersions hindered encounters between an excited pyrene and oxygen resulting in a pronounced increase in τ_M observed in Figure 1 at NAF(56) concentrations larger than 10 g/L.

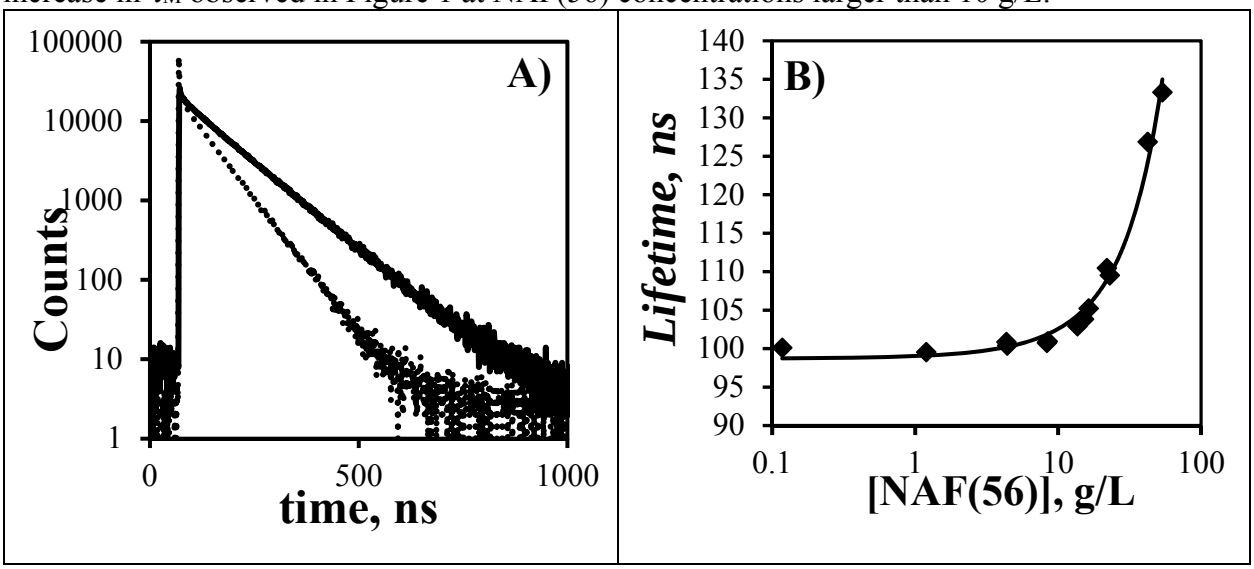

Figure 1. A) Fluorescence decays of (…) 0.1 wt% and (—) 54.6 wt% of Py(0.004)-NAF(56) and NAF(56) dispersions in DMSO and B) plot of pyrene lifetime as a function of the combined NAF(0.004)-NAF(56) and NAF(56) concentration in DMSO.

PEF can occur in two different ways, either by direct excitation of pyrene aggregates or diffusive encounters between an excited and a ground-state pyrene. The former and latter pathway will dominate for viscous and fluid samples having a high and low starch concentration, respectively. Excimer produced from pre-aggregated pyrenes shows a fluorescence maximum at a lower wavelength in the fluorescence spectrum than the excimer formed by diffusion as shown in Figure 2A. The combined observation of an increase in the lifetime of pyrene and a blue shift in the excimer emission spectrum reflected an increase in the viscosity of the local environment probed by pyrene as the starch concentration in the Py(0.004)-NAF(56) dispersions increased.

