Un- and refolding kinetics of WT staphylococcal nuclease

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Introduction

Globular proteins in solution are only marginally stable and represent complex macromolecular systems whose structure and function is determined by the sum of hydrophobic, electrostatic, and hydrogen bond interactions. Because folding in a natural environment is very complex and often happens too fast, the experimental approaches for studying the responsible forces have focused mainly on the un- and refolding of small proteins or enzymes using rapid-mixing, temperature-jump, and (only recently developed) pressure-jump techniques. The advantage of using pressure jumps as a perturbation technique is guided by the ability to provide fast and bidirectional unfolding as well as refolding environments for the polypeptide in solution. Pressure propagates homogeneously through the system and usually causes no disruption of intramolecular covalent bonds below 20 kbar. In general, pressure is a rather mild perturbation technique for studying biomolecular phase transformations. In the case of protein un- and refolding studies, pressure perturbation often has the additional advantage of significantly slowing down the reactions due to positive activation volumes. In this study, we present pressure-jump kinetic studies of the tertiary structural evolution in the folding/unfolding reactions of WT staphylococcal nuclease (SNase) using time-resolved synchrotron small-angle x-ray scattering (SAXS) techniques.

Methods and Materials

Recombinant staphylococcal nuclease (SNase) with the sequence of nuclease A from the V8 strain of *Staphylococcus aureus* was obtained using the λ-expression system in the *Escherichia coli* strain Arλ9 expressed by the group of C. Royer. The experiments were carried out at the BESSRC-CAT beamline 12-ID of the Advanced Photon Source (APS). The experimental setup consists of a thermostatable, home-built, high-pressure cell with diamond windows allowing pressure experiments in the range from ambient pressure up to 7 kbar at temperatures up to 80°C. The pressure jumps were achieved by using a pressure-jump apparatus with pneumatically driven high-pressure valves allowing pressure jumps with a maximum amplitude of 7 kbar and a response time of 5 ms. Due to the strong absorption of the diamond windows of the high-pressure cell, only synchrotron x-ray sources with high photon intensity, such as APS, allow for taking diffuse small-angle scattering data with high accuracy and sufficiently short exposure times. We performed temperature-dependent un- and refolding experiments on 1 % (w/w) SNase in Bis-Tris-buffer solution (pH = 5.5) with a variable pressure-jump amplitude in up- and downward directions. Figure 1 shows a typical small-angle scattering pattern. The data were recorded with a time resolution of 1 s.

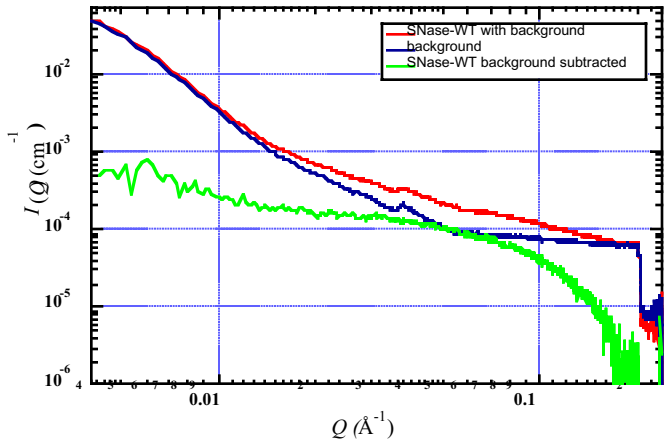

Figure 1: Small-angle scattering data of 1 % (w/w) SNase WT in Bis-Tris-buffer solution (pH 5.5) taken 100 s after a pressure jump from 3000 to 1400 bar. The exposure time was 1 s.

Results

The unfolding and refolding reaction of SNase is fully reversible under the conditions chosen and no radiation damage occurred during the time scale of the experiments. We obtained the time-dependent changes of the radius of gyration R_g and the pair distribution function $p(r)$ upon un- and refolding of the protein for different pressure-jump amplitudes at a few selected temperatures. The refolding of SNase occurs on a time scale of seconds. Figure 2 shows the extracted kinetic data of a series of 60 pictures of a pressure jump from 4 kbar to 0.8 kbar at 22.5 °C.

