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

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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.

Graphical Abstract

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Keywords

Bence-Jones; Light chain amyloidosis; Kinetic stability; Thermal unfolding; Protein aggregation; Circular dichroism

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].

Experimental evidence suggests that isolated V_L domains refold rapidly, but undergo delayed folding when in the context of a full length light chain [13]. It is interesting to note that in most of the studies, the thermal unfolding of Bence-Jones Proteins, free MM LCs,

AL LC, and recombinant LCs (encompassing the V_L and the C_L domain, also called FL) has been described as an apparent 2-state process, showing a single irreversible sigmoidal transition, often followed by protein aggregation [7–11, 14–16]. There is only one report of multiple unfolding transitions for the LC thermal unfolding performed at pH 3.23 [17].

Tischer and Auton describe the use of scan rate-dependent thermal unfolding of proteins to calculate thermodynamic parameters [18]. This method is particularly helpful for proteins with irreversible unfolding that is kinetically controlled. We have successfully characterized recombinant, full-length immunoglobulin light chains with this method. We found that AL-09 LC is less thermodynamically stable than $\kappa 1$ LC [10], which is consistent with results obtained for their corresponding V_L domains. Interestingly, the presence of the κC_L domain did not have any stabilizing effect. Our results differ from studies of a $\lambda 6$ (IGLV 6–57) full-length protein [8]. Klimtchuk and co-workers characterized the thermal unfolding properties of the V_L , C_L components, and the FL of protein AL-01-095 (comparing their results to the urine-derived BJP) [8]. Their results show that the C_L domain (LC3*04 or IGLC3) was more stable than any of the other variants, suggesting that the C_L domain in this $\lambda 6$ (IGLV 6–57) FL protein is destabilized by the V_L domain.

In this study, we have characterized the thermodynamics of 9 BJPs from 5 patients with AL amyloidosis and 4 MM patients previously described for their differential ability to be recruited in amyloid fibril elongation experiments [19]. From the five AL BJPs included in this study, partial unfolding and solubility issues precluded the analysis of two of them, so we conducted scan rate dependency experiments on the remaining seven proteins. Using this new approach, we determined thermodynamic parameters to compare their behavior and try to draw correlations with disease state. We modeled and analyzed the location of the mutations found in these proteins and compared it with their thermodynamic and fibril formation recruitment properties. λ BJPs are in general more stable than κ BJPs regardless of the disease state with which they are associated. While all BJPs present scan rate dependency of thermal unfolding, the extent of the scan rate dependency varies among them. Two BJPs show 3-state unfolding transitions and 2-state partial refolding, suggesting a sequential unfolding and incomplete refolding of the two domains. This sequential unfolding of BJPs has not been observed before to the best of our knowledge.

Materials and Methods

Patient-derived Bence Jones Proteins

Monoclonal serum BJP proteins were isolated from patients with an initial diagnosis of MM ($n = 4$) or AL amyloidosis ($n = 4$), or in one case, a patient initially presenting with MM later developed light chain amyloidosis (patient AL2 κ ; Table 1). These components were isolated from urine specimens as previously described [20]. The V_L -subgroup classification was determined serologically using polyclonal antihuman V_L -subgroup-specific antisera: antihuman total κ light chain (14-6E4) and total λ light chain (21-3F4) monoclonal antibodies [20, 21]; or by amino acid sequence analysis [19, 22]. Isolated BJPs were lyophilized and stored at room temperature until used. Protein concentrations were determined using the theoretical extinction coefficient of $\epsilon_{280\text{nm}} = 24,660 \text{ M}^{-1}\text{cm}^{-1}$ for AL1 κ ; $\epsilon_{280\text{nm}} = 24,660 \text{ M}^{-1}\text{cm}^{-1}$ for AL2 κ ; $\epsilon_{280\text{nm}} = 33,140 \text{ M}^{-1}\text{cm}^{-1}$ for AL3 κ ; $\epsilon_{280\text{nm}} =$

