QUASI-ELASTIC NEUTRON SCATTERING STUDIES OF PROTEIN DYNAMICS

Progress Report

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In the previous technical report, we presented results on our experiments conducted at ONRL in February of 1991. We were the first users of the triple-axis spectrometer after the reactor start-up, and although we did obtain useful data, we had to correct it for an extremely large background. In January of 1992, we were able to conduct quasi-elastic scattering experiments at the H8 triple-axis spectrometer at Brookhaven National Laboratory. The data obtained was quite interesting. The background was low, and the results confirmed some of the indications provided by the ORNL experiments and showed that the objectives outlined in our proposal are attainable. A summary of the data collected at BNL is given below.

1. Collaborators.

Experiments were conducted on the H8 triple-axis spectrometer by H. Cao, C. F. Hazlewood, and H. E. Rorschach of Rice University in collaboration with S. Shapiro and T. Thurston of BNL.

2. Sample.

Dialyzed trypsin. Quasi-elastic scattering studies were conducted on the dry powder, 20% trypsin-D₂O solutions, and pure D₂O as a function of temperature. The following scans were made:

- a) 20% trypsin-D₂O solution: $S(Q, \omega)$ was measured for $0.5 < Q < 2.5 \text{ \AA}^{-1}$ for $T = 200, 280, 300 \text{ K}$. An $S(Q)$ scan was made at 300 K for $0.5 < Q < 4.6 \text{ \AA}^{-1}$.
- b) Powdered trypsin: $S(Q, \omega)$ was measured for $0.5 < Q < 2.5 \text{ \AA}^{-1}$ at 300 K, and an $S(Q)$ scan was made at 300 K for $0.5 < Q < 4.6 \text{ \AA}^{-1}$.
- c) D₂O: $S(Q, \omega)$ and $S(Q)$ were measured at 300 K for the above Q ranges.
- d) The spectrometer resolution function was measured at fixed Q .

3. Summary of data analysis.

The $S(Q, \omega)$ data for D₂O was nearly flat, except at low Q , where there is a small quasi-elastic peak. This data was fitted with a Voight function and subtracted from the solution data to obtain the scattering from the trypsin protons. This D₂O-corrected data was then fitted with a Voight function using the measured spectrometer resolution function. A fit at $Q = 2.0 \text{ \AA}^{-1}$ and $T = 280 \text{ K}$ is shown in Figure 1. (The experimental data has error bars; the points are from the fitting function.) From these fits, we obtained the linewidth and the integrated intensity, from which we can obtain information on the diffusive properties of the protons and the mean-squared thermal amplitude, $\langle u^2 \rangle$.

The line width as a function of Q^2 for the solution at 300 K and 280 K are shown in Figure 2a&b. This data is linear at small Q^2 and levels off at large Q^2 , which is typical of a jump-diffusion process. We do not yet have a good model for this type of diffusion for the segments of the trypsin, but it may correspond to jumps between microconformational states, as suggested by Frauenfelder. If the water molecules that are closely associated with the protein are part of the protein structure, then chain motions would be associated with rearrangements of these molecules. If these molecules are tightly bound, then their resulting diffusion during the rearrangement would be jump diffusion with a very small diffusion coefficient, which would be reflected in a small value of the chain diffusion

coefficient. The initial slope of the curve in Figure 2a gives $D = 0.14 \times 10^{-5} \text{ cm}^2/\text{sec}$, and the asymptotic value corresponds to a residence time of approximately $4.4 \times 10^{-11} \text{ sec}$. (For pure water, $D = 2.5 \times 10^{-5} \text{ cm}^2/\text{sec}$, and the residence time is about $2 \times 10^{-12} \text{ sec}$.) The measurements at 300 K and 280 K are consistent, with a slightly smaller value of D and a slightly larger value of the residence time at the lower temperature.

A Debye-Waller fit to the integrated intensity at 300 K is shown in Figure 3. The dotted line is a fit with $\langle u^2 \rangle / 3 = 0.33 \text{ \AA}^2$, which is consistent with the average thermal amplitude to be expected in a typical protein. (It is, however, somewhat larger than the value obtained from some previous measurements of $S(Q)$ at ORNL.)

Data was collected on the frozen solution at 200 K. This data is similar to the powder data. The lines are not broadened by any diffusive motion and have a width practically equal to the resolution function. We have data at only three values of Q , and the Debye-Waller plot has a large scatter. (See Figure 4.) The value for the thermal amplitude is $\langle u^2 \rangle / 3 = 0.28 \text{ \AA}^2$, but there is a large uncertainty in this value.

The powder data could be well fitted with a Voight function with a width only slightly larger than the resolution function. (See Figure 5.) A plot of the intensity vs. Q^2 (see Figure 6) has the right qualitative behavior, but there is much more scatter in this data, and we cannot make a good estimate of $\langle u^2 \rangle$.

The $S(Q)$ data did not prove particularly enlightening. The pure D_2O showed a broad peak at about $2 \text{ \AA}^{-1}$, as expected, and this same peak appeared in the trypsin- D_2O solution (See Figures 7 and 8.) The intensity due to the proton scattering decreases with Q^2 , as expected from the Debye-Waller factor, but it is hard to extract a good numerical value for $\langle u^2 \rangle$ due to uncertainties associated with the unknown background corrections. The powder did not show any structural features in an $S(Q)$ scan.

4. Conclusions.

We are very encouraged by these preliminary results. They agree with our expectations based on earlier work at ORNL.

1. The trypsin protons exhibit a large diffusive motion in liquid solution. This diffusive motion is absent in the powder and in the frozen solution.
2. This diffusive motion has the character of a jump diffusion, with a value of D significantly reduced from the value for pure water and a residence time significantly larger than that for pure water.
3. The high-frequency thermal motion obtained from the Debye-Waller factor is consistent with earlier measurements, and there is little difference between the liquid and the frozen solution.

4. Suggestions for further study.

a) We need to have more data on the trypsin- D_2O solution. We must verify the leveling-off of the line-width at high Q , since the present interpretation that the line-width levels off is based on only one point at $Q^2 = 6.25$. Data at a larger number of Q -values will permit a better comparison with jump diffusion theories.

b) We need better data on the powder, since we do not have good Debye-Waller fits for the intensity vs. Q^2 . The powder should be studied over a wider temperature range, $50 \text{ K} < T$

< 300 K, to see if $\langle u^2 \rangle$ is proportional to T . These measurements would also be useful for the frozen solution.

c) The $S(Q)$ measurements are puzzling but intriguing. We had originally thought that we could use this data to subtract off the D_2O contribution to the trypsin solution data. There was also the possibility that the D_2O "bump" in the solution data at $2 \text{ \AA}^{-1}$ might have a different shape or location than for the pure D_2O , which would indicate an interesting interaction between the protein and the solvent. We should repeat these measurements with better statistics.

d) We will ultimately need to do studies on single crystals of trypsin. This is a longer range study, since we must first grow the crystals. We have discussed crystal-growing techniques with B. Schoenborn, and we believe that we can obtain the necessary help to grow the crystals here at Rice from members of our biochemistry department who are involved in X-ray studies of proteins (Prof. George Phillips).

Q=2.0 T=280K TRYPsin D2O

FIGURE 1

FIGURE 2a

FIGURE 2b

FIGURE 3

FIGURE 4

TRYP POW T=300K Q=2.0

FIGURE 5

DW POWDER T=300K VOIGHT FIT

FIGURE 6

D2O S(Q) 3-AXIS T=300K 14.7EV

FIGURE 7

TRYP D20 S(Q) 3-AXIS T=300K 2.67EV

FIGURE 8