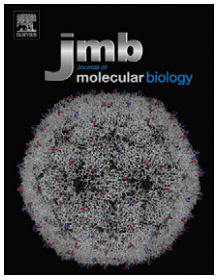

The Kinetic Stability of a Full-Length Antibody Light Chain Dimer Determines whether Endoproteolysis Can Release Amyloidogenic Variable Domains

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Abstract

Light chain (LC) amyloidosis (AL amyloidosis) appears to be caused by the misfolding, or misfolding and aggregation of an antibody LC or fragment thereof and is fatal if untreated. LCs are secreted from clonally expanded plasma cells, generally as disulfide-linked dimers, with each monomer comprising one constant and one variable domain. The energetic contribution of each domain and the role of endoproteolysis in AL amyloidosis remain unclear. To investigate why only some LCs form amyloid and cause organ toxicity, we measured the aggregation propensity and kinetic stability of LC dimers and their associated variable domains from AL amyloidosis patients and non-patients. All the variable domains studied readily form amyloid fibrils, whereas none of the full-length LC dimers, even those from AL amyloidosis patients, are amyloidogenic. Kinetic stability—that is, the free energy difference between the native state and the unfolding transition state—dictates the LC's unfolding rate. Full-length LC dimers derived from AL amyloidosis patients unfold more rapidly than other full-length LC dimers and can be readily cleaved into their component domains by proteases, whereas non-amyloidogenic LC dimers are more kinetically stable and resistant to endoproteolysis. Our data suggest that amyloidogenic LC dimers are kinetically unstable (unfold faster) and are thus susceptible to endoproteolysis that results in the release amyloidogenic LC fragments, whereas other LCs are not as amenable to unfolding and endoproteolysis and are therefore aggregation resistant. Pharmacologic kinetic stabilization of the full-length LC dimer could be a useful strategy to treat AL amyloidosis.

Introduction

There is strong genetic and pharmacologic evidence that the misfolding and/or aggregation of initially soluble proteins can cause dysfunction in post-mitotic tissue(s) in the systemic amyloidoses [1–3]. A relatively common systemic amyloid disease is immunoglobulin (Ig) light chain (LC) amyloidosis (AL amyloidosis) [3], a fatal condition if untreated, which affects 10 patients per million per year [4]. AL amyloidosis is caused by the misfolding, or misfolding and misassembly of an Ig LC or fragment thereof, whose sequence is generally unique to each patient [5]. These LCs are secreted from neoplastic monoclonal plasma cells, often without an Ig heavy chain. Most LCs are efficiently removed from the blood by the kidneys [6,7]. However, some LCs are amyloidogenic—they can misfold and aggregate after secretion from plasma cells to form a variety of structures including amyloid fibrils, causing proteotoxicity in organs, most commonly, the heart and kidneys. In AL amyloidosis, the population of clonal plasma cells is generally small, and proteotoxicity occurs without fully developed cancer symptoms, although amyloid deposition can also be a symptom of multiple myeloma, a more aggressive plasma cell cancer [8,9]. The underlying cancer can often be treated with chemotherapy and/or stem cell replacement therapy, which removes the source of amyloidogenic LCs, stopping active aggregation and proteotoxicity [10]. However, many AL amyloidosis patients present with advanced cardiomyopathy, which makes it difficult for them to tolerate chemotherapy. Thus, there is an urgent need for both earlier diagnosis and therapeutic strategies that reduce LC cardiotoxicity [10,11]. A

better understanding of whether a given LC sequence will be amyloidogenic and why some are especially cardiotoxic could allow potentially dangerous LCs to be identified earlier [4].

The two classes of LCs, κ and λ, each consist of an N-terminal variable (V) Ig domain attached to a C-terminal constant (C) Ig domain; 22–25 kDa (Fig. 1a). Each Ig domain is stabilized by an intrachain disulfide bond (Fig. 1a). The variable domains (V-domains) have diverse sequences used for antigen recognition, whereas the constant domains (C-domains) are much more conserved. LCs from the λ6a subclass are more commonly observed in amyloid deposits than in mature antibodies, so the presence of a λ6a clone seems to represent a risk factor for AL amyloidosis [12]. λ LCs are typically detected in the blood as disulfide-linked dimers (Fig. 1a), exhibiting a half-life of 3–6 h [13].

