Altered Dimer Interface Decreases Stability in an Amyloidogenic Protein*³

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Amyloidoses are devastating and currently incurable diseases in which the process of amyloid formation causes fatal cellular and organ damage. The molecular mechanisms underlying amyloidoses are not well known. In this study, we address the structural basis of immunoglobulin light chain amyloidosis, which results from deposition of light chains produced by clonal plasma cells. We compare light chain amyloidosis protein AL-09 to its wild-type counterpart, the xI O18/O8 light chain germline. Crystallographic studies indicate that both proteins form dimers. However, AL-09 has an altered dimer interface that is rotated 90° from the xI O18/O8 dimer interface. The three non-conservative mutations in AL-09 are located within the dimer interface, consistent with their role in the decreased stability of this amyloidogenic protein. Moreover, AL-09 forms amyloid fibrils more quickly than xI O18/O8 in vitro. These results support the notion that the increased stability of the monomer and delayed fibril formation, together with a properly formed dimer, may be protective against amyloidogenesis. This could open a new direction into rational drug design for amyloidogenic proteins.

Amyloidoses are a group of protein misfolding diseases characterized by amyloid fibril deposition. Although different proteins with widely varying native structures are linked to these diseases, they all form morphologically similar fibrils that are straight, unbranched assemblies of cross β-sheets (1). In light chain amyloidosis (AL), a population of monoclonal plasma B cells proliferates and secretes immunoglobulin (Ig) light chains that aggregate and form amyloid fibrils in the extracellular space of vital organs, causing fatal organ failure (2, 3).

A normal Ig pairs two light chains (LCs) with two heavy chains (HCs), the products of gene rearrangement and somatic hypermutation, generating a heterotetramer that is secreted from a plasma B cell. Within the heterotetramer, the variable domain of the LC (V_L) and the variable domain of the HC (V_H) typically join noncovalently to form a dimer interface. Although the specific amino acid residues involved in this interface vary widely between LC proteins, the tertiary and quaternary structure of the dimer interface is well conserved. Structural studies of LC dimers (Bence Jones proteins) show that V_L·V_L domains associate with the same dimer interface as V_H·V_H domains (4). Because 85% of AL patients secrete free LC from the plasma cell in the form of an LC dimer (5), the study of V_L·V_L domain interactions is pertinent to AL.

AL LC proteins share structural homology with normal Igs, where V_L structures consist of two β-sheets with three and four antiparallel β-strands packed together forming a Greek key β-barrel (6–12). Despite this structural conservation, AL LC proteins have been shown to be thermodynamically less stable than non-amyloidogenic multiple myeloma (MM) LC proteins, possibly because of the nature of somatic mutations (13–15). The increased rate of amyloidogenicity in the AL LC proteins has largely been attributed to this decreased stability (14). In addition to instability, the loss of the Ig heterotetramer may also contribute to the amyloidogenicity of AL proteins. Based on these observations, we hypothesize that mutations of residues within the dimer interface in AL proteins may maintain the same monomeric structure but disrupt the dimeric interactions, causing instability leading to amyloidogenesis.

Comparing an AL protein with its corresponding unmutated germline protein will help us understand the contributions of individual mutations to the protein structure and thermodynamic stability. Moreover, certain germline subtypes have

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3 The abbreviations used are: AL, light chain amyloidosis; LC, light chain; HC, heavy chain; V_L, light chain variable domain; MM, multiple myeloma; T_m NaS, melting temperature; T_m NaS, melting temperature with 500 mM Na_2SO_4; C_α, concentration of denaturant where 50% of protein is unfolded; PDB, Protein Data Bank; EM, electron microscopy; ThT, thioflavin T; r.m.s., root mean-square.

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proven to be highly represented among amyloidogenic proteins (16–18), possibly contributing to protein instability. In this study, we characterize a protein generated from a highly amyloidogenic germline, κ O18/O8, and compare its structure and thermodynamic parameters with an amyloidogenic protein, AL-09, derived from the same germline subtype. AL-09 Vlc comes from a patient with cardiac AL and differs from the κ O18/O8 germline by seven residues: S30N, N34I, K42Q, N53T, D70E, I83L, and Y87H (supplemental Fig. S1) (19). Notably, all of the non-conservative mutations in AL-09 (N34I, K42Q, and Y87H) are located in the Vlc-Vlc dimer interface. The comparisons between AL-09 and its germline, κ O18/O8, are unique, because a κ LC germline protein has never previously been described.

