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A Single Mutation at the Sheet Switch Region Results in Conformational Changes Favoring λ6 Light-Chain Fibrillogenesis

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Systemic amyloid light-chain (LC) amyloidosis is a disease process characterized by the pathological deposition of monoclonal LCs in tissue. All LC subtypes are capable of fibril formation although λ chains, particularly those belonging to the λ6 type, are overrepresented. Here, we report the thermodynamic and *in vitro* fibrillogenic properties of several mutants of the λ6 protein 6aJL2 in which Pro7 and/or His8 was substituted by Ser or Pro. The H8P and H8S mutants were almost as stable as the wild-type protein and were poorly fibrillogenic. In contrast, the P7S mutation decreased the thermodynamic stability of 6aJL2 and greatly enhanced its capacity to form amyloid-like fibrils *in vitro*. The crystal structure of the P7S mutant showed that the substitution induced both local and long-distance effects, such as the rearrangement of the V_L (variable region of the light chain)–V_L interface. This mutant crystallized in two orthorhombic polymorphs, $P2_12_12_1$ and $C222_1$. In the latter, a monomer that was not arranged in the typical Bence–Jones dimer was observed for the first time. Crystal-packing analysis of the $C222_1$ lattice showed the establishment of intermolecular β–β interactions that involved the N-terminus and β-strand **B** and that these could be relevant in the mechanism of LC fibril formation. Our results strongly suggest that Pro7 is a key residue in the conformation of the N-terminal sheet switch motif and, through long-distance interactions, is also critically involved in the contacts that stabilized the V_L interface in λ6 LCs.

Keywords: AL amyloidosis; crystal structure; fibrillogenesis; λ6 subgroup; thermodynamic stability

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Abbreviations used: ASU, asymmetric unit; BJ, Bence–Jones; CDR, complementarity-determining region; FR, framework region; LC, light chain; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; ThT, thioflavin T; V_L, variable region of the light chain; WT, wild type.

Introduction

Amyloidosis derived from amyloid light chain (LC) is a systemic disease characterized by the extracellular deposition of a fibrillar proteinaceous material made of immunoglobulin LC fragments, comprising the variable domain (V_L) either alone or with a portion of the constant domain (C_L).[1–4] The mechanisms involved in fibril formation remain unclear. Several lines of evidence point to thermodynamic stability as one of the major factors that influence the amyloidogenic behavior of the LCs.[5–9] Point mutations that destabilize the folding of the LC, extreme values of pH and temperature, and the presence of chemical denaturants induce the formation of amyloid-like fibril *in vitro*.[10–16] These data suggest that misfolded species play a key role in the aggregation route.[6,11,14,17] Although ample data support this model, little is known about the nature of the structural changes leading to the amyloid aggregation of LC.

The V_L adopts the typical immunoglobulin fold characterized by the topological organization of nine strands (**A**, **B**, **C**, **C′**, **C″**, **D**, **E**, **F**, and **G**) as a Greek key motif.[18] As in other β-sheet proteins, the LCs make use of "negative design" features to prevent the edge strands from forming promiscuous β interactions, thereby preventing aggregation of the native state. The V_L makes use of several edge-protection strategies, including the favorable placement of prolines and charged residues, a β-bulge on edge strand **G** (C-terminal region), and a loop comprising residues 40–60 that caps edge strands **C″** and λ LCs, N-terminal strand **A** (residues 1–14) adopts a characteristic conformation known as the "sheet switch," which has been proposed to protect the two edge strands **B** and **G** from developing spurious intermolecular interactions that lead to aggregation.[19]

Consistent with the misfolding hypothesis of protein aggregation, it could be rationally predicted that the V_L amyloidogenesis proceeds via conformational intermediates that compromise the effectiveness of these protective motifs (e.g., the N-terminal sheet switch). Consequently, aggregation-prone regions, such as the edge strands that are normally buried in the native state, may partially access the molecular surface. If this assumption is correct, the substitution of residues at position 7 and/or position 8 will disturb the interaction network that stabilizes the N-terminal sheet switch in λ LC, thereby favoring its aggregation into amyloid-like fibrils.

To confirm this hypothesis, we used the 6aJL2 protein as a model. This V_L contains the sequences encoded by the λ6 germ-line genes.[21] In this work, we report the thermodynamic and *in vitro* fibrillogenic properties of 6aJL2 mutants in which Ser substitutes Pro7 and/or His8. Serine is present in other V_Lλ gene segments in both positions. The effect of substituting His8, a residue characteristic of the λ6 proteins, by Pro was also studied. We observed that the P7S mutation decreased the thermodynamic stability of 6aJL2 and enhanced its fibrillogenic potential *in vitro*. In contrast, H8P and H8S mutants were almost as stable as the wild-type (WT) protein and were poorly fibrillogenic. The crystal structures of the native 6aJL2 and the point mutant P7S were determined to aid in the interpretation of the biophysical data. Our structural data clearly indicate that Pro7 is a key residue that affects the stability of the sheet switch at the N-terminus, thus supporting its protective function. Additionally, this residue is involved in long-distance interactions that stabilize the whole domain, in particular, the conformation of the V_L–V_L interface.

Results

Thermodynamic stability of mutants and the kinetics of amyloid fibril formation

The thermodynamic parameters of the different mutants (P7S, H8P, H8S, and P7S–H8S), as determined from thermal and chemical equilibrium

Table 1. Thermodynamic and kinetic parameters for the chemical and thermal unfolding and the aggregation of the λ6 proteins

	Thermal unfolding			GdnHCl unfolding				*In vitro* fibrillogenesis		
								Without seed	With seed	
Protein	$\Delta G_{25\,°C}$[b] (kcal/mol)	T_m[c] (°C)	ΔH_m[c] (kcal/mol)	ΔG_{H_2O} (kcal/mol)	$-m$ (kcal/mol)	C_m (M)	$\Delta\Delta G$[a] (kcal/mol)	t_{lag} (min)[d]	A_{ThT}[e]	K[f] (s^{-1})
6aJL2	5.2	49.9	86.2	5.1	3.6	1.41	—	900	1	5.3×10^{-5}
P7S	3.7	43.0	79.5	2.5	3.0	0.84	2.1	50	1.6	1.1×10^{-4}
H8P	4.4	50.1	84.8	4.5	3.0	1.48	−0.3	1000	0.5	6.1×10^{-5}
H8S	5.0	49.5	84.4	4.5	3.5	1.28	0.5	500	0.3	4.8×10^{-5}
P7S–H8S	3.8	43.7	79.4	N.D.	N.D.	N.D.	N.D.	130	2.0	1.2×10^{-4}

N.D. means not determined.
[a] Cumulative free energy change relative to 6aJL2: $\Delta\Delta G_{unf} = (m^{6aJL2} \times C_m^{mut}) - (m^{6aJL2} \times C_m^{6aJL2})$.
[b] Calculated as described by Pace *et al.*[39]
[c] Calculated from the plot of ΔG *versus* T.
[d] Calculated by extrapolating the linear region of the hyperbolic phase back to the abscissa.
[e] Asymptotic ThT fluorescence value of the elongation reaction, normalized to 6aJL2.
[f] Pseudo-first-order growth rate constant fits from the data shown in Fig. 1d.