35,660 M-1cm-1 for AL1 λ ; $\epsilon_{280\text{nm}} = 37,150$ M-1cm-1 for AL2 λ ; $\epsilon_{280\text{nm}} = 26,150$ M-1cm-1 for MM1 κ ; $\epsilon_{280\text{nm}} = 24,660$ M-1cm-1 for MM2 κ ; $\epsilon_{280\text{nm}} = 35,660$ M-1cm-1 for MM1 λ and $\epsilon_{280\text{nm}} = 24,660$ M-1cm-1 for; $\epsilon_{280\text{nm}} = 35,660$ M-1cm-1 for MM2 λ . Integrity of the LCs was determined by mass spectrometry. The purity of the protein was determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

Circular Dichroism Spectroscopy

To ensure the quality of the reconstituted pure proteins, the global secondary structure after protein purification was determined by circular dichroism (CD). Experiments were conducted using 2 μM protein in phosphate buffered saline (PBS), pH 7.4 using 1.0 cm path-length cuvettes. Far UV-CD spectra between 260 and 200 nm were acquired at 4°C, on a Jasco Spectropolarimeter 810 (Jasco, Inc., Easton, MD) prior to thermal denaturation. Temperature was regulated within $\pm 0.002^\circ\text{C}$ using a Peltier system. A wavelength of 216 nm (indicative of β sheet content) was used to follow protein unfolding and refolding within the range of 4–90°C. Data were collected every 0.5°C for each of the five scan rates: 0.5, 1.0, 1.5, 2.0, and 2.5°C min⁻¹, with continuous stirring to avoid gradients of temperature within the solution and decrease the protein aggregation. Each set of scan rate data was collected in triplicate.

Scan rate dependent thermal unfolding

The analysis of the thermal unfolding transitions was carried out using two different approaches as we previously reported [10, 18]:

- A. Measurement of scan rates for the determination of T_m values at zero scan rate. This approach requires the determination of the transition midpoint of the thermal unfolding at different scan rates. The apparent transition midpoints, $T_{m,app}$ obtained from spectroscopic data at each scan rate were calculated by nonlinear least-squares fitting using the integrated van't Hoff equation as described previously[23].

The T_m values at zero scan rate (called $T_{m,eq}$) were determined empirically from linear extrapolation of the thermal scan rate dependence of the apparent T_m to zero scan rate.

- B. Fitting the data to an irreversible 2-state model. For an irreversible 2-state transition, unfolding is kinetically controlled by a first order rate constant that varies with temperature according to the Arrhenius equation 3 [18]. Our fitting protocol assumes 2-state unfolding. However, we observed apparent three-state unfolding with two of our proteins (AL2 λ and MM2 λ , supplementary figure 1). The first unfolding transition is observed between 40–50°C with a second transition between 50–60°C. Refolding is not fully reversible, but we observe 2-state partial refolding.

- C.

The activation energy of an unfolding reaction defined as $N \rightarrow U$ is $G^\ddagger = -RT \ln k^\ddagger$; we expand $k^\ddagger$ as a function of temperature defining $\beta = (\beta - \beta^*) = (1/RT - 1/RT^*)$ using a Taylor series, where T^* is the temperature at which $G^\ddagger = 0$.

$$\ln k^\ddagger(T) = \ln k_{T^*}^\ddagger + \Delta\beta \left(\frac{\partial \ln k^\ddagger}{\partial \beta} \right)_{T^*} + \frac{\Delta\beta^2}{2} \left(\frac{\partial^2 \ln k^\ddagger}{\partial \beta^2} \right)_{T^*} \quad (1)$$

Where we define $\ln k_{T^*}^\ddagger = 0$, and $(\partial \ln k^\ddagger / \partial \beta)_{T^*} = -\Delta H_{T^*}^\ddagger$. The latter term corresponds to the enthalpy of activation and $(\partial^2 \ln k^\ddagger / \partial \beta^2)_{T^*} = RT^{*2} \Delta C_{PT^*}^\ddagger$ is the relationship that defines the heat capacity of activation. The inclusion of the second order parameter in this Taylor expansion is given only for the sake of completeness.