**EXPERIMENTAL PROCEDURES**

*Site-directed Mutagenesis*—Because the germline protein is not expressed naturally, the κ O18/O8 germline DNA was generated by mutating the cDNA of AL-103, another protein derived from the κ O18/O8 germline that differs from the germline by only 4 codons. These codons were mutated to the germline sequence using the QuikChange® Multi Site-directed Mutagenesis kit (Stratagene). The Mayo Clinic DNA Sequencing Core facility confirmed the mutagenesis.

*Cloning, Expression, Extraction, and Purification*—The AL-09 protein sequence has previously been deposited in GenBank™ with the accession number AF490909 (16). Recombinant AL-09 protein was expressed and purified as described previously (19). κ O18/O8 (sequence deposited under GenBank™ accession number EF640313) protein was extracted from the periplasmic space of *Escherichia coli* BL21 (DE3) Gold cells following freeze-thaw and washing with phosphate-buffered saline. The protein was purified by size exclusion chromatography (HiLoad 16/60 Superdex 75 column) on an AKTA FPLC (GE Healthcare) system. Pure protein was verified by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis.

*Circular Dichroism Spectroscopy (CD)*—Protein secondary structure was monitored at 4 °C by far UV-CD (Jasco spectropolarimeter 810) from 260–200 nm. Samples contained 20 µM protein in a 0.2-cm cuvette, and measurements were taken every 1 nm with a scanning speed of 50 nm/min. Thermal denaturation experiments followed the ellipticity at 218 nm over a temperature range of 4–90 °C. The temperature was increased by 30 °C/h with a response time of 32 s. Protein refolding was also measured immediately after the denaturation using the above parameters from 90 to 4 °C. The thermal denaturation curves were analyzed as described previously (19) to calculate a T_m (melting temperature, where 50% of the protein is unfolded).

Chemical denaturation with urea was carried out by equilibrating 20 µM protein samples overnight at 4 °C in either 0 or 8 M urea. Subsequent samples were generated by exchanging equal volumes of the two stock solutions of 0 and 8 M urea to create a range of urea concentrations while keeping the protein concentration constant. Each sample was equilibrated for 10 min at each urea concentration, and then the denaturation experiment was followed by CD with a 60 s scan at 218 nm or by Trp fluorescence, with excitation at 294 nm and an emission scan from 310–400 nm. Urea concentration was calculated using a hand refractometer (20). The denaturation curves were analyzed by the same method as described for the thermal denaturation experiment. The C_m is the concentration of denaturant where 50% of the protein is unfolded. ΔG_folding was determined from chemical denaturation data. The enthalpy (∆H) was determined from the thermal denaturation data using the van’t Hoff equation, as described in Ref. 14.

*Fibril Formation*—Fibril seeds were formed with κ O18/O8 and AL-09 (20 µM protein) by shaking 750-µl samples in 1-ml polypropylene tubes at 30 rpm with 500 mM Na_2SO_4 and 0.02% NaN_3 in 10 mM Tris-HCl (pH 7.4) buffer. Temperature for fibril formation was 68 and 51 °C for κ O18/O8 and AL-09, respectively, which represents the melting temperature in the presence of 500 mM Na_2SO_4 (T_mNaSO_4) of each protein. Thioflavin T (ThT) fluorescence was monitored to follow fibril formation. A 5-µl fibril sample was added to 5 µl ThT, and the fluorescence emission was measured (PTI-QM2001 fluorometer). The excitation wavelength was 450 nm, and the emission was scanned from 470 to 530 nm. Before they were used to seed further reactions, the fibrils were washed three times with buffer to remove Na_2SO_4. The concentration of seeds was determined by pelleting the fibrils and measuring the concentration of the soluble protein. This concentration was subtracted from the initial protein concentration to find the fibril concentration.

Fibril formation kinetics were followed (with each protein in triplicate in a 96-well plate) by measuring ThT fluorescence on a plate reader ( Analyst AD, Molecular Devices) with an excitation wavelength of 430 nm and an emission wavelength of 485 nm. Plates were incubated at 37 °C in a temperature-controlled incubator and shaken continuously on a Lab-Line titer plate shaker (speed setting 3). Each well contained 20 µM protein, a 1:20 ratio of seeds to soluble protein, 150 mM NaCl, 0.02% NaN_3, and 5 µM ThT in 10 mM Tris-HCl buffer (pH 7.4). The total volume for each reaction was 260 µl.

