

India signs Ocean Charter

The oceans sustain life on planet Earth; if the oceans die, we die. We are barely aware of this fact because we live on land. So the United Nations declared 1998 as the International Year of the Ocean (IYO) to provide a window through which governments, organizations and individuals can become aware of the oceans and to consider the actions needed to pursue our responsibility to sustain this fragile common heritage without which we cannot exist.

The idea of designating 1998 as IYO emanated from the 17th Session of the Assembly of the Intergovernmental Oceanographic Commission (IOC) of UNESCO held in March, 1993. The Twenty Seventh Session of the General Conference of UNESCO endorsed the

proposal in November, 1993. The United Nations General Assembly formally adopted the proposal through a Resolution in December, 1991.

The Intergovernmental Oceanographic Commission (IOC) prepared a text for the Ocean Charter for adoption by all the member states of IOC. The Charter is a statement of principles and is not a legally-binding document. It is a declaration of intent of belief for and commitment to the future of the ocean and marine and coastal environment. The aim of the Charter is to combine political and public commitment as a powerful means of creating awareness. It is expected that this Ocean Charter would be signed by the heads of the State of the member countries. At a

function on 9 November 1998 Secretary, Department of Ocean Development signed the Charter on behalf of India. Records of the signatories, dates and signing-events will be kept by Canada for publication after 1998. The function also marked the release of 23 million inland letters which will carry the message of the International Year of Ocean in English, Hindi and regional languages.

The IOC has also designed another Charter for signing by individuals in the member countries. The Department of Ocean Development is planning to approach Universities, national laboratories, research agencies, NGOs, etc. for getting individual scientists and general public to sign.

RESEARCH NEWS

Protein folding intermediates – Are we seeing too many of them

Anil K. Lala

Several years ago, Levinthal predicted that the vast conformational space available to an unfolded protein makes it highly unlikely that it will use random search method to reach its native state¹. This led to the initial suggestion that a protein folds via distinct intermediates and evidence in favour of a folding transition involving structured intermediates has been growing²⁻⁵. Towards this end, two state (where no intermediate is involved and clear examples in this category are available^{6,7}), three state and multiple state models involving intermediate states have been proposed for native (N) to unfolded (U) protein. These structured intermediates involved in three state and higher state folding have many features of the secondary structure of the native state. Experimental conditions can be found *in vitro* to observe and characterize such intermediates, e.g. the molten

globule state (MG), and they are often referred to as the equilibrium intermediates. These intermediates have been structurally characterized for many proteins^{1-4,8}. The most common of these states, the molten globule state⁹, is characterized by partial retention of the secondary structure of the native state but with loss of the tertiary structure, giving rise to a compact structure without rigid packing in the protein interior³. There is also a substantial increase in the fluctuations of side chains leading to increased hydrophobic exposure. Further in the $N \leftrightarrow MG \leftrightarrow U$ transition, pre-MG states have been reported^{4,10}. Similarly highly ordered MG states have been described as late intermediates in the folding process^{11,12}. The MG state is thus more like a general description of the equilibrium intermediate in the $N \leftrightarrow U$ transition and there are likely to be more than one type of ordered inter-

mediates in the multiple-state folding process. Finally with recent reports on conformational characteristics of many proteins when bound to chaperones, being similar to MG state has increased the possibility of such intermediate states being involved in *in vivo*¹³⁻¹⁵.

Of late however, there has been considerable interest in detecting intermediate states formed in the early stages of folding which are referred to as burst phase intermediates or kinetic folding intermediates. These intermediates in many cases are formed in the millisecond time range and their characterization has been a subject of intensive research in the last few years^{7,16}. The results obtained in many cases indicate that the kinetic intermediates often resemble the equilibrium intermediates and MG like states serve as useful models of the kinetic intermediates^{4,16}. At this stage, one wondered how critical

it was to pursue detection and characterization of kinetic intermediates, specially as their detection was often challenged by dead time of the stopped-flow unit linked to an optical or some other detection unit. However, it is not unusual to see, as in many other scientific endeavours, the limits of instrumentation being pushed to an extreme in order to detect the rapidly-forming intermediates in the submilli second region^{7,17}. The experiments often involve rapid addition of a protein in concentrated denaturant like 8M guanidine hydrochloride to none or low denaturant concentration solution, so that the denatured protein folds to its native conformation. The rapid mixing provided by the stopped flow apparatus in conjunction with the detection units then looks for the early intermediates formed in the folding process, the dead time of the stopped flow unit being often the limiting factor. Recent studies on detection of a characteristic optical signal during the burst phase of folding suggests that one may be actually observing an ensemble of partially-folded conformers rather than a distinct kinetic intermediate^{18,19}. A very recent study using ribonuclease A as a model protein effectively establishes this point²⁰. Ribonuclease A has been one of the well studied proteins where pulse labelling NMR studies on folding have provided sufficient evidence for the presence of a stable secondary structure prior to formation of the native protein²¹. The recent study²⁰ takes advantage of the fact that on denaturation, reduced ribonuclease A does not fold back to the native state, but the normal ribonuclease A with its disulphide bonds intact does.

