Role of Complementarity-Determining Regions 1 and 3 in Pathologic Amyloid Formation by Human Immunoglobulin k1 Light Chains

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Introduction

Immunoglobulin light chain (LC) amyloidosis is a life-threatening disease complicated by vast numbers of patient-specific mutations. Here we explored 14 patient-derived and engineered proteins related to k1-family genes IGKVLD-33’/01 and IGKVLD-30’/01.

Methods

LCs related to germline genes KXVLD-33’/01 (Part 1) and IGKVLD-30’/01 (Part 2) were expressed in E. coli and purified using Ni-NTA beads. The HDX study was performed at 120 °C in 10 mM sodium phosphate buffer, pH 7.4, 150 mM NaCl, using labeling time points ranging from 10 seconds to 4 hours. Digestion was performed using an in-gel column. Deamination incorporation was measured with a Waters HSD system and a Waters Synapt XSi ion mobility mode. PLGS was used for peptide identification and Dynamic NMR to probe the dynamic deamidation incorporation. The results were mapped on the structures of native and heavy-pairing LCs (PDB: 1B0D).

HDX MS analysis was integrated with studies of thermal stability, proteolytic susceptibility, and amyloidogenic sequence propensity. See also ref. [7].

Results

Part 1: Proteins analyzed in k1 sub-family IGKVL3-33’/01

Secondary structure, thermal stability and proteolytic susceptibility of patient-derived proteins related to IGKVLD-33’/01

Part 2: Proteins analyzed in k1 sub-family IGKVL3-30’/01

Secondary structure, thermal stability and proteolytic susceptibility of patient-derived proteins related to IGKVLD-30’/01

Conclusions

- LC stability varied among patient-derived proteins, with one order of stability being AL1 = AL2 = GL1. Engineered LCs showed a different stability order, AL2 (GL2) = AL1 (GL2) = GL1 (AL2).
- LCs related to the area of dialysis formation in the setting of LC-secreting multiple myeloma showed LCs with a high density of AL1 compared to GL1, potentially serving as a trigger for amyloid formation.

Part 1

Engineered LCs (AL2 and GL2) related to the k1 germline gene (KXVLD-33’/01) showed major changes in stability compared with patient LCs related to germline genes (KXVLD-33’/01), with the order of stability being AL2 = GL2 = AL1.

Secondary structure, thermal stability and proteolytic susceptibility of patient-derived proteins related to IGKVLD-33’/01

No data

References

- Desiree A. Demers, Olga Gursky, Esther Bullitt, and John R. Engen
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