*Electron Microscopy (EM)*—A 3-µl fibril sample was placed on a 300 mesh copper formvar/carbon grid and air-dried. The sample was negatively stained with 4% uranyl acetate, washed, air-dried, and inspected on a Philips Technai T12 transmission electron microscope.

*Crystallization/X-ray Data Collection*—Purified κ O18/O8 and AL-09 proteins were concentrated to 890 µM and 1.4 mM, respectively, in 10 mM Tris-HCl buffer (pH 7.4). Crystals of both proteins were obtained in hanging drops using vapor diffusion against 30% w/v polyethylene glycol 4000 and 0.2 M Li_2SO_4 in 0.1 M Tris buffer (pH 7.9–8.9) at 22 °C. A 2-µl aliquot of the protein solution was mixed with an equal volume from each reservoir. The equilibrated conditions were suitable for cryoprotection of crystals by flash-freezing in liquid N_2. Table 2 summarizes the statistics for the crystallographic diffraction data collections and structural refinement. These data were collected at beamline 19BM (Structural Biology Consortium, Advanced Photon Source (APS), Argonne National Laboratory). The data sets were collected at 70 K.

*Structure Determination*—Diffraction data were processed with HKL2000 and SCALPACK (21). Both structures were solved by molecular replacement using PHASER (22, 23). Monomeric probe structures were used, first the κ LC BRE (1BRE.pdb) for the κO18/O8 diffraction data and then the
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RESULTS

Secondary Structure and Thermodynamic Stability of kO18/O8 and AL-09—Far UV-CD spectra confirmed that the germline kO18/O8 and the amyloidogenic AL-09 proteins assumed the typical Ig β-sheet secondary structure (Fig. 1). Both kO18/O8 and AL-09 have β-sheet structure, with the characteristic minimum near 218 nm (Fig. 1a). A second minimum at 235 nm is attributed to the interaction of the 11 (AL-09) or 12 (kO18/O8) aromatic residues in the proteins (34, 35). These residues cause the lone tryptophan in each protein (Trp-35) to be optically active in the far UV region, creating the second minimum.

Thermal and chemical denaturation experiments assessed the comparative thermodynamic stability between kO18/O8 and AL-09. Both proteins refold reversibly, and the \( T_m \) for kO18/O8 is 56.1 °C, whereas for AL-09 it is only 41.1 °C (Fig. 1b). Similarly, chemical denaturation with urea results in a C_m for kO18/O8 of 4.0 M, compared with 1.9 M for AL-09 (Table 1). When comparing the free energy of folding, kO18/O8 also shows significantly increased stability over AL-09, with a \( \Delta G_{\text{folding}} \) of -6.1 kcal/mol compared with -3.5 kcal/mol for the amyloidogenic protein (Table 1). Enthalpy calculations reflect the same trend, with \( \Delta H \) values of -95.7 and -62.8 kcal/mol for kO18/O8 and AL-09, respectively. Taken together, these data indicate that mutations from the germline sequence may be causing AL-09 to be less thermodynamically stable and increasing its propensity to misfold and form amyloid fibrils.

AL-09 Presents Faster Amyloid Fibril Formation Kinetics in Vitro—Previously, AL-09 was incubated in 10 mM Tris-Cl buffer (pH 7.4) with 50 mM NaCl at 37 °C for one month without any sign of fibril formation (19). In an effort to induce fibril formation in both proteins, we incubated kO18/O8 and AL-09 with 500 mM NaSO_4 at their corresponding \( T_m \) in the presence of 500 mM Na_2SO_4 (Table 1). Na_2SO_4 has been shown to stabilize proteins and folding intermediates (36, 37) and also...
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37 °C in the presence of 150 mM NaCl in 10 mM Tris-HCl buffer (pH 7.4) to mimic physiological conditions. The samples were continually agitated to induce fibril formation and monitored by ThT fluorescence. AL-09 shows a significant increase in ThT fluorescence over κl O18/O8 within 24 h (p value = 0.05), indicating more rapid fibril formation for the amyloidogenic protein (Fig. 2a). An increase in κl O18/O8 ThT fluorescence does not occur until after 215 h (supplemental Fig. S2). The presence of fibrils was confirmed by EM (Fig. 2, b and c). These data indicate that under identical conditions, AL-09 has a significantly shorter lag time for fibril formation compared with κl O18/O8, confirming increased amyloidogenicity for the disease-causing protein.

