Supplementary Material (ESI) for Chemical Communications

Supplementary information for "Formation and dissolution of hen egg white lysozyme amyloid fibrils in protic ionic liquids"

1. Fibrilization

Amyloid fibrils are thread-like protein aggregates which have a highly repetitive beta sheet structure. It has been established that this is the common and distinguishing structure for amyloid fibers regardless of the starting conformation of the protein. It is also well accepted, following the work of Dobson and coworkers cited in text, that any protein will fibrilize under the "correct conditions". The correct condition however, will depend on the protein and there are a great many reports on this issue. The fibrilization mechanism is not at all well understood, but is generally believed to require an initial misfolding of the protein to enhance β-sheet character followed by a nucleation of the fibril itself. Issues of parallel vs antiparallel stacking of the β-sheets remain to be fully clarified, though the antiparallel mode seems to be more stable and lead to greater insolubility. The initial destabilization of the protein to induce misfolding can be achieved in a number of ways, most frequently by chemical destabilization (e.g. by alcohol, excess acidity or excess basicity) or by physical destabilization using heat or pressure to take the protein to conditions near denaturation. The presence of mutations, i.e. an internal destabilization of the normal protein, is involved in many inherited tendencies to folding diseases (Jacob-Kreutz, for instance). Some of the most sophisticated studies of fibrilization have involved the synthesis of proteins with specific residue substitutions at points in the chain that destabilize the normal folded structure.

Additional references

1. Carulla, N. Caddy, G. L. Hall, D. R, Zurdo, J. Gairi, M. Feliz, M. Robinson, C. V. and Dobson, C. M. Nature 436 2005 554-558
2. Thomas, L. K. PNAS. 100(2). 2003. 383-385
3. Makin. S, Atkins, E. Sikorski, P. Johansson. J and Serpell. PNAS 102(2) 2005. 315-320
4. Markin. S and Serpell L. C. FEBS. J. 272. 2005. 5950-5961.

.

2. Character of ionic liquids used

In this work only three ionic liquids have been used, and all have involved simple amine-based cations, namely, ammonium, ethylammonium, and triethyl ammonium, which are then charge-neutralized by simple anions. We avoid cations with large alkyl groups that can have surfactant properties. As far we can tell, the behavior of interest (fibrilization on the one hand and fibril dissolution on the other), can be controlled by just one quantity, the proton activity, though no doubt use of heavily fluorinated or, alternatively, polyprotic anions, or long side chain cations, would reveal other forces at work. The proton activity is adjustable by change of anion at fixed cation, and can be monitored by the proton NMR resonance frequency. The most acid of these, the bisulfate, was neutralized by a simple ammonium cation, but probably an alkylated ammonium cation would have performed the same way. Our

interest lay in the fibrilization phenomenon, more than in the chemical details of what would cause it. These details are not without interest and will be examined in follow-up work.

3. **Additional solutions studied by Th T fluorescence**

ThT binding was recorded on a R551 fluorescence spectrophotometer. The excitation was set at 450 nm and the emission recorded at 485 nm. 20 μM Thioflavin T was added to each sample. Protein concentration to 10 mg μl^{-2}. Results quoted are the average of 5 measurements.

Several additional solutions were tested for indications of fibrilar material (stacked β-sheet) by Th T fluorescence but were not listed in Table 2 because they showed no intensity, for completeness we list them below.

1	Native HWL diss. in 80EAN20H_20
2	Native HWL diss. in 80EAN20H_20 after thermal denaturation
3	Native HW in neat EAN
4	Native HWL diss. in 80TEATf20H_20
5	Native HWL diss. in 80TEATf-20H_20 after thermal denaturation and refold

4. **The bioactivity test details**. Enzyme activity assay of HWL purchased from Sigma Aldrich product number L6876 was conducted according to (i) the product information provided on the Sigma Aldrich website and (ii) the reference cited within the communication, ref. 31, (Shugar). Live cells of micrcoccus lysodeikiticus bacterium absorb strongly at 450 nm. Figure S1 shows the decrease in absorbance at 450 nm as the bacterial cells are lysed by the enzyme. The rate for the fresh enzyme is significantly greater than that for the case in which the enzyme being assayed was recovered from the fibrilized state by dissolution in EAN. The latter nevertheless lyses live cells at a rate 46% of the normal rate of decrease (as already tabulated in Table 3). For inactive lysozyme, the absorbance vs time plot would be flat.

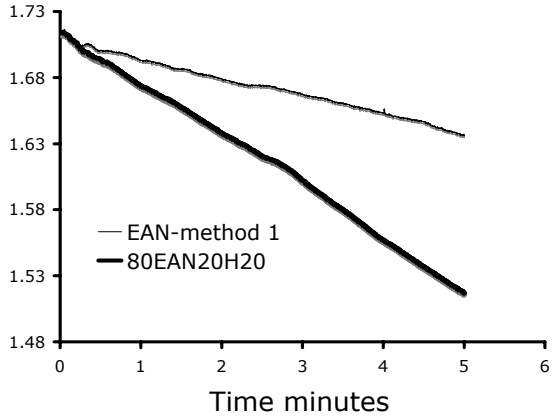

Figure S1: Enzyme Assay for native HWL dissolved in 80EAN20H_20 (thick line) compared with that for the sample obtained by dissolving Fibrils from Method1 in EAN. Both were diluted to normal aqueous solution strengths before conducting assay.