Using stopped-flow CD, the authors measured the signal for the denatured ribonuclease A and did detect a significant signal in the burst phase which finally reached the CD of the native protein over several seconds, as the protein nucleates to its native conformation. However, the reduced ribonuclease A also showed similar burst phase optical signal, even though it did not fold to the native state. The current study thus emphatically proves that one is observing an ensemble of rapidly folding conformers with very similar energy rather than an early kinetic intermediate²⁰. It is quite likely that one is observing a solvent effect (sudden change from 8M guanidine hydrochloride to a very low concentration) in various burst phase studies, rather than formation of any specific kinetic intermediate. This study thus, hopefully, puts to rest what has been very intensively studied in the last few years. It must be emphasized here that this study²⁰ does not argue against the formation of folding intermediates, but cautions regarding what might just be a reflection of drastic and rapid solvent effect change being confused as a kinetic intermediate.

1. Levinthal, C., *J. Chem. Phys.*, 1968, **85**, 44-45.
2. Kim, P. S. and Baldwin, R. L., *Annu. Rev. Biochem.*, 1990, **59**, 631-660.
3. Matthews, C. R., *Annu. Rev. Biochem.*, 1993, **62**, 653-863.
4. Ptitsyn, O. B., *Adv. Protein Chem.*, 1995, **47**, 83-229.
5. Miranker, A. D. and Dobson, C. M., *Curr. Opin. Struct. Biol.*, 1996, **6**, 31-42.

6. Schindler, T., Herrier, M., Marahiel, M. A. and Schmid, F. X., *Nat. Struct. Biol.*, 1995, **2**, 663-673.
7. Jackson, S. E., *Folding and Design*, 1998, **3**, R81-R91.
8. Ptitsyn, O. B., *Curr. Opin. Struct. Biol.*, 1995, **5**, 74-78.
9. Ohgushi, M. and Wada, A., *FEBS Lett.*, 1983, **164**, 21-24.
10. Lala, A. K. and Kaul, P. K., *J. Biol. Chem.*, 1992, **267**, 19914-19918.
11. Redfield, C., Smith, R. A. G. and Dobson, C. M., *Nat. Struct. Biol.*, 1994, **1**, 23-29.
12. Dobson, M., *Curr. Biol.*, 1994, **4**, 636-640.
13. Martin, J., Langer, T., Boteva, R., Schramel, A., Horwich, A. L. and Hartl, F. U., *Nature*, 1991, **352**, 36-42.
14. Rajaraman, K., Raman, B. and Rao, C. M., *J. Biol. Chem.*, 1996, **271**, 27595-27600.
15. Lindner, R. A., Kapur, A. and Carver, J. A., *J. Biol. Chem.*, 1997, **272**, 27722-27729.
16. Roder, H. and Colon, W., *Curr. Opin. Struct. Biol.*, 1997, **7**, 15-28.
17. Burton, R. E., Huang, G. S., Dogherty, M. A., Fulbright, P. W. and Oas, T. G., *J. Mol. Biol.*, 1996, **263**, 311-322.
18. Sosnick, T. R., Shülerman, M. D., Mayne, L. and Englander, S. W., *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 8545-8550.
19. Jennings, P. A., *Nat. Struct. Biol.*, 1998, **5**, 846-848.
20. Qi, P. X., Sosnick, T. R. and Englander, S. W., *Nature Struct. Biol.*, 1998, **5**, 882-884.
21. Udgaonkar, J. B. and Baldwin, R. L., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 8197-8201.

Anil K. Lala is in the Department of Chemistry and Biotech Center, IIT Bombay, Powai, Mumbai 400 076, India.

Cambrian life explosion in fray: Evidence from more than 1 b.y. old animal body fossils and skeletonization event

Rajesh K. Vishwakarma

Putative outburst of animal phyla in the Cambrian age, mainly, evidenced by the 'absence' of fossils of triploblastic metazoans from rocks predating the Cambrian¹, now appears to be inadapted following a recent discovery of more than 1-Ga-old triploblastic meta-

zoans from India². But another simultaneous and independent discovery of ancient brachiopods and the shelly fossils indicating an earliest Cambrian age³ is likely to reiterate the Cambrian fossil explosion about 540 million years ago. The brachiopods and shelly fossil

fauna (for convenience read B-Sf as brachiopods and shelly fossils respectively), in particular are so far considered as a useful tool for biostratigraphic correlation of the Precambrian-Cambrian boundary in chert-phosphorite Member of the Tal Formation, lesser Himalaya,