Crystal Structures Reveal Novel Dimer Interface for AL-09—Although the same crystallization conditions were used to produce both LC crystals, the molecular packing creates different space group symmetries: P6₁ for κl O18/O8 and P4₁32 for AL-09. The structure of κl O18/O8 was solved by molecular replacement (MR) with AL protein BRE (1BRE.pdb) (8). The asymmetric unit of the κl O18/O8 crystal contains one dimer, while that of AL-09 has two dimers. The κl O18/O8 structure was refined to 1.3-Å resolution, with Rfactor and Rout values of 11.9 and 14.8%, respectively (Table 2), electron density Fig. 3e). The AL-09 structure was determined by MR with κl O18/O8 and was refined to 2.5-Å resolution with an Rout of 16.5% and Rout of 20.6% (Table 2). Both structures have the characteristic immunoglobulin fold (Fig. 3, a and b).

The most striking difference between κl O18/O8 and AL-09 is in the dimer interface (compare Fig. 3, a and b). The AL-09 interface is rotated 90° relative to κl O18/O8, significantly altering the interacting residues in the interface (Fig. 3e and supplemental Table S1).

To evaluate the biological significance of all protein-protein interactions within the asymmetric unit, we utilized the Protein Interfaces, Surfaces and Assemblies (PISA) service (40). A detailed rationale of the PISA interface selection is included as a supplemental note. When the relevant κl O18/O8 interface was searched against the Protein Data Bank (PDB), the results indicated that ≈80% of its interface residues occupy equivalent positions in all other AL and MM LC protein structures, including those named WAT, REI, LEN, DEL, and BRE (6–8, 12, 41). The same interfacial analysis for AL-09 returned no match to any known structure in the PDB (threshold of ±60% similarity).

According to these analyses, κl O18/O8 has 7 residues (Tyr-49 (monomer B)/Asp-50 (monomer A), Glu-55, Thr-56, Tyr-87, Gly-99, and Gln-100) in its dimer interface that are not

to catalyze fibril formation reactions (19, 38, 39). By incubating at the TmNaSo4, we were able to compare fibril formation of both proteins where ΔGfolding = 0 (39), even though the Tm values were different for each protein. In this case, both κl O18/O8 and AL-09 were able to form ThT-positive fibrils, confirmed by EM (data not shown).

To gauge whether κl O18/O8 has delayed fibril formation compared with the amyloidogenic AL-09 under identical conditions, we carried out self-seeded reactions similar to those described previously (19). The reactions were seeded with a dilution of preformed fibrils (from the fibril formation assays using Na2SO4) in which the two proteins were incubated at

| Table 2: Data collection and model refinement statistics |

|          | κl O18/O8 | AL-09 |
|----------|-----------|-------|
| Space group | P6₁       | P4₁32 |
| Cell a, b, c (Å) | 74.27, 74.27, 99.05 | 176.05 |
| Resolution (Å) | 1.30 (1.33-1.30) | 176-2.55 (2.64-2.55) |
| Completeness (%) | 99.7 (96.3) | 99.3 (93.0) |
| Redundancy | 12.2 (5.6) | 98.7 (68.3) |
| R| <1/σ| | 0.046 (0.53) | 0.095 (0.47) |
| R| <1/σ| | 53.2 (2.3) | 82.5 (9.8) |
| Rfactor | 0.139 (0.218) | 0.165 (0.242) |
| Rout | 0.148 (0.218) | 0.206 (0.376) |
| No. reflections | 74839 (5311) | 29819 (2094) |

R.m.s deviations

| Bond length (Å) | 0.019 | 0.020 |
| Bond angle (°) | 1.78 | 1.83 |

Ramachandran plot

| Most favored regions (%) | 96.24% | 93.22% |
| Outliers (%) | 0.00% | 0.23% |

* Highest resolution shell shown in parenthesis.