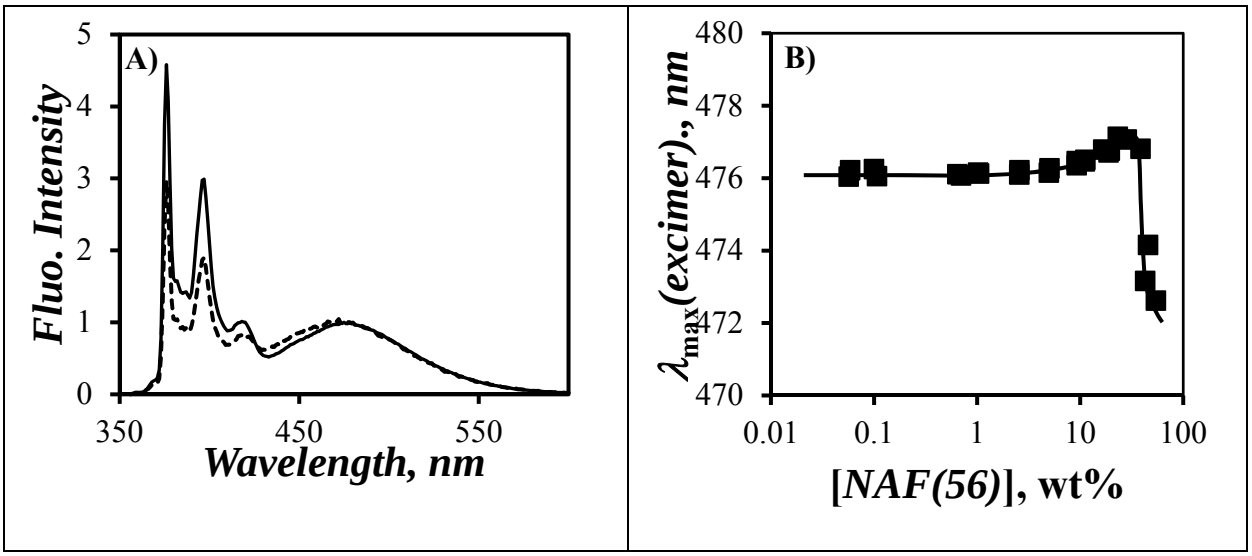

Figure 2. A) Fluorescence spectra of Py(6)-NAF(56) dispersions at an overall starch concentration of (—) 0.05 wt% and (---) 54.4 wt% of NAF(56) in DMSO and B) plot of the wavelength at the excimer fluorescence maximum (λ_{max}) of Py(6)-NAF(56) plotted as a function of NAF concentration.

The number-average hydrodynamic diameter, D_h, of the NAFs in DMSO was obtained by dynamic light scattering while the D_h of amylopectin was estimated to equal 200 nm. The fluorescence spectra of the Py-NAFs were acquired and their I_E/I_M ratios were determined and plotted as a function of NAF(56) concentration in Figure 3A. The I_E/I_M ratio remained constant at low starch concentration for all samples studied before showing a steep increase with starch concentration at concentration above about 10 g/L. The increase in I_E/I_M occurred at concentrations above the overlap concentration of the samples. Indeed C^* for amylopectin, NAF(56), NAF(17) and NAF(8) was determined by intrinsic viscosity measurements to equal 0.7, 1.5, 3.1, and 3.9 wt%, respectively.

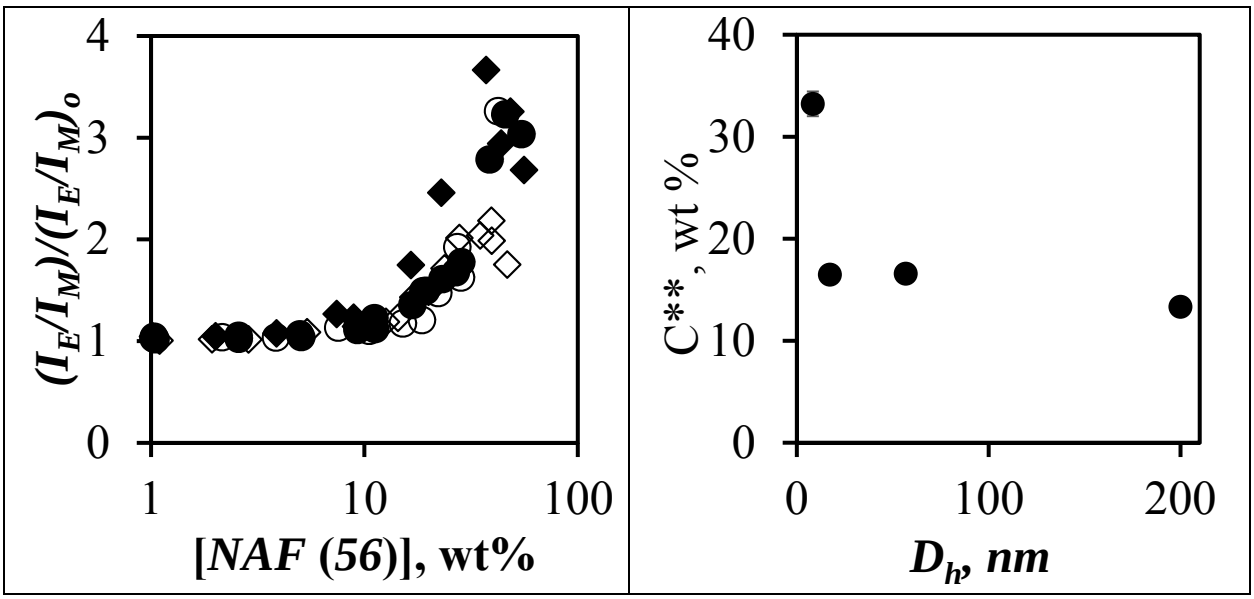

Figure 3. Plots of A) $(I_E/I_M)/(I_E/I_M)_o$ as a function of NAF(56) concentration for (◆)Py(9)-Amylopectin, (●) Py(6)-NAF(56), (◇) Py(7)-NAF(16), and (○)Py(6)-NAF(8) and B) C^{**} as a function of D_h.

