Conformational changes during β_2-microglobulin amyloid assembly

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Introduction

Numerous studies of amyloid assembly using different protein systems under a variety of conditions have indicated that partially unfolded states are responsible for initiating aggregation *in vitro* and *in vivo*; however, little is known about the structure of key amyloid intermediates in atomic detail. Here we use ΔN6, a truncation variant of the naturally amyloidogenic protein β_2-microglobulin (β_2m), to determine, for the first time, the structure of a non-native amyloidogenic intermediate at high resolution in solution using nuclear magnetic resonance (NMR)

Real-time NMR refolding studies confirm the structural analogy of ΔN6 and I_T

In order to validate whether the non-native slow folding intermediate I_T shares a common structure with ΔN6, wild-type β_2m was denatured in 8 M urea and then refolded by ~10-fold dilution of the denaturant in 25 mM sodium phosphate pH 7.5 at 25°C. The re-equilibration back to the native state via the trapped amyloidogenic intermediate I_T state was monitored using SOFAST- ^{1}H-^{15}N HMQC spectra at 25°C acquired ~2 min after dilution. Figure 1A shows the superposition of the ^{1}H-^{15}N spectra of ΔN6 and the kinetic intermediate I_T. After ~2 min of refolding the spectrum (Figure 1) is predominantly (>75%) I_T (Eichner *et al.*, 2009) and the spectra reveals 76 cross peaks corresponding to the I_T state 68 of which overlay well with those measured for ΔN6 (N_{WT}) (^{1}H/^{15}N within ± 0.05/0.5 ppm)

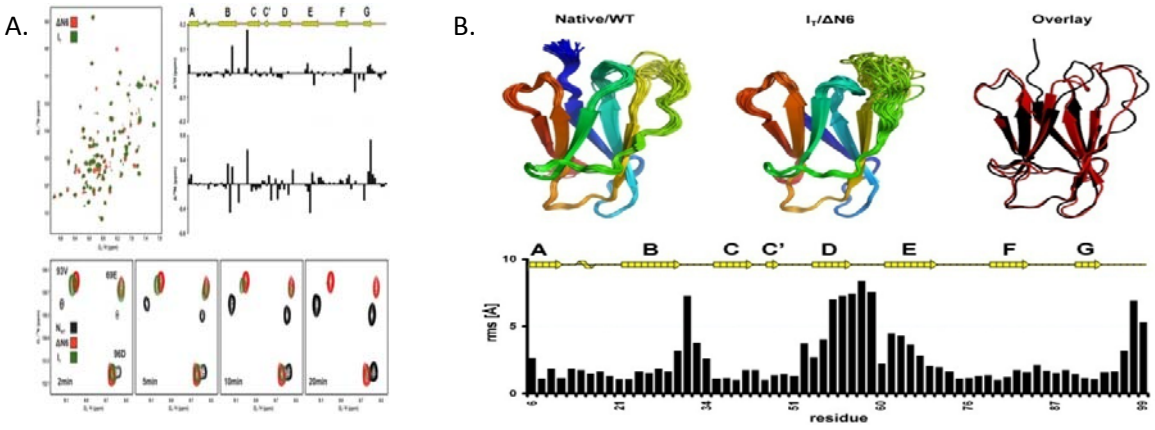

Figure 1: (A) ^{1}H-^{15}N-HSQC spectra of WT I_T state and ΔN6 β2M. **(B)** NMR solution structures of WT (left) and ΔN6 β2M (right).

The high-resolution solution structure of I_T reveals a native-like Ig fold

After having validated that ΔN6 mimics structurally the amyloidogenic intermediate I_T a full chemical shift assignment and structure calculation of the wild-type protein and ΔN6 was carried out at pH 7.5, and 25°C. The resulting structural ensembles (Figure 1B) revealed that ΔN6 has a native-like Ig β-sandwich fold, which is quite similar to the native fold with some loss of secondary structure elements and rearrangement of residues around Phe 30.

Publications

Eichner, T. and Radford, S. (2009) A Generic Mechanism of beta2-Microglobulin amyloid assembly at neutral pH involving a specific proline switch. *J Mol Biol*, **386**:1312-1326.

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