* Ramachandran outlier for AL-09 is glycine residue 41 in chain B with phi, psi angles 37.1°, 88.4°.
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Comparing the dimer interface residues illustrates conformational changes linked to the mutations present in AL-09 (Fig. 3d). Among the interface residues, the Y87H mutation in AL-09 is of particular interest, because Tyr-87 is >95% conserved across all κ and λ V_{L} germline sequences. Based on the PISA analysis, His-87 is not included as part of the AL-09 dimer interface because of the 90° rotation, whereas Tyr-87 is included in the κ O18/O8 interface. The His-87 side chain presents extremely strong electron density in the AL-09 crystal, defining a clear rotamer conformation. In addition, to accommodate the N34I mutation in AL-09, both Tyr-36 and Phe-98 side chains are repositioned (Fig. 3d). Given the position of Phe-98 in AL-09, a germline-like dimer interface is sterically impossible. Because the monomer backbones are unaltered, the conformational changes observed in the dimer interface residues suggest that the Ile-34 and His-87 mutations are a driving force in changing the altered interface.

Dimer Dissociation of κ O18/O8 and AL-09—Delving further into the implications of differing dimer interfaces between κ O18/O8 and AL-09, analytical ultracentrifugation (AUC) experiments assessed the monomer-dimer dissociation of κ O18/O8 and AL-09 under non-denaturing conditions at 4 °C.

AUC data show that κ O18/O8 has about a 10-fold higher dimer dissociation constant (217 ± 70 μM) compared with AL-09 (23 ± 8.8 μM) when all the speeds and protein concentrations are averaged together (Table 3). The corresponding ΔG_{dissociation} values describing the dimer to monomer transition for κ O18/O8 and AL-09 are 4.6 and 5.9 kcal/mol, respectively (Fig. 4). The presence of 100 mM NaCl decreased the dimer affinity slightly for both proteins. Overall, the altered dimer interface of AL-09 appears to change its affinity of dimerization with respect to that observed for κ O18/O8.

We also performed analytical size exclusion chromatography to assess the oligomerization of the proteins. These data indicate that both κ O18/O8 and AL-09 populate monomeric species at about 2 μM (supplemental Fig. S3).

Because we were expecting the altered dimer interface of AL-09 to have a weaker affinity, the AUC results were somewhat surprising. This led us to compare the thermodynamic stability of both proteins at two concentrations, allowing us to evaluate different concentrations of dimer in solution.

FIGURE 3. Crystal structures revealed different dimer interfaces for κ O18/O8 (a) and AL-09 (b). c, superposition of κ O18/O8 (blue and cyan) and AL-09 (brown and salmon) dimers illustrated that AL-09 had a 90° rotation from the canonical (germline-like) interface. d, arrangement of key interface residues was significantly disrupted upon superposition of κ O18/O8 (blue) and AL-09 (brown) monomers. The presence of the second monomers for κ O18/O8 (cyan) and AL-09 (salmon) showed that a canonical dimer interface in AL-09 was sterically impossible, given the conformation of F98 (yellow highlight). e, stereo images of κ O18/O8 2Fo-Fc electron density (at 1 σ contouring). The images show the electron density around Trp-35.

TABLE 3
Analytical ultracentrifugation analysis to determine K_d

| Protein        | Speed | [Protein] | K_d |
|-----------------|-------|-----------|-----|
| κ O18/O8        | 15    | 50        | 132 ± 1.99 |
|                 | 13    | 17        | 189 ± 6.4  |
|                 | 15/13 | 50        | 289 ± 2.85 |
| Average with NaCl | 15   | 50        | 217 ± 70.2 |
| Without NaCl    | 13    | 50        | 305.5 ± 3.3 |
| AL-09           | 15    | 33        | 17.8 ± 0.11 |
|                 | 13    | 50        | 7.6 ± 0.07 |
|                 | 13    | 33        | 30.6 ± 0.30 |
|                 | 50    | 18.6 ± 0.20 |
|                 | 50    | 25.6 ± 0.28 |
|                 | 13/10 | 50        | 29.2 ± 0.32 |
| Average with NaCl | 15   | 50        | 23 ± 8.8 |
| Without NaCl    | 13    | 50        | 34.7 ± 0.28 |

All samples were run in 10 mM Tris, pH 7.4, 100 mM NaCl (unless noted otherwise), temperature: 4 °C. Speed and protein concentration were as indicated. Model used is M to D, reversible association.