Amyloid fibrils isolated from AL amyloidosis patients often contain LC fragments, which appear to result from endoproteolysis. Early reports identified V-domains as being the main constituent of fibrils [14], as reviewed in Refs [8,15], but amyloid can also comprise C-domains and full-length, two-domain LCs [8,16–20]. It is not clear which fragment(s) play a role in the deposition or toxicity of LCs. To date, research on the mechanisms of LC amyloid formation has mainly focused on isolated V-domains [5,21–26]. The propensity of V-domains to form amyloid generally correlates with their thermodynamic stability, suggesting that aggregation is driven by populating partially folded states [5,21–30]. The role of the C-domain and the amyloidogenic potential of full-length LCs have recently received more attention [28,31–34], but to date, there has been no systematic survey of how the structure and stability of full-length LCs relate to their amyloid propensity.

Herein, we investigate the kinetic stability (unfolding rates) and amyloidogenicity of recombinant λ LCs. Under native-like conditions, all full-length λ LC dimers remained soluble and non-amyloidogenic, whereas both the V-domains studied formed amyloid fibrils. Full-length amyloidogenic LC dimers were less kinetically stable and therefore more susceptible to endoproteolysis than full-length non-amyloidogenic LC dimers. Thus, endoproteolysis of kinetically

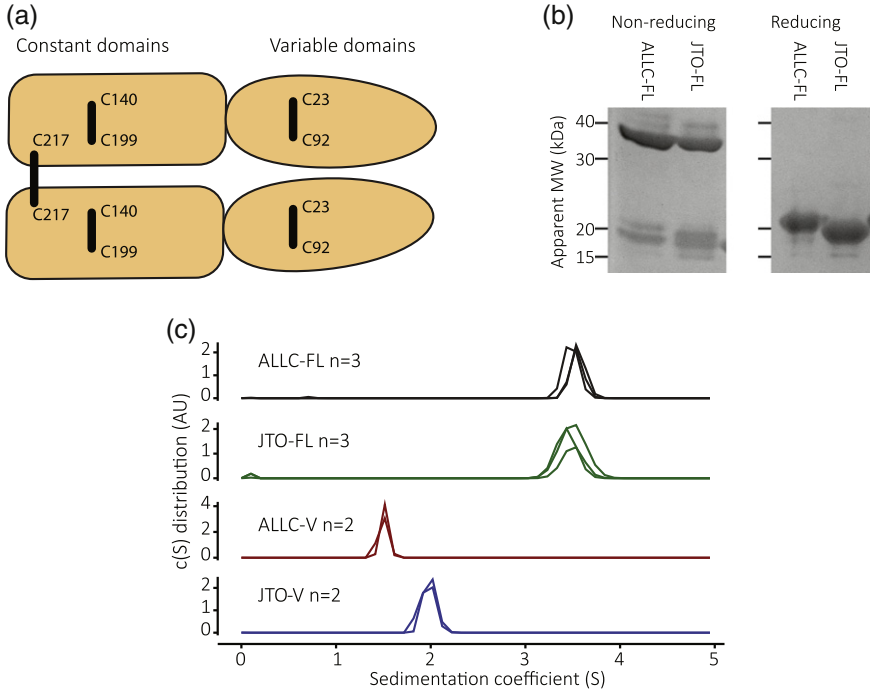

Fig. 1. Recombinant full-length LCs form interchain disulfide-linked dimers. (a) Schematic diagram of an LC homodimer, where each monomer has a variable and constant Ig domain. Each domain is stabilized by an intramolecular disulfide bond, and the two chains are linked by an interchain disulfide bond (shown by black lines). The cysteine residues forming each bond are indicated by their residue numbers in the germline sequence. (b) Reducing and non-reducing SDS-PAGE of ALLC-FL and JTO-FL, indicating the presence of the native interchain disulfide bond. Both ALLC-FL and JTO-FL have a higher apparent molecular weight (MW) when the interchain disulfide is reduced. Positions of MW markers are shown. (c) Sedimentation velocity AUC analysis of ALLC-FL and JTO-FL and their respective V-domains at 20 °C, OD_{280} = 0.4 in PBS buffer (pH 7.4). Colors represent different LCs: ALLC-FL, black; ALLC-V, red; JTO-FL, green; JTO-V, blue. The sedimentation behavior of the full-length LCs is consistent with a dimeric quaternary structure. ALLC-V sediments more slowly than JTO-V, indicating that ALLC-V has a lower propensity to form dimers in solution.