For all samples studied, the I_E/I_M ratio began to increase at a starch concentration much higher than the overlap concentration. For example, I_E/I_M increased at 13.3 (± 0.1), 16.6 (± 0.5), 16.5 (± 0.2) and 33.2 (± 1.2) wt% for amylopectin, NAF(56), NAF(17), and NAF(8), respectively. Since PEF depends on the local concentration of pyrene $[Py]_{loc}$ and viscosity of the local environment of pyrene, a larger $[Py]_{loc}$ and lower local viscosity result in enhanced PEF. However, the increase in τ_M in Figure 1 demonstrated that the local viscosity experienced by pyrene increased as the NAF(56) concentration increased. Consequently, the increase in the I_E/I_M ratio observed in Figure 3A can only be due to an increase in $[Py]_{loc}$. Increasing the NAF(56) concentration increases the osmotic pressure applied to Py-Amylopectin and the Py-NAFs resulting in their compression as interpenetration of these highly branched polysaccharides is not possible.[1] The compression increased $[Py]_{loc}$ which resulted in enhanced PEF in Figure 3A.

The concentration where the I_E/I_M ratio began to increase, referred to as C^{**}, was plotted as a function of D_h in Figure 3B. The larger NAF and amylopectin showed a lower C^{**} value than the smaller NAFs. This trend could be rationalized with the Solution-Cluster Model (SCM) that predicts that the interior of amylopectin and NAF is constituted of dense clusters of helices separated by low density linear oligosaccharides.

Osmotic pressure triggers the compression of the fragments by reducing first the excluded volume between the clusters of helices (Stage 1). Amylopectin, NSF(56), and NSF(16) experienced the compression of the excluded volume between the clusters of helices as the osmotic pressure built up. But after the excluded volume between clusters of helices has been fully compressed, the distance between helices decreased bringing the helices closer to each other. This constitutes Stage 2 in the plot of I_E/I_M as a function of starch concentration. NAF(8), lacking excluded volume between clusters of helices, skips Stage 1 and shows an increase in I_E/I_M at a higher starch concentration. These considerations were illustrated in Figure 4 that depicts the compression of clusters of helices experienced by amylopectin when the starch concentration is increased.

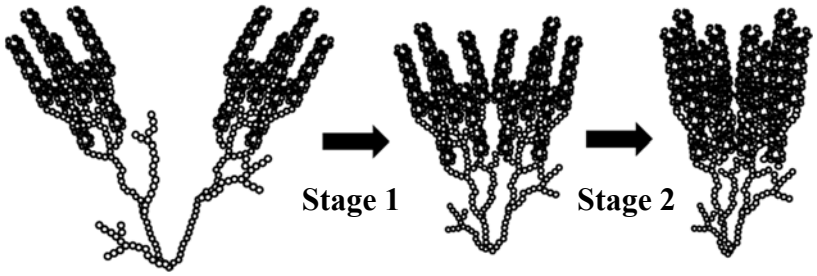

Figure 4. Illustration of the compression experienced by clusters of helices. Stage 1: Disappearance of excluded volume between clusters of helices. Stage 2: Compression of the helices within a cluster of helices.

CONCLUSIONS

The compressibility of amylopectin was studied by monitoring PEF as a function of starch concentration. An increase in the starch concentration built up the osmotic pressure applied to

amylopectin which resulted in the shrinkage of amylopectin, as probed by an increase in PEF. The shrinkage of amylopectin was predicted to take place in two stages. Stage 1 described the reduction of the excluded volume generated by linear oligosaccharides that link clusters of helices. A further increase in starch concentration, and thus osmotic pressure, eliminated all excluded volume between the clusters of helices and brought the helixes of amylopectin closer to each other. The fraction of excluded volume in the polysaccharide increases with the size of the polysaccharides and, therefore, the compression of amylopectin was observed at lower concentration compared to the compression of smaller NAFs. These observations are consistent with the SCM.

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