included in the interface of AL-09. Conversely, AL-09 includes Asn-93 and Tyr-97 in its dimer interface, and these residues are not found in the dimer interface of κ O18/O8 (supplemental Table S1).
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A recent report by Qin et al. (42) asserts that the amyloidogenic protein SMA is less stable in its monomeric form (5 μM) than in its predominantly dimeric form (180 μM). This suggests a potential protective effect for the canonical dimer. Our previous chemical denaturation experiments (Table 1) used 20 μM protein, a concentration where (based on affinity data) κl O18/O8 and AL-09 are both predominantly monomeric (96 and 70% monomer, respectively). In order to evaluate a possible increase in stability for the dimers, we increased the concentration to 200 μM. This did not affect the Cm value for AL-09, which was 1.9 m at both concentrations. A similar result was observed for κl O18/O8, with little change observed in the Cm value. However, it is notable that at 200 μM, κl O18/O8 is still 70% monomer. A much higher (experimentally prohibitive) concentration may be needed to fully evaluate the potential protective effects of the dimer in this case.

DISCUSSION

The molecular features that cause a protein to become amyloidogenic are enigmatic, and by examining the three-dimensional structure, biochemical and biophysical properties of an amyloidogenic protein and its germline counterpart, we attempt to determine some of the underlying factors involved in amyloidogenicity. Of all our results, finding a novel dimer interface for amyloidogenic AL-09 is the most unexpected and enlightening. The altered interface includes all three non-conservative mutations in AL-09, implicating this region in the decreased protein stability. Coupled with the thermodynamic data, the crystal structures illustrate clear differences in the properties of κl O18/O8 germline and AL-09 dimers.

As expected, κl O18/O8 is much more stable than AL-09. Previous studies comparing AL and MM proteins show that MM proteins GAL and Wil are more stable than their amyloidogenic counterparts BIF and Jto (13, 14). The thermodynamic stability of AL-09 compares well with other reported AL proteins, resulting in similar Tm values between 38.3° and 45.1° C. κl O18/O8 has a Tm of 56.1° C and the lowest ΔGfolding value reported, making it more stable than any of the disease-associated Vl proteins studied to date.

Fibril formation by recombinant AL proteins is well characterized, but the propensity of a κ germline protein to form fibrils has not been tested. Despite the significantly higher stability of κl O18/O8, we are able to induce the protein to form fibrils by incubation at its TmNaS. Although most proteins can be induced to form fibrils under harsh conditions (43), fibril formation by κl O18/O8 may also reflect the overrepresentation of this germline in AL. κl O18/O8 is among the germlines found more frequently in AL (16), and it is possible that this germline has a higher natural tendency to form fibrils compared with other germline sequences that are less frequently or never observed in AL patients.

The kinetics of amyloid fibril formation with seeded reactions under physiological conditions affirm that κl O18/O8 has a significantly longer lag time prior to fibril formation compared with AL-09. The structural differences between these two proteins may be partially responsible for the variation in kinetics.

Because of somatic hypermutations, the amino acid sequence of the pathogenic AL protein differs in each patient. Previous studies examining sequence databases of AL patients in search of commonalities among the mutations resulted in identification of four risk factors for κL Cs (44). While these factors are useful indicators of potential amyloidogenicity, a sequence analysis cannot account for all disease-causing proteins. We recently conducted a structural modeling study with AL sequences and found that the most common site of mutations in AL patients are mutations in the dimer interface (45). AL-09 is representative of this group of proteins. The study described in this paper implicates tertiary and quaternary structural factors in pathogenesis, which may correlate with the location of mutations in the structural modeling studies.

Studies of other LC proteins have revealed less drastic structural changes than the difference in dimer interface that we observe for AL-09. A comparison of two κ MM proteins, WAT and REI, reveals that an 11.8° rotation is necessary to superimpose the two structures (6). Upon superposition with the κl
O18/O8 germline structure, however, no deviation is observed, indicating that while WAT and REI may deviate from each other, they still retain the canonical LC interface. MM protein RHE also has mutations that alter its monomeric structure, resulting in an unusual dimer interface. This interface does not resemble the structure of either κl O18/O8 or AL-09 interfaces, however, and Novotny and Haber (4) postulate that the RHE structure may be altered because of crystallization at low pH. In another MM protein, single point mutations in LEN (Q38E and K30T) form flipped dimers, which are rotated 180° compared with the native protein (46). The flipped domain is attributed to a change in the electrostatic potential in the mutant dimer interfaces. Ionic interactions are also critical in the MM protein Jto, where an ion bridge between Asp-29 and Arg-68 is critical in stabilizing the protein and preventing amyloidogenicity, as compared with AL protein Wil that contains neutral residues Ala-29 and Ser-68 and lacks the stabilizing electrostatic interaction (47).