Adapting the calculations from Tischer *et al.*, 2013, we assume that the rate equation for the irreversible formation of U with respect to β is in terms of the population, P_U .

$$\frac{\partial P_U(T)}{\partial \beta} = -\frac{\partial P_N(T)}{\partial \beta} = \frac{\partial P_U}{\partial t} \frac{\partial t}{\partial T} \frac{\partial T}{\partial \beta} = \frac{-RT^2}{v} \frac{k^\ddagger(T)}{v} (1 - P_U(T)) \quad (2)$$

where $P_U/t = k(1 - P_U)$; t/T is the inverse of the thermal scan rate (v) and $T/\beta = -RT^2$. Separation of variables followed by numerical integration results in the following expression for the unfolded state population, where $(P_N + P_U = 1)$.

$$P_U(T) = 1 - \exp \left(\frac{1}{Rv} \int \frac{k^\ddagger(T)}{\beta^2} \partial \beta \right) \quad (3)$$

For spectroscopy we used the following function for the observed CD or fluorescence change.

$$Obs(T) = Obs_N(T) (1 - P_U(T)) + Obs_U(T) P_U(T) \quad (4)$$

Where $Obs_N(T)$ and $Obs_U(T)$ are linear baseline functions for the native and unfolded state observables.

This last equation was used to fit all thermal transitions using a Microsoft Excel script written by Tischer *et al.*, 2013 that incorporates Boole's method for the numerical integration [24]. Fitting results were minimized to the total standard deviation of the sum of squared residuals. The goodness of fit was calculated via the R^2 coefficient of determination from the calculated total sum of squares $SS_{tot} = \sum_{ij} (y_i - \bar{y})^2$, regression of squares $SS_{reg} = \sum_i (\hat{f}_i - \bar{y})^2$ and the residual sum of squares $SS_{err} = \sum_i (y_i - \hat{f}_i)^2$, where y_i and $\hat{f}_i$ are individual observed and fit values at any given temperature and $\bar{y} = (\sum_{i=1}^n y_i) / n$ is the mean experimental observable for the entire transition.

$$R^2 = 1 - \frac{s_{err}}{s_{tot}} = 1 - \frac{\sum_i (y_i - f_i)^2}{\sum_i (y_i - \bar{y})^2} \quad (5)$$

The analysis yields the transition temperature T^* at which the rate of unfolding is equal to unity and the enthalpy of the transition at T^* that describes the temperature dependence of the rate constants for both heat-induced unfolding and refolding by cooling. Using this method it is possible to compare the T^* and H^* values which are independent of the applied scan rate.

Sequence alignment of BJP protein sequences

The protein sequences were initially determined by tryptic digestion followed by amino acid sequencing [25]. The sequences of these proteins were previously reported [26, 27]. Tryptic digestion of the proteins was used to confirm the sequences by the proteomics core facility at Mayo Clinic.

Sequences from each BJP were aligned with the 73 human light chain germline sequences using an automated alignment tool created by Roman Zenka and M. Ramirez-Alvarado (unpublished). Non-conservative mutations were defined in our earlier report [28]. The mutations were then mapped on the X-ray structures. For IGKV 1–33 and 1–39, we used 1B6D.pdb; for IGLV 3–21 and 4–69, we used 1LIL.pdb; for IGLV 6–57, we used 2CD0.pdb

Results

Secondary Structure Analyses

Table 1 includes the sequence alignment of the proteins used in this study. Two of the nine BJPs (AL3 κ and AL1 λ) were not fully folded at 4°C or soluble at the concentrations required for our study, respectively, while the rest were soluble and fully folded. Figure 1 shows the comparison of the native far-UV CD spectra of all the proteins tested in this study. In all cases, the soluble BJPs exhibited the expected β -sheet secondary structure of immunoglobulin proteins, as evidenced by a characteristic far UV-CD spectrum, with a minimum observed at 218 nm, typical of a β -sheet. Detailed inspection of the far-UV CD spectra showed small differences between the κ (Fig. 1A & C) and λ proteins (Fig. 1C & D), without significant differences between BJP from MM or AL patients belonging to the same isotype. We confirmed that the 7 BJPs studied did not refold reversibly, in agreement with previous reports from our laboratory and others [7–11].

