The use of hydrogen-deuterium exchange to investigate the role of structural conformation and protein folding of the amyloidogenic protein b_2-microglobulin.

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Overview

Studies into the conformational dynamics of β_2-microglobulin (β_2m) using hydrogen deuterium exchange (HDX) coupled to electrospray ionisation mass spectrometry (ESI-MS)[1] have been initiated. A comparison of the rate of exchange with variations in pH and temperature has been made.

Introduction

β_2-microglobulin (β_2m) is an 11,860.4 Da protein comprising seven β-sheet strands (Figure 1). In its physiological role, β_2m forms the light chain of the major histocompatibility complex and is important in cellular immunity. However it is also the building block of debilitating amyloid deposits which are found in the joints of dialysis-related amyloidosis sufferers[2].

Catabolism of free β_2m by the kidney normally regulates the blood concentration of the protein. However in kidney failure this mechanism is lost and β_2m concentrations in the blood can increase 60-fold. Dialysis does not remove β_2m and over the course of years β_2m monomers aggregate to form tissue specific amyloid fibrils. Clinical symptoms of DRA are seen in 100% of dialysis patients after 15 years of treatment.

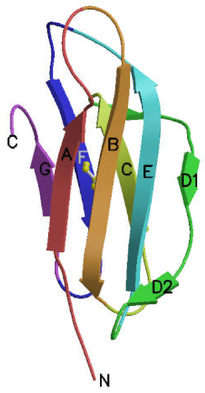

Figure 1: b_2m

The mechanism by which the amyloid fibrils are formed is not known nor is the structure of the fibrils. Our previous ESI-MS work has provided interesting insights into the conformational properties of β_2m[3,4]. Here we are using HDX to investigate the conformational dynamics of the protein.

Methods

β_2m was deuterated by dissolving in D_2O (5 mg mL^{-1}); after 7 days the solution was lyophilised and diluted in D_2O to 5 mg mL^{-1} (Figure 2). HDX was initiated by a 1:25 dilution of this solution into a 5mM ammonium acetate/ 5 mM ammonium formate buffer adjusted to the required pH with HCl or ammonium hydroxide. Aliquots were removed at time intervals and analysed by ESI-MS.

ESI-MS on the Platform II (Waters Corp., Manchester, UK) used a flow rate of 10 µL min^{-1}, a capillary voltage of 3.5 kV and a cone voltage of 40V. Nano-ESI-MS on the LCT Premier (Waters Corp., Manchester, UK) was achieved with a NanoMate temperature controlled interface (Advion, Ithaca, NY, USA) using a spray voltage of 1.7 kDa and a cone voltage of 40V.

Results

The extent of β_2m deuteration was measured by ESI-MS (Figure 2). The mass difference of 197 Da indicates complete deuteration.

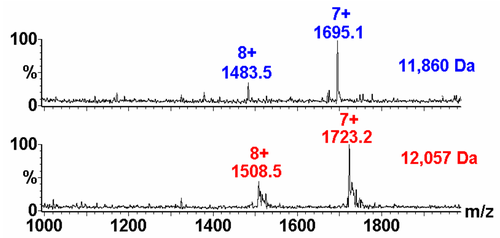

Figure 2: ESI-MS m/z spectra of deuterated (lower) and non-deuterated b_2m (upper) acquired at pH 7.

HDX of deuterated β_2m was carried out at pH 5 at 4ºC, and at pH 7 at 4ºc and 22ºC. The reaction was initiated, temperature controlled and sampled using the NanoMate interface on the LCT Premier (Figure 3). At 2 mins, the fastest exchange was at pH 7 (22ºC) where 140 deuteriums had been replaced with hydrogens (Table 1). By 60 mins, all three reactions indicated a protected core of ~35 deuterated sites. This is consistent with EX1 kinetics, while peaks due to both EX1 and EX2 kinetics can be seen between 3 and 5 hours (Figure 4).

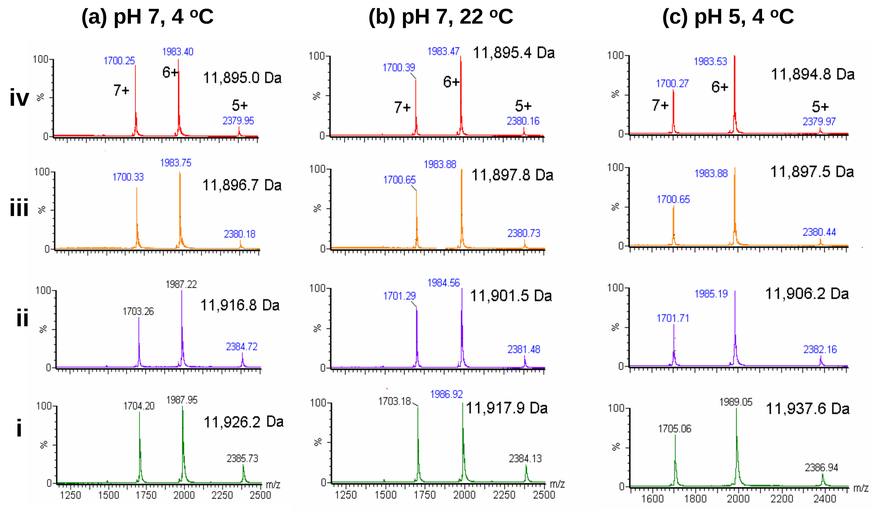

Figure 3: HDX of b_2m monitored by nanoESI-MS at time: (i) 2 mins; (ii) 20 mins; (iii) 40 mins; (iv) 60 mins.

Time (mins)	pH 7 4ºC	pH 7 22ºC	pH 5 4ºC
60	-162	-163	-163
30	-161	-161	-160
20	-141	-157	-151
2	-131	-140	-120

Table 1: HDX of b_2m monitored by nano-ESI-MS showing loss of deuterium with time

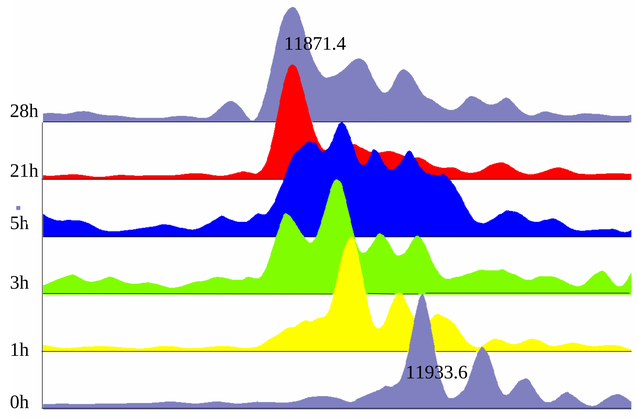

Figure 4: Mass profile time course of b_2m HDX at pH 5 monitored by ESI-MS. The reaction was cooled to 4ºC for the first minute to slow the initial rate and then continued at 22ºC.

Conclusion

NanoESI-MS with the NanoMate can be used effectively for temperature controlled HDX. Further work planned includes confirming the EX1/EX2 exchange kinetics of β_2m HDX mechanisms and studying the HDX properties of a series of β_2m variants with different amyloidogenic properties.

References

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Acknowledgements

We thank the BBSRC, the Wellcome Trust and the University of Leeds for financial support, and ASMS for a Travel Grant for JPH.