

Investigation into the role of lysosomal proteolysis in Dialysis Related Amyloidosis

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Introduction

β_2 -microglobulin (β_2 m) is one of approximately twenty proteins that aggregate to form highly ordered amyloid fibrils *in vivo*. β_2 m is a small 99 residue soluble protein, which is non-covalently bound to a membrane-integrated heavy chain, forming a major histocompatibility complex (MHC) class I molecule. MHC class I molecules are expressed on the surface of all nucleated cells and present peptide fragments, derived from intracellular proteins, to cytotoxic T lymphocytes. *In vivo*, β_2 m is continuously shed from nucleated cells as part of its normal catabolic cycle, into the serum. β_2 m is then transported to the proximal tubule of the kidney, where it is degraded and excreted. As a consequence of renal failure the β_2 m serum concentration increases by up to 60-fold. This high β_2 m serum concentration causes free β_2 m to associate, forming insoluble amyloid fibrils, which typically accumulate in the musculoskeletal system. As a consequence uremic patients who have been dialysed for 10-15 years develop dialysis-related amyloidosis (DRA), a debilitating arthritic-like condition.

Hypothesis

How β_2 m fibrils form *in vivo* is unknown, but *in vitro* studies have shown that incubation of β_2 m at acidic pH or the removal of the N-terminal six residues ($\Delta N6\beta_2$ m) induces rapid fibril formation. We hypothesise that cells within the joint capsule internalise β_2 m, which enters the endocytic pathway and accumulates in the lysosomes. We propose that the acidic microenvironment of lysosomes (pH 4.5), coupled with its high concentration of proteases, may stimulate fibril formation.

Internalisation of β_2 m by a model cell line

HeLa cells were used as a model cell line to analyse the internalisation of human β_2 m. These cells were incubated with monomeric human β_2 m at the concentration typically seen in the serum of uremic patients (50 μ g/mL) (Fig. 1). Internalisation of β_2 m was analysed using immunofluorescence microscopy, and localisation of internalised β_2 m in the endosomal pathway was analysed by co-staining with antibodies specific for early endosomal antigen-1 (EEA-1) and vesicle associated membrane protein-7 (VAMP7). Internalised human monomeric β_2 m accumulates in perinuclear regions and colocalises with both EEA-1 and VAMP7.

These data are consistent with sorting of the internalised β_2 m to lysosomes.

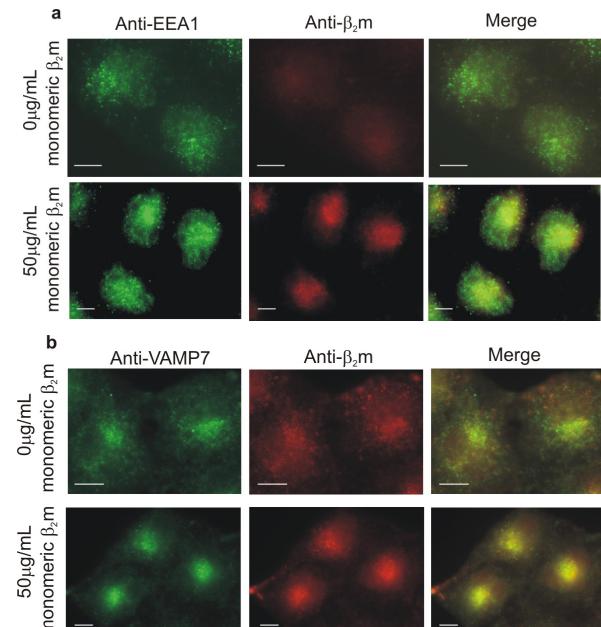


Fig. 1 – Immunofluorescence microscopy images of HeLa cells which have been incubated in the presence and absence of 50 μ g/mL monomeric human β_2 m for 1 hour at 37°C/5%CO₂. β_2 m was detected by staining with a β_2 m specific antibody and its localisation determined by co-staining with antibodies to (a) EEA-1 and (b) VAMP7. 10 μ m scale bar.

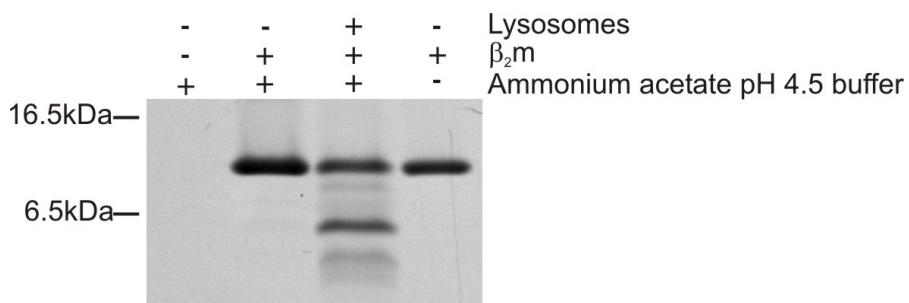


Fig. 2 – Tris-tricene gel of $\beta_2\text{m}$ digested by lysosomes, isolated by subcellular fractionation from HeLa cells.

Lysosomal digestion of $\beta_2\text{m}$

To determine the effect that the lysosomal environment has on $\beta_2\text{m}$, lysosomes were isolated from HeLa cells by subcellular fractionation and these fractions used to digest monomeric human $\beta_2\text{m}$ (Fig. 2). We are currently identifying the discrete bands generated and investigating whether these truncated $\beta_2\text{m}$ species form amyloid fibrils both *in vivo* and *in vitro*.

Publications

Morten, I.J., Hewitt, E.W. and Radford, S.E. (2004) β_2 -microglobulin and dialysis-related amyloidosis in *Protein Misfolding, Aggregation and Conformational Diseases*, Eds. Uversky, V.N. and Fink, A.L., Kluwer Academic/Plenum Publishers.

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