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The compactness of chemically denatured lysozyme

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Introduction

Hen egg lysozyme is a well studied protein with its sequence and three-dimensional structure. As a kind of α alpha-helix protein, it is composed of two subunits, alpha unit and small β unit. Four disulfide bonds maintains the architecture of the native state. Lysozyme denatured in 8M urea or 5M GuHCl was used to study the folding process with the implicit assumption that the denatured protein is completely lost its structure [1]. It is well known that the disulfide bond is covalently bonded, which keeps intact at chemical denatured protein. Characterizing the structure of lysozyme at denatured state is critical to elucidate the folding mechanism of lysozyme.

Result

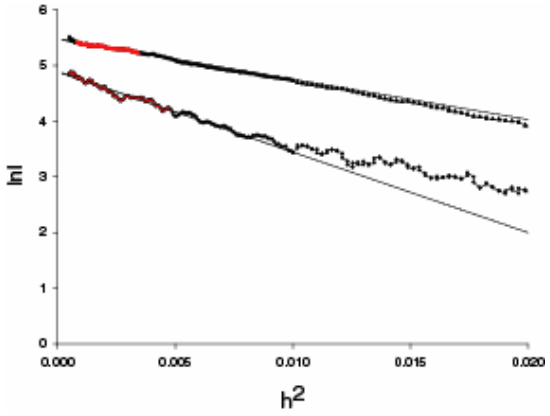
We performed x-ray solution scattering of urea dependence of lysozyme at 4 Å, in 50mM sodium phosphate buffer at pH 2.0. To prevent association, we use low protein concentration, 2mg/ml. From the Guinier approximation, radius of gyration (Rg) of native hen egg lysozyme (in 0M urea) is 13.7 Å. It is in good agreement with our estimation from lysozyme crystal structure [2]. This result indicates the solution structure of lysozyme is similar with its crystal structure. Kratky plot of the native lysozyme showed a bell shape (Fig 2). It is well known that the curve shape is used to judge the compactness of the polypeptide chain. The bell shape curve indicates the lysozyme is globule at the native state. Rg obtained from the peak of Kratky plot was 14 Åvery close to the Rg of the native lysozyme.

The Rg of urea-denatured lysozyme (8M urea) increased to 21.5 A, clearly smaller than the estimation from the length of lysozyme. This indicates some structure was still remained in 8M urea solution. Further, the Kratky plot also showed a peak (Fig 2). The Rg estimated from Kratky plot is about 22 Å. It is in good agreement with that obtained from Guinier plot. This indicates lysozyme still keeps compactness at urea-denatured state even though the circular dichroism and fluorescence showed the structure of the lysozyme was totally desrupted in 8M urea.

The result indicates four disulfide bonds of lysozyme play a key role to keep the compactness of chemically denatured lysozyme. On the other hand, the denatured protein was not as compact as the native state due to denaturant

It is also useful to explore the folding mechanism of lysozyme. The result indicates the folding process of lysozyme from chemically denatured state probably lack hydrophobic collapse process.

Fig 1 Gunier plot of native and unfolded hen egg lysozyme



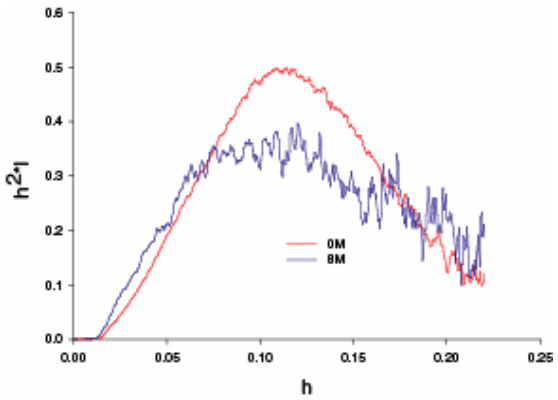


Fig 2 Kratky plot of native and unfolded hen egg lysozyme

References

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