Our AUC data show that the amyloidogenic protein has a slightly higher dimer affinity than κl O18/O8. However, Stevens et al. (48) report a range of over 1000-fold for the $K_d$ values of κl LCs ($10^{-3}$–$10^{-6}$ M), indicating that the 10-fold difference that we observe is within a normal range for these proteins.

Our results comparing $\Delta G_{\text{unfolding}}$ and $\Delta G_{\text{dissociation}}$ suggest that AL-09 may have a slightly more stable dimer but a much less stable monomer compared with κl O18/O8 (Fig. 4). These findings separate dimer affinity and unfolding processes under the experimental conditions used (20 μM protein concentration), where both proteins are mostly monomeric. The diversity of mutations in AL proteins may affect the free energy of dissociation and folding independently. Between κl O18/O8 and AL-09, the difference in $\Delta G_{\text{unfolding}}$ values (monomer to unfolded) is greater than the difference in $\Delta G_{\text{dissociation}}$ (dimer to monomer). Because the $K_d$ values of LCs encompass such a large range (as noted above), a wide variance in $\Delta G_{\text{dissociation}}$ values would also be expected. Thus, the $\Delta G_{\text{unfolding}}$ makes the most significant contribution to free energy, and comparing these values clearly indicates that κl O18/O8 is more stable than AL-09.

The hypothesis put forth by Qin et al. (42) indicates a potential pathologic effect for the monomer and a protective effect for the canonical dimer. In the report by Qin et al. that examines dimer stability, the AL protein SMA not only has an increased stability at a higher concentration, but also shows decreased fibrillation as protein concentration increases. Although we could not comprehensively evaluate a possible protective effect of the κl O18/O8 dimer, the increased stability of the monomer and delayed fibril formation do not preclude the possibility that this dimer structure may protect against amyloidogenesis. Moreover, the unusual dimeric structure of AL-09 could sample partially unfolded states that may be amyloidogenic.

Other amyloid precursor proteins adopt stabilizing native quaternary structures with multiple subunits; these proteins also have an extremely destabilized monomer prone to aggregation. One example is transthyretin (TTR), which is a tetrameric protein linked to familial amyloidosis. Destabilizing point mutations cause the TTR tetramer to dissociate, and the resulting monomer triggers fibril formation (49, 50). Small molecules that stabilize the tetramer have protective effects, preventing misfolding and amyloid formation (51).

Because quaternary structure can confer stability and prevent amyloid formation, as shown for TTR, it is possible that the loss of the lg heterotetramer due to the excess free light chain secreted by AL patients could play a role in the misfolding that results in fibril deposition in AL. Qin et al. (42) report that the $V_{A}^{-1}$-$V_{L}$ dimer has protective effects that prevent misfolding and amyloid formation, suggesting a common mechanism by which TTR and LC dissociation may cause amyloidogenesis. The effects of AL LC dimer interface mutations may be comparable to the effects of destabilizing mutations in TTR-related amyloidosis.

Although AL proteins have similar behavior with regard to stability, the mutational variability among these proteins makes it challenging to pinpoint a single causative factor. Both the amyloidogenic LC protein SMA and AL-09 show decreased stability, even though they have mutations in different regions. SMA mutations are primarily located in the top and bottom of the β-barrel, while the AL-09 mutations are concentrated in the dimer interface region. The characteristics of these two AL proteins suggest the possibility that mutations in different regions destabilize the protein differently, but still lead to amyloidogenesis. Examining other cohorts of AL proteins will lead to a more complete understanding of the relative importance of location of mutations, dimer stability, and interface structure for amyloid formation. Further studies investigating the possible role of dimer formation in disease pathogenesis would be particularly informative, as forming and/or stabilizing an LC dimer may prevent fibrillation in AL patients. Rational drug design aimed at stabilizing the protein conformation may yield promising therapeutic advances to treat AL.

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