Protein stability

Recent experimental evidence [8, 10, 11] indicate that the folding of free light chains (and BJPs) is kinetically controlled, facilitating sampling/populating amyloidogenic conformations. To determine whether the stability of the BJPs is governed by kinetic or thermodynamic stability, we measured scan rate dependency of the unfolding reactions.

Figures 2 and 3 show thermal unfolding transitions at different scan rates for the 7 proteins analyzed. The λ proteins were shown to be more stable than κ for both MM and AL

proteins. When comparing all κ BJPs, there is not a large difference in the melting temperatures between AL and MM proteins. The range of scan rate dependency on the difference in apparent melting temperature (T_{app}) varies between 2.03 °C for MM2 λ to T_{app} = 7.99°C for MM1 λ . Within the group of κ proteins the T_{app} range is 1.73°C for AL2 κ to 4.55°C for MM1 κ . Figures 4–6 show the thermodynamic parameters calculated from the scan rate thermal unfolding experiments for all proteins. The H_{T^*} values do not vary as a function of scan rate in any of the BJP proteins analyzed

Overall, the data indicates that BJP unfolding is kinetically controlled. However, the scan rate dependency is not uniform or as large as reported for the amyloidogenic protein AL-09 FL [10].

3-state unfolding transitions

Heretofore, all of the BJPs and recombinant full length proteins thermal unfolding characterization presented 2-state unfolding (from folded to unfolded). For the first time, we observed 3-state unfolding transitions (populating an unfolding intermediate) with two of the proteins in this study: AL2 λ and MM2 λ , which are among the most stable proteins in our study (Figure 7). Cooling (refolding) experiments performed using AL2 λ and MM2 λ show partial reversibility and 2-state refolding (see supplementary figure 1).

Location of mutations and scan rate dependency on protein unfolding

Mass spectrometry was conducted to determine the identity of the constant domain for all proteins, which revealed that all λ proteins shared the same germline gene product, IGLC2. We then mapped the mutations found in these two proteins unto known crystal structures (see methods section for details) and compared them with the location of the mutations found in the other BJPs in this study with 2-state unfolding transition. The mutations in AL2 λ and MM2 λ are in similar regions of the protein as indicated on figure 7.

Both AL2 κ and MM2 λ BJPs showed very small scan rate dependency on protein unfolding. The AL2 κ BJP has a T_{app} = 1.73°C, which falls within the error of our measurements (0.627–2.34°C). MM2 λ has a small scan rate dependency; T_{app} = 2.03°C

AL2 κ BJP, the only glycosylated protein, has non-conservative mutations in six secondary structure elements: **β -strand C**, **loop C–C'**, **CDR2**, β -strand D, CDR3, **β -strand G** (Figure 7). MM2 λ has non-conservative mutations in eight secondary structure elements: loop A–B, β -strand B, CDR1, **β -strand C**, **loop C–C'**, **CDR2**, β -strand C'', and **β -strand G**. AL2 κ and MM2 λ share 4 secondary structure elements with non-conservative mutations (in **bold**).

AL2 λ and MM1 λ present the largest scan rate dependency on protein misfolding (T_{app} = 6.14 and 7.99°C, respectively). AL2 λ presents non-conservative mutations in nine secondary structure elements: **β -strand A**, **β -strand B**, **CDR1**, β -strand C, **loop C–C'**, β -strand D, loop E–F, **CDR3**, **β -strand G**. MM1 λ presents non-conservative mutations in nine secondary structure elements: **β -strand A**, **β -strand B**, **CDR1**, **loop C–C'**, loop C'–D, loop D–E, strand E, **CDR3**, **β -strand G**. AL2 λ and MM1 λ share six common regions of mutagenesis (in **bold**). Comparing the location of mutations for the proteins with no scan rate dependency and the largest scan rate dependency, we note that the proteins with the

large scan rate dependency have 4 unique common areas with mutations: **β -strand A, β -strand B, CDR1, and CDR3** (Table 1). The mutations found in these regions for AL2 λ are: N1D, T20I, S27D, S31aG, N31bY, S93R, and S94D; for MM1 λ are: V3E, S7P, S27R, H29Y, S30D, and G94D.