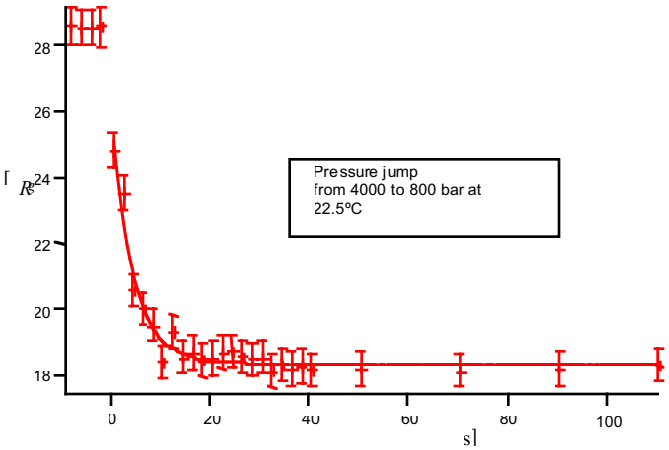

Figure 2: Time evolution of the radius of gyration R_g after a pressure jump from 4 kbar to 800 bar. The protein refolds with a time constant of 4.5 s.

The unfolding of SNase WT occurs on the time scale of minutes. Figure 3 presents a typical example.

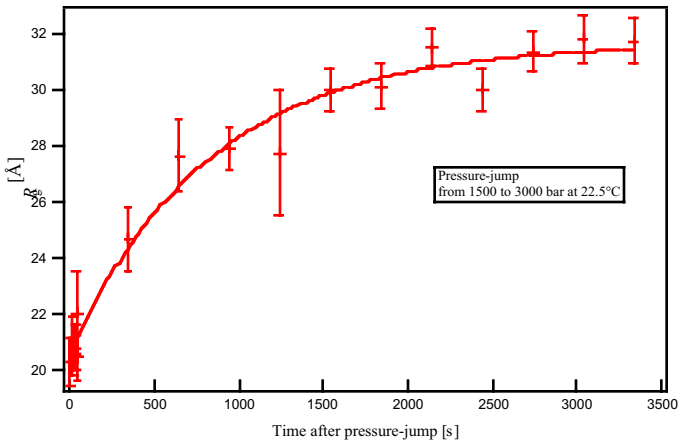

Figure 3: Time-dependent changes of R_g after a pressure jump from 1500 to 3000 bar. The protein unfolds with a time constant of 14 min.

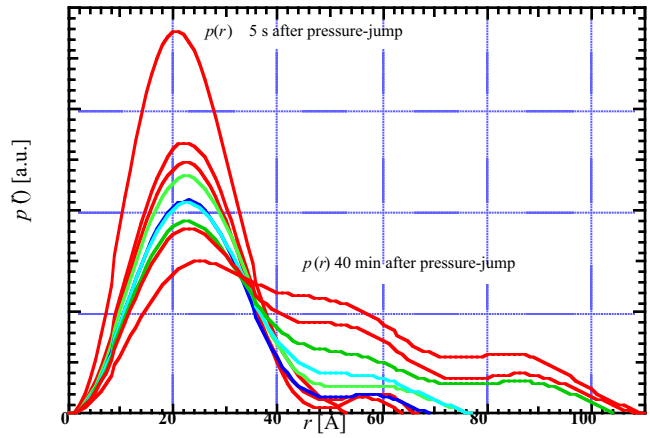

Figure 4: *p*(r)-plot for SNase WT obtained after a pressure jump from 1000 to 3000 bar. The first scan was 5 s after the pressure jump, the following scans were recorded in steps of 5 min.

Discussion

SNase WT has been found to be essentially a globular molecule of $R_g \approx 17$ Å, in agreement with NMR and crystallographic structural studies of this protein. Application of high pressure (e.g., 4000 bar) leads to an approximate two-fold increase of the R_g value ($R_g \approx 32$ Å). The denaturated state achieved by urea as denaturant leads to a similar value of R_g. The unfolding and refolding reaction of SNase seems to conform to a two-state, first-order transition. The pressure-induced unfolding of SNase follows relatively slow kinetics on the time scale of minutes. During the unfolding, extended (ellipsoidal) structures are formed as indicated by the corresponding pair-distance distribution functions (Figure 4). The refolding of the protein is much faster and occurs on the time scale of seconds. An increase in temperature at similar pressure-jump amplitudes leads to a drastic increase of the re- and unfolding reactions. Corresponding pressure-jump FTIR studies performed in our laboratory reveal that the reversible first-order changes in β-sheet, α-helical, and random structure occur on a similar time scale [1].

Acknowledgments

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Reference

[1] G. Panick, R. Malessa, R.Winter, G. Rapp, K.J. Frye, and C.A. Royer, *J. Mol. Biol.* **275**, 389–402 (1998).