"Control of protein folding and misfolding in ionic liquid media, and a conjecture on early earth biology".

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We describe recent studies [1-4] in which many of the complex features of biomolecule behavior, such as protein folding and fibrillization, are reproduced (with little change from biological behavior) in hydrated (10-20 wt % water) protic ionic liquid solutions (PILs) in which the water activity is only a small fraction of its normal value. An outstanding finding of these studies has been the stabilization against the normal deteriorating influences of aggregation and hydrolysis, of model proteins like lysozyme and ribonuclease A, when in the PILs environment. To characterize the protein stability we define a "refoldability", or "refolding index" (RFI), which is the percentage of a protein that refolds after an unfolding scan conducted in a differential scanning calorimeter at 20K/min, followed by intrument cooling. This percentage is determined by comparison of first and second unfolding endotherms, RFI $=100\Delta H_{d2}/\Delta H_{d1}$ and, for the above proteins in their stable zones, is 97-99.

We then show how the proton activity in the (H_3O^+-free) PIL can be characterized by the NMR chemical shift of the lone proton on a PIL cation, such as diethylmethyl-ammonium, δ(N-H). This varies greatly as the anion is changed from acetate to perchlorate. High RFI zones are found for certain δ(N-H) ranges[4] , and fibrillization is observed when δ(N-H) of the solvent PIL lies far outside these stable ranges [3].

The fact that these high concentration ionic media stabilize proteins against deterioration (and loss of bioactivity as determined on dilution) strikes a chord of interest in the light of Phillip Ball's commentary, in his book "Life's Matrix: a biography of water" [5], on the difficulties confronting the common "primordial soup" theories of the origin of life in which complex biomolecules are considered to form by spontaneous association in the organic precursor-containing, aqueous, matrix. How could molecules such as the proteins and RNAs arise in the face of their unfavorable hydrolysis rates, ask critics. With an interest in this question we enquire into whether such syntheses could arise in ambient temperature media that are friendly to biomolecules by virtue of the presence of water only at low activity. We find that the simplest protic ionic liquids, ammonium nitrate and ammonium acetate, PILs whose component acid and base members can form by primitive natural routes, are ambient temperature liquids in the presence of 20 wt% water, and that the proteins of our study dissolve in them readily, without denaturing. While they do not denature at ambient temperature, they are not stabilized in these simple media: once thermally unfolded, they do not refold (i.e. the RFI = 0). However, on introduction of just 10% of the simple organic cation salt ethylammonium nitrate, the stability, assessed by the RFA, becomes as high as any observed in the previous studies [6], suggesting that such non-hydrolyzing liquids could have served as biogenesis media in the distant past.

1. N. Byrne, L.-M. Wang, J.-P. Belieres, CAA, Chem. Commun. 2714, (2007)
2. N. Byrne and C. A. Angell, J. Mol. Biol, 378,. 707. (2008)
3. N. Byrne and C. A. Angell, (Chem. Commun. In press)
4. Angell, C. A. Byrne, N., and Belieres, J.-P. Acc. Chem. Res. 40, 1228, (2007)
5. Ball, P. "Life's Matrix: a biography of water", 1999.
6. N. Byrne, J-P. Belieres and C. A. Angell.. (Aust. J. Chem. submitted).