Discussion

In this study we have conducted a systematic study of the kinetic stability of BJPs from MM and AL patients. We have observed that λ LCs seems to be more stable than κ LCs, regardless of the disease state, in agreement with Andrich *et al.*, 2017 [11]. There was a trend suggesting MM proteins are more stable than AL proteins. In this regard, thermodynamic and kinetic stability are not sufficient to explain the high amyloidogenicity of the light chains evaluated in this study. This suggests that the precise location and nature of somatic mutations and the presence of post-transcriptional modifications may alter the folding pathway by affecting the kinetic stability. In addition, the modulating effect of the constant domain, and host factors may play important role in amyloid aggregation. Previously, we have reported that both MM and AL BJPs were recruited by amyloid-like V_L fibrils; however the extent of recruitment of AL-derived BJPs was significantly greater, allowing discrimination of the two disease populations [19].

Immunoglobulin domains are rich in proline residues. These make up approximately 5–10% of their primary sequence and are generally found in turns connecting the β -strands. Thus, very often immunoglobulin domain folding is complicated by peptidyl–prolyl isomerization. In the absence of structural constraints, e.g. in the unfolded state of a protein, proline residues undergo cis/trans isomerization. In the native state, however, generally only one isomer is compatible with the native structure often giving rise to slow folding reactions caused by peptidyl–prolyl isomerization reactions.

Protein folding studies of immunoglobulin light chain domains indicate a kinetically controlled process, ascribed to cis/trans isomerization of some proline residues, resulting in kinetic traps allowing the protein to populate partially folded intermediates [13, 29–32]. Experiments conducted with one of the immunoglobulin light chain domains associated with amyloidosis found that kinetic stability (possibly influenced by the presence of cis proline 95a) plays a major role in the AL-103 amyloid fibril formation process [23].

Structural and sequence data allows us to determine the possible role of proline isomerization in the scan rate dependency and protein unfolding properties of the BJPs analyzed in this study. The λ proteins in this study have more proline residues than the κ proteins, particularly in the constant domain. The proteins that have the most stable, 3-state unfolding transitions (AL2 λ and MM2 λ supplementary figure 1) are the ones that have the largest number of proline residues in the study.

AL1 κ and MM2 λ are the proteins that presented similar rate constants of recruitment into amyloid fibrils in our previous study on recruitment of light chains [19]. However, their %LC bound to fibrils is dramatically different. AL1 κ is the protein that presented the largest ability to bind to amyloid fibrils while MM2 λ presents the second lowest percentage. AL1 κ

has a pseudo 3-state unfolding, not as clear as AL2 λ and MM2 λ . However, it is the least stable protein we characterized in this study. We propose that the 3-state unfolding makes AL1 κ and MM2 λ slow recruiters for amyloid elongation, while the partially folded states populated at 37°C make AL1 κ very efficient to be bound to the fibrils, just not in a fast way. AL1 λ showed the lowest rate constant among the AL BJPs and the lowest %LC bound to fibrils. In our study, AL1 λ was one of the two proteins that we could not characterize due to its poor solubility at the concentrations required for the current study (~50 μ g) compared to the ones required for the recruitment report (5–10 ng) [19]. Our data confirms AL1 λ and MM2 λ have very different stability and kinetic properties for folding and that the %LC bound is the right parameter to discriminate between amyloidogenic and non-amyloidogenic proteins (The location of non-conservative mutations for AL1 λ and MM2 λ is shown in supplementary figure 2).

The two proteins with the large scan rate dependency in protein unfolding are λ BJPs. This scan rate dependency may be attributed in part to cis/trans proline isomerization, particularly proline residues in the constant domains CL2/CL3. For this reason, we examined the proline residue isomers in two crystal structures from BJP.

For the κ structure, we analyzed 1B6D.pdb and found the following (common isomerization in **bold**): **P8-Cis**, **P40-Trans**, **P44-Trans**, **P59-Trans**, P95-Cis, P111-Trans, **P117P118-TransTrans**, **P141-Cis**, P204-Trans (figure 8).

