Investigation of the Effects of Novel Kinetic Stabilizers on the Structural Dynamics of Amyloidogenic Immunoglobulin Light Chain (IgG LC) Using Hydrogen Deuterium Exchange Mass Spectrometry (HDMS)

Daniele Peterle1, Nicholas L. Yan2, Elena Klimchuk3, Olga Gursky3, Gareth J. Morgan3, Thomas E. Wales1, Jeffery W. Kelly2 & John R. Engen1

1Department of Chemistry & Chemical Biology, Northeastern University, Boston, MA, USA  
2Department of Chemistry, The Scripps Research Institute, La Jolla, CA, USA  
3Amyloidosis Center, Boston University School of Medicine, Boston, MA, USA

Introduction

A major obstacle to the study of amyloidogenesis is the formation of high molecular weight (MW) fibrils that are usually precipitated in vitro at pH 4–5. Small molecules (e.g. acrylamide) are capable of disrupting amyloid self-assembly in vitro; however, their effects are not well studied in cells. Thus, an understanding at the molecular level of how small molecules can stabilize the α-helical structure and inhibit fibrillation is a major challenge. In this study, we used hydrogendeuterium exchange mass spectrometry (HDMS) to determine the structural dynamics of amyloidogenic light chains (LCs) in the presence of three small molecules: MnCl2, MgCl2, and D-penicillamine (DPA).

HDMS Methods

HDMS was used to evaluate the effects of amyloidogenic LCs on the conformational stability of amyloidogenic light chains. Two different HDMS methods were used: (1) M83 and M83/100 in solution; and (2) M83 and M83/100 in membrane-bound LCs. The M83 study was performed at 20°C in pH 7.4 buffer (L), pH 7.4. Using labeling time points of 10 seconds to 1 hour, a gradient was performed with a 0.1% acetonitrile (ACN) buffer. HDMS were used for peptide identification and DPA was used to promote the deuterated exchange reactions.

Results

Figure 1. HDMS screening of 4 different kinetic stabilizers that show MB3 is the most effective compound.

Figure 2. MB3 promotes dimer formation.

Figure 3. MB3 inhibits fibril formation.

Figure 4. MB3 reduces tryptophan fluorescence in vitro.

Conclusions

The kinetic stabilizer MB3 had the greatest impact on reducing LC flexibility, consistent with it being the most potent stabilizer reported in a fluorescence polarization assay (Fig. 1). MB3 was shown to stabilize the highly amyloidogenic domain (Δ) and promote its fibrillation (Fig. 2). MB3 was also shown to reduce the rate of fibril formation induced by peptide (Fig. 3). MB3 was shown to reduce the rate of fibril formation induced by peptide (Fig. 4). MB3 was shown to reduce the rate of fibril formation induced by peptide (Fig. 5).

Supporting Information

The Supporting Information includes a more detailed description of the HDMS methods, a comparison of the HDMS analysis with previously published data, and additional HDMS results. The Supporting Information is available in the online version of the article.