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The concurrency of several biophysical traits links immunoglobulin light chains with toxicity in AL amyloidosis

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Background

Light chain amyloidosis (LC-AL) is the most common systemic AL. It is caused by the overproduction and the aggregation of toxic and monoclonal immunoglobulin LCs in target organs. Among all the organs injured by the pathology, the heart is the most affected one. In particular, the ventricular compliance is reduced, resulting in a symptomatic congestive heart failure [1]. It is extremely relevant that due to the genetic rearrangement and somatic hypermutation, a high variability among LCs' sequences is generated. This means, virtually, that each AL patients present different amyloidogenic LCs [2], stressing in the necessity to investigate a large set of cases. To date, it is particularly interesting the observation described by Milani et al. [1], where it was observed that the severity of heart symptoms is linked with the blood concentration of full-length LCs, which are the major circulating species. Specifically, these facts highlight a crucial role for full-length LC in the pathology beyond the amyloid deposits. However,

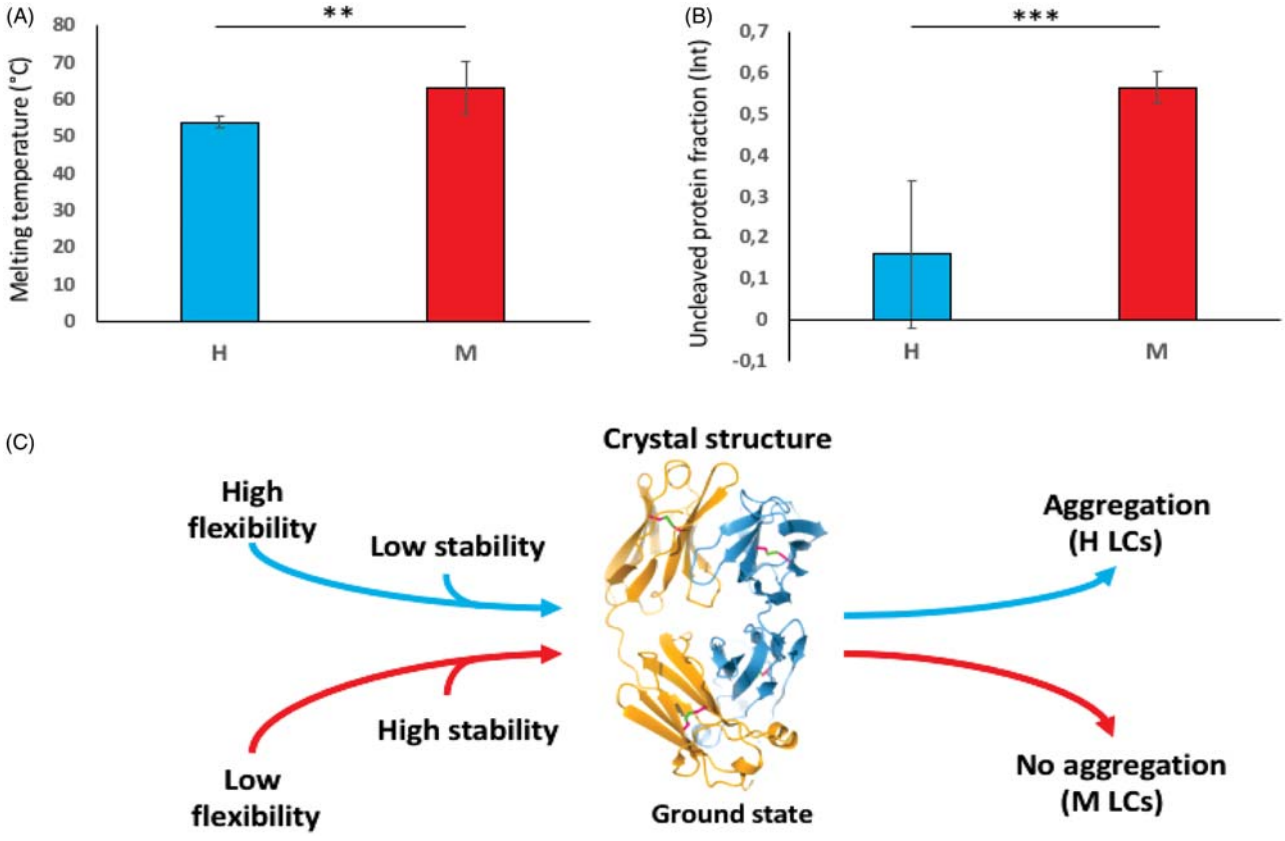

Figure 1. (A) Average of H LC and M LC melting temperatures. (B) Average of the H and M uncleaved protein fraction at 180 min after limited proteolysis; ***p values $<.0005$; **p values $<.005$. (C) Graphical representation of the biophysical properties contributing in the amyloidogenic properties.

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up to now, despite the several works conducted, the molecular bases of proteotoxicity and the aggregation mechanism(s) are still unclear.

Material and methods

Our approach consisted in a biophysical and structural characterization. LCs were purified from patients' urine, or by the recombinant expression in *Escherichia coli* [3]. Protein stability was evaluated by circular dichroism (CD) and by anilinonaphthalene-1-sulfonic acid (ANS) fluorescence, while flexibility and dynamics were studied by limited proteolysis [3]. The X-ray diffraction experiments, on LCs crystals, were carried out at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France.

Results

In order to achieve as generalizable as possible data, our study is based on a large pool of thirteen κ full-length LCs divided in two groups. In particular, eight LCs are amyloidogenic and responsible of severe cardiac symptoms in AL patients (H LCs); five are non-amyloidogenic LCs and selected from patients affected by multiple myeloma, and used as control (M LCs). In order to highlight potential determinants of LCs proteotoxicity and aggregation, all proteins were extensively structurally and biophysically characterized. First, X-ray diffraction of seven LCs crystals (five H LCs and two M LCs) reveals that H and M LCs structures match very closely. From the biophysical point of view, the melting temperature measurements assessed by three independent spectroscopic techniques demonstrate that H LCs tend to be less stable than M LCs (Figure 1(A)). Moreover, limited proteolysis by trypsin and protease K strongly indicate that a more pronounced flexibility for H LCs compared to M LCs (Figure 1(B)). These findings suggest thermodynamic properties and protein dynamics to have a role in the molecular proteotoxicity mechanism(s). In particular, since the crystal structures are conserved, it is very likely that dynamics plays a critical role in defining the propensity of a LC to be toxic or not.

Recently, Diomede et al. [4] described H LCs toxicity *in vivo* to be increased by the presence of copper ions. Along this line, we are investigating potential interactions between LCs and bivalent cationic metal ions, including copper. In particular, we observe a direct and specific binding between H LCs and Cu^{2+}. Our findings show that the LC-copper interaction destabilizes the proteins and it seems to turn H LCs in a more flexible state, underling the importance of dynamics in determining LC toxicity.

Discussion and conclusions

The new contribution of this work highlights the concurrency of different biophysical traits to be linked with LCs amyloid propensity. Our data suggest that thermal stability and flexibility/dynamics correlate with the proteotoxicity LCs tendency, whereas the overall structural determinants are conserved between H and M LCs (Figure 1(C)).

Disclosure statement

The authors report no conflict of interest.

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