For the λ structure, we analyzed 1LIL.pdb and found: P7-Trans, **P8-Trans**, P15-Trans, **P40-Trans**, **P44-Trans**, P55-Trans, **P59-Trans**, P109-Trans, P114-Trans, **P119P120-TransTrans**, **P141-Cis**, P154-Trans, P164-Cis, P183-Trans, P211-Trans (figure 8). The study by Aldrich *et al.*, 2017 did not provide sequence information of the proteins studied and therefore a comparative analysis of their results in the context of the location of the mutations and the proline content is not possible [11].

Our previous studies with κ I V_L and κ I LC demonstrated that the κ I LC forms fibrils faster and under all conditions compared to κ I V_L [10, 33]. We later demonstrated that an excess of V_L over LC in the same reaction mixture accelerates amyloid fibril formation. These results suggest that κ I LC is a better nucleator while κ I V_L is a better elongator and that the constant domain is modulating the amyloid formation reaction [34]. A possible way in which the constant and variable domains of LC can modulate amyloidogenic properties is by altering the unfolding/folding pathways, becoming a complex, often irreversible process. These alterations can happen without a significant effect on the apparent thermodynamic stability of the protein [13, 32, 35].

A possible model for the apparent 3-state unfolding of AL2 λ and MM2 λ may involve sequential unfolding of one domain after the other, with partial refolding of only one of those domains occurs under the experimental conditions. In the case of the LCs that presented the 3-state unfolding, we speculate that the pI of one of the domains is closer to the physiological pH used in this study, and therefore is possible to observe a small first unfolding transition. We hypothesize, based on previous studies indicating that the C_L may be more thermodynamically stable than its V_L counterpart; that the constant domain is able

to refold, but further studies are needed to confirm this. Notably, only MM BJPs exhibited partial reversibility.

Notably AL2 κ , a BJP derived from a patient who presented with MM and later developed AL amyloidosis, behaved like other AL-derived LC, with a high rate and extent of recruitment by synthetic amyloid-like fibrils [19]. AL2 κ has no scan-rate dependence of the thermal unfolding, indicating the absence of kinetic control in the folding process, probably due to its glycosylation acquired through somatic mutations (N30-N31-S32). Glycosylation is one of the most common post-translational modifications (PTM) to occur in protein biosynthesis, yet its role on the thermodynamic and kinetic stability of proteins is poorly understood. However, it is a positive risk factor for amyloidogenicity in κ LC proteins [36].

Oligosaccharides have been found in both murine κ type and human λ light chains synthesized by tumor cells [37]. It has been shown that PTMs cannot only adjust structural features of proteins, but also the thermodynamic, and kinetic stability [38]. Carbohydrates can, in principle, stabilize proteins by affecting their underlying energy landscapes [37, 38], most probably by increasing the energy of the unfolded state compared to the native state, and affecting the height of the folding barrier. We propose that the delayed *in vivo* amyloidogenic character of AL2 κ LC could be due the effect of glycosylation, kinetically stabilizing the AL protein without altering its ability to recruit monomeric protein into amyloid fibrils. Future studies understanding the role of glycosylation in the amyloidogenic properties of light chains are ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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

Figure 1.

Far-UV CD spectra of (A) MM1 λ (green) and MM2 λ (brown), (B) MM1 κ (purple) and MM1 κ (orange), (C) AL2 λ (blue), (D) AL1 κ (red) and AL2 κ (black); in PBS (pH 7.4) at 4 °C, before (filled symbols) and after irreversible thermal unfolding (open symbols). In all cases, samples were prepared at 2.0 μ M and measured using a 1 cm cuvette. Both heating and cooling were carried out at 0.5 °C min⁻¹.

Figure 2.

Thermal denaturation curves of (A) MM1 λ and (B) MM2 λ , following changes by circular dichroism as a function of thermal scan rate. In all cases, melting curves were measured continuously from 4 °C to 90 °C every 0.5 °C. Heating scan-rates employed 0.5 °C min⁻¹ (black), 1.0 °C min⁻¹ (blue), 1.5 °C min⁻¹ (green), 2.0 °C min⁻¹ (orange) and 2.5 °C min⁻¹ (red). Samples were prepared at 2.0 μ M and measured using a 1 cm cuvette with continuous stirring. Error bars correspond to the standard error obtained from 3 independent experiments.

