HDX-ESI-MS reveals enhanced conformational dynamics of the amyloidogenic protein β_2-microglobulin upon release from the MHC-1

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Introduction

The light chain of the major histocompatibility complex class 1 (MHC-1) (Figure 1), the protein β_2-microglobulin (β_2m), has amyloidogenic properties that only arise upon its dissociation from the MHC-1. Here hydrogen/deuterium exchange electrospray ionisation mass spectrometry (HDX-ESI-MS) has been used to compare the solution dynamics of β_2m in its MHC-1 bound state compared with those of β_2m as a free monomer. The capability of tandem mass spectrometry to dissociate the MHC-1 into its individual constituents in the gas phase following deuterium incorporation in solution has permitted the direct observation of the exchange properties of MHC-1 bound β_2m for the first time.

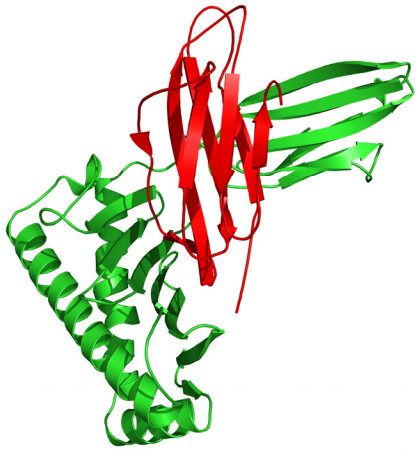

Figure 1. Ribbon diagram of the MHC-1 (PDB 2VLL) showing the heavy chain (α1, α2, α3; green ribbon) and the light chain (β_2m; red ribbon). The peptide binds in the cleft formed by the two helical regions (α1 and α2) of the heavy chain.

Results

ESI-MS is a powerful technique with which to monitor protein conformational dynamics using HDX. HDX-ESI-MS showed clearly that when β_2m is bound in the MHC-1, its H$\rightarrow$D exchange follows EX2 kinetics with an exchange rate of 0.002 ± 0.0003 min^{-1}, and indicates that ~20 protons remain protected from exchange after 17 days.

In comparison, when in an unbound state free from the MHC-1, β_2m exhibits very different characteristics exemplified by HDX mechanisms which encompass both EX1 and EX2 kinetics. The EX2 kinetics show a ten-fold increase in the exchange rate (0.023 ± 0.002 min^{-1}) compared with MHC-1 bound β_2m, and that ~10 highly protected protons exchange only via an EX1 mechanism. The EX1 data observed for unbound β_2m are consistent with unfolding of the protein's exchange-protected core, with a $t_{½}$ of 68 mins.

The comparison between MHC-1 bound and unbound β_2m highlights a remarkable change in the conformational dynamics of β_2m on its release from the MHC-1. When bound to the MHC-1, there is a significant damping of the conformational dynamics of β_2m, consistent with stable, macromolecular, protein complex architecture, whilst upon dissociation from the stabilising influence of the MHC-1, free β_2m becomes highly dynamic and undergoes unfolding transitions which result in an aggregation-competent protein.

These observations are of significance as partial, or more complete, unfolding is considered to be the key initiating step in protein aggregation processes which lead to disease-related fibril formation.

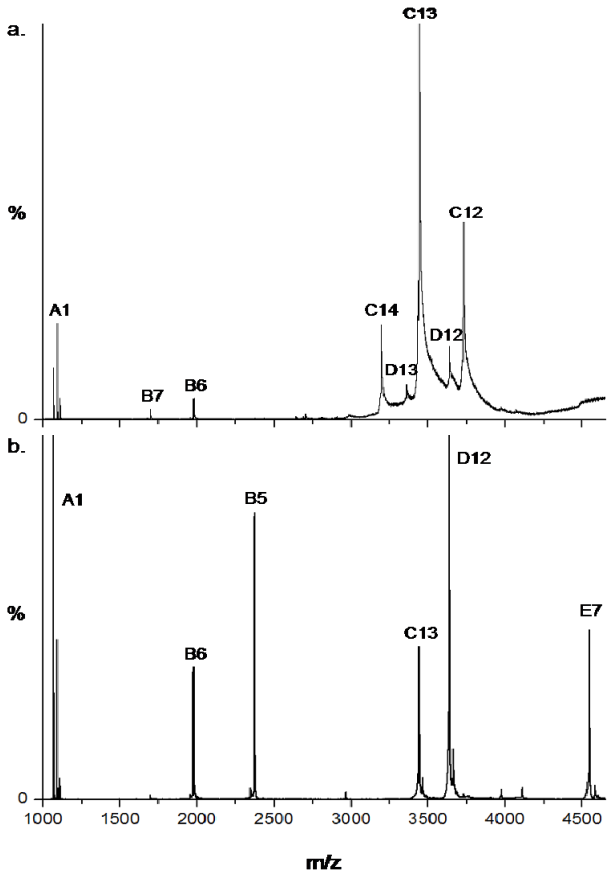

Figure 2.
(upper) ESI-MS m/z spectrum of the MHC-1 (pH 7.0), showing predominantly intact MHC-1 (C), together with traces of free β_2m (B), peptide (A) and MHC-1 without peptide (D);

(lower) ESI-MS/MS m/z spectrum showing dissociation of the intact MHC-1 (C; +13 charge state ions, m/z 3442) to yield: MHC-1 heavy chain (E), free β_2m (B), peptide (A) and MHC-1 without peptide (D).

The numbers adjacent to the letters relate to the charge states of those ions.

Collaborators

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