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Kinetic stability and sequence/structure studies of urine-derived Bence-Jones proteins from multiple myeloma and light chain amyloidosis patients

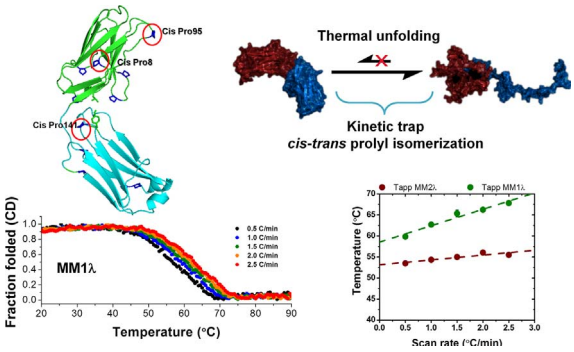
Luis M. Blancas-Mejía[a], Emily B. Martin[b], Angela Williams[b], Jonathan S. Wall[b,c], Marina Ramirez-Alvarado[a,d,*]

[a] Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA
[b] Department of Medicine, The University of Tennessee Medical Center, Knoxville, TN, USA
[c] Department of Radiology, The University of Tennessee Medical Center, Knoxville, TN, USA
[d] Department of Immunology, Mayo Clinic, Rochester, MN, USA

HIGHLIGHTS

- Bence Jones (BJP) kappa light chain proteins are less stable than lambda BJPs.
- The most stable BJPs in our study present 3-state unfolding transitions.
- The location of the mutations and the BJPs' folding properties correlate.
- The BJPs kinetic control in protein folding could be attributed to proline residues.

GRAPHICAL ABSTRACT



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ABSTRACT

It is now accepted that the ability of a protein to form amyloid fibrils could be associated both kinetic and thermodynamic protein folding parameters. A recent study from our laboratory using recombinant full-length (encompassing the variable and constant domain) immunoglobulin light chains found a strong kinetic control of the protein unfolding for these proteins. In this study, we are extending our analysis by using urine-derived Bence Jones proteins (BJPs) from five patients with light chain (AL) amyloidosis and four patients with multiple myeloma (MM).

We observed lower stability in κ proteins compared to λ proteins (for both MM and AL proteins) in agreement with previous studies. The kinetic component of protein stability is not a universal feature of BJPs and the hysteresis observed during refolding reactions could be attributed to the inability of the protein to refold all domains.

The most stable proteins exhibited 3-state unfolding transitions. While these proteins do not refold reversibly, partial refolding shows 2-state partial refolding transitions, suggesting that one of the domains (possibly the variable domain) does not refold completely. Sequences were aligned with their respective germlines and the location and nature of the mutations were analyzed. The location of the mutations were analyzed and compared with the stability and amyloidogenic properties for the proteins in this study, increasing our understanding of light chain unfolding and amyloidogenic potential.

* Corresponding author at: Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA.
E-mail address: ramirezalvarado.marina@mayo.edu (M. Ramirez-Alvarado).

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1. Introduction

There are currently 35 extracellular amyloid fibrils in humans [1]. Systemic light chain-associated amyloidosis (AL) is characterized by the abnormal proliferation of monoclonal plasma cells that secrete free light chains that misfold and aggregate as amyloid fibrils in vital organs causing organ failure and death [2]. It is now widely accepted that the amyloid deposits found in affected tissues of AL amyloidosis patients include, in addition to variable domain fragments, full length immunoglobulin light chains [3,4], although it is unclear if the amyloid fibrils are formed of mixtures of full length (FL) and fragments comprising the variable domain (V_L) and the constant domain (C_L) [5].

Bence Jones proteins (BJPs) are monoclonal light chains excreted in urine by patients with plasma cell dyscrasias such as AL amyloidosis and multiple myeloma (MM). Bence Jones proteins are monoclonal and readily isolated from patients' urine, and they are available in sufficient quantities for detailed biophysical analysis. Because of this, these proteins have been an invaluable research material for biochemical and biophysical investigation of immunoglobulin molecules [6–11]. Early studies assessing BJP stability were conducted studying protease resistance of these proteins [12]. Bence Jones proteins from patients with AL amyloidosis or MM have been compared for their thermodynamic stability. Consistent results from these comparative studies have indicated that the former are less thermodynamically stable than MM BJPs from the same or similar germline sequence with a high level of sequence identity [7,9,11]. One important characteristic of the thermodynamic properties of BJPs is that these proteins do not refold reversibly, in contrast to the reversible behavior observed for immunoglobulin light chain variable domains (V_L) from AL amyloidosis proteins (for a review, please see [2]). This irreversible behavior has also been observed using recombinant full length immunoglobulin light chains [8,10].

Table 1
Summary of the biological and biophysical properties and sequence alignment of the BJPs. Germline sequences are represented in bold font. Yellow or red font highlights the somatic mutations, while green highlight indicate tryptic peptides identify by mass spectrometry.

Protein	Germline	Tm app (0.5 °C/min)	Δtapp (2.5°C/min-0.5°C/min)	Total number of mutations	Non conservative mutations/total mutations	2 state vs. 3-state unfolding	location of non-conservative mutations
AL1κ (CRO)	Kappa I O12 (IGKV 1-39)	48.80 ± 0.07	3.86	15	0.5	2-state unfolding	
AL2κ (HIG)	Kappa I O18 (IGKV 1-33)	49.85 ± 0.43	**-1.73**	13	0.7	2-state unfolding	***β-strand C***, ***loop C-C'***, ***CDR2***, β-strand D, CDR3, ***β-strand G***
AL3κ (CAB)	Kappa 4 B3 (IGKV 4-1)	---	---	12	0.9	2-state unfolding	
AL1λ (GIO)	Lambda 6a (IGLV 6-57)	---	---	8	0.6	2-state unfolding	
AL2λ (SUT)	Lambda 6a (IGLV 6-57)	50.07± 0.18	**6.14**	17	0.8	**3-state unfolding**	***β-strand A***, ***β-strand B***, ***CDR1***, β-strand C, ***loop C-C'***, β-strand D, loop E-F, ***CDR3***, ***β-strand G***
MM1κ (GAL)	Kappa I O18 (IGKV 1-33)	49.32 ± 0.12	4.55	6	0.7	2-state unfolding	
MM2κ (WAT)	Kappa I O18 (IGKV 1-33)	52.17 ± 0.19	3.88	11	0.4	2-state unfolding	
MM1λ (HAG)	Lambda 4b (IGLV 4-69)	59.83 ± 0.03	**7.99**	22	0.69	2-state unfolding	***β-strand A***, ***β-strand B***, ***CDR1***, ***loop C-C'***, loop C'-D, loop D-E, strand E, ***CDR3***, ***β-strand G.***
MM2λ (JRH)	Lambda 3h (IGLV 3-9)	53.49 ± 0.03	2.03	19	0.74	**3-state unfolding**	loop A-B, β-strand B, CDR1, ***β-strand C***, ***loop C-C'***, ***CDR2***, β-strand C", and ***β-strand G***