Figure 3.

Thermal denaturation curves of (A) MM1κ and MM2κ, and (B) AL2κ and AL1κ following changes by circular dichroism as a function of thermal scan rate. In all cases, melting curves were measured continuously from 4 °C to 90 °C every 0.5 °C. Heating scan-rates employed 0.5 °C min⁻¹ (black), 1.0 °C min⁻¹ (blue), 1.5 °C min⁻¹ (green), 2.0 °C min⁻¹ (orange) and 2.5 °C min⁻¹ (red). Samples were prepared at 2.0 μM and measured using a 1 cm cuvette with continuous stirring. Error bars correspond to the standard error obtained from 3 independent experiments.

Figure 4.

Changes in the enthalpy reference independent of the applied scan rate $H^\ddagger$, as a function of the scan rate calculated for (A) MM1 λ (green) and MM2 λ (brown), (B) MM1 κ (purple) and MM2 κ (orange), (C) AL2 λ (blue), (D) AL1 κ (red) and AL2 κ (black). Continuous represent the averaged $H^\ddagger$ value. Data are the average of triplicates obtained from fitting each thermal unfolding transition using Eq. (4). Error bars correspond to the standard error obtained from 3 independent experiments.

Figure 5.

Comparison of the stability parameters (T^* values) obtained from fitting the thermal transitions in figures 2 and 3, for: (A) MM1 λ (green) and MM2 λ (brown), (B) MM1 κ (purple) and MM1 κ (orange), (C) AL2 λ (blue), (D) AL1 κ (red) and AL2 κ (black). Proteins were grouped as function of the disease and isotype to facilitate comparisons. Continuous line represent averaged T^* values, and error bars represent the standard error obtained from 3 independent experiments.

Figure 6.

Comparison of the scan rate dependence of the apparent T_m values (T_{mapp}), obtained from fitting the thermal transitions in figures 2 and 3, for: (A) MM1 λ (green) and MM2 λ (brown), (B) MM1 κ (purple) and MM1 κ (orange), (C) AL2 λ (blue), (D) AL1 κ (red) and AL2 κ (black). Proteins were grouped as function of the disease and isotype to facilitate comparisons. Dashed lines represent the fitting to a linear function. Error bars correspond to the standard error obtained from 3 independent experiments.

Figure 7.

Location of the mutations found in the most stable proteins and the only ones with 3-state unfolding behavior (top); proteins with small scan rate dependency (middle), and proteins with the largest scan rate dependency (bottom). Red highlights and text denotes non-conservative mutations. 1b6d.pdb was used for the κ proteins, 1lil.pdb was used for the λ proteins.

Figure 8.

Comparison of the relevant structural elements of the κ and λ BJPs. The variable (V_L) and constant (C_L) domains are connected by the join region (~10 aa), and each of the domains has one buried disulfide bond that stabilizes the beta-sandwich fold. Proline residues within V_L and C_L fragment are shown in a stick representation and highlighted in blue. cis- prolines are indicated by the red circles, and the rest are trans- prolines.

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	T _m app (0.5 °C/min)	tapp (2.5°C/min-0.5°C/min)	Total number of mutations	Non conservative mutations/tot al mutations	2 state vs. 3-state unfolding	location of non-conservative mutations
AL1κ (CRO)	Kappa IO12 (IGKV 1-39)	48.80 ± 0.07	3.86	15	0.5	2-state unfolding	
AL2κ (HIG)	Kappa IO18 (IGKV 1-33)	49.85 ± 0.43		13	0.7	2-state unfolding	
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		17	0.8		
MM1κ (GAL)	Kappa IO18 (IGKV 1-33)	49.32 ± 0.12	4.55	6	0.7	2-state unfolding	
MM2κ (WAT)	Kappa IO18 (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		22	0.69	2-state unfolding	
MM2λ (JRH)	Lambda 3h (IGLV 3- 9)	53.49 ± 0.03		19	0.74		