Amyloid fibrils from muscle myoglobin

Even an ordinary globular protein can assume a rogue guise if conditions are right.

The sequence of amino acids in a natural protein determines the way in which it folds up into its unique biologically active 'native' conformation[1]. But we show here that myoglobin, the archetypal globular protein[2], can convert into an alternative and radically different structure that closely resembles the amyloid and prion aggregates seen in pathological conditions such as Alzheimer's and Creutzfeldt–Jakob diseases[3–6]. This most striking example of such a structural transition provides compelling evidence for the idea that amyloid represents a generic form of polypeptide conformation, but one that evolution has ensured is not normally formed in living systems[7,8].

Myoglobin (Fig. 1a) is a compact and highly soluble protein without any native-state properties to suggest that it has a predisposition to form amyloid fibrils. Whereas the latter are characteristically rich in β-sheets, native myoglobin lacks any elements of such structure and has most of its sequence arranged in well-defined α-helices. Moreover, all partially folded states of the protein characterized so far are significantly helical[9], although, as with most proteins, there is evidence for the presence of more extended conformations in aggregates and precipitates[9,10].

In a screening process, in which pH, temperature and buffers were varied, we found that incubation of apomyoglobin (the protein without its haem group) in 50 mM sodium borate, pH 9.0 at 65 °C, conditions under which the native fold is substantially destabilized, resulted in the formation of large quantities of fibrillar structures (Fig. 1b). Solutions of these fibrils gave rise to a far-ultraviolet circular-dichroism spectrum containing a single minimum at 215 nm (Fig. 1c), which is diagnostic for the presence of β-structure. Moreover, an anisotropic X-ray diffraction pattern was obtained with characteristic 'cross-β' reflections at 4.63 ± 0.08 and 10.11 ± 1.23 Å (Fig. 1d).

These results indicate that myoglobin fibrils contain β-strands that are oriented perpendicular to the main fibre axis[5] and, together with the properties of the fibril-containing solutions in tinctorial assays using thioflavin T and Congo Red, indicate that these fibrils are indistinguishable in their core structure from disease-related amyloid fibrils[5].

Our findings for myoglobin provide strong evidence that the sequences of polypeptides associated with amyloid or prion diseases need not be fundamentally distinct from those of other proteins by having any explicit coding for the cross-β structure[7]. But how can a polypeptide chain fold into two different, well-ordered states? In the native state of a globular protein such as myoglobin (Fig. 1a), side-chain interactions are crucial in defining the unique and characteristic main-chain fold[1]. We suggest that, in contrast, the cross-β conformation is dominated by main-chain interactions that are common to different polypeptides; hence sequence effects are much less significant in determining the amyloid core structure. The latter is instead likely to be favoured by the inherent physical-chemical properties of the otherwise disordered polypeptide chains as they aggregate slowly under specific solution conditions in which the native state is unstable.

We believe that the ability of natural protein sequences (which represent only a small fraction of all possible sequences) to fold efficiently into cooperative globular structures, together with other strategies such as employing molecular chaperones, is a very effective evolutionary adaptation to suppress amyloid formation *in vivo*. Conditions compromising such protective mechanisms, including ageing or mutational changes, may sometimes allow even a highly selected natural sequence to revert to its alternative conformation.

This proposal enables the basic principle of protein folding, namely that there is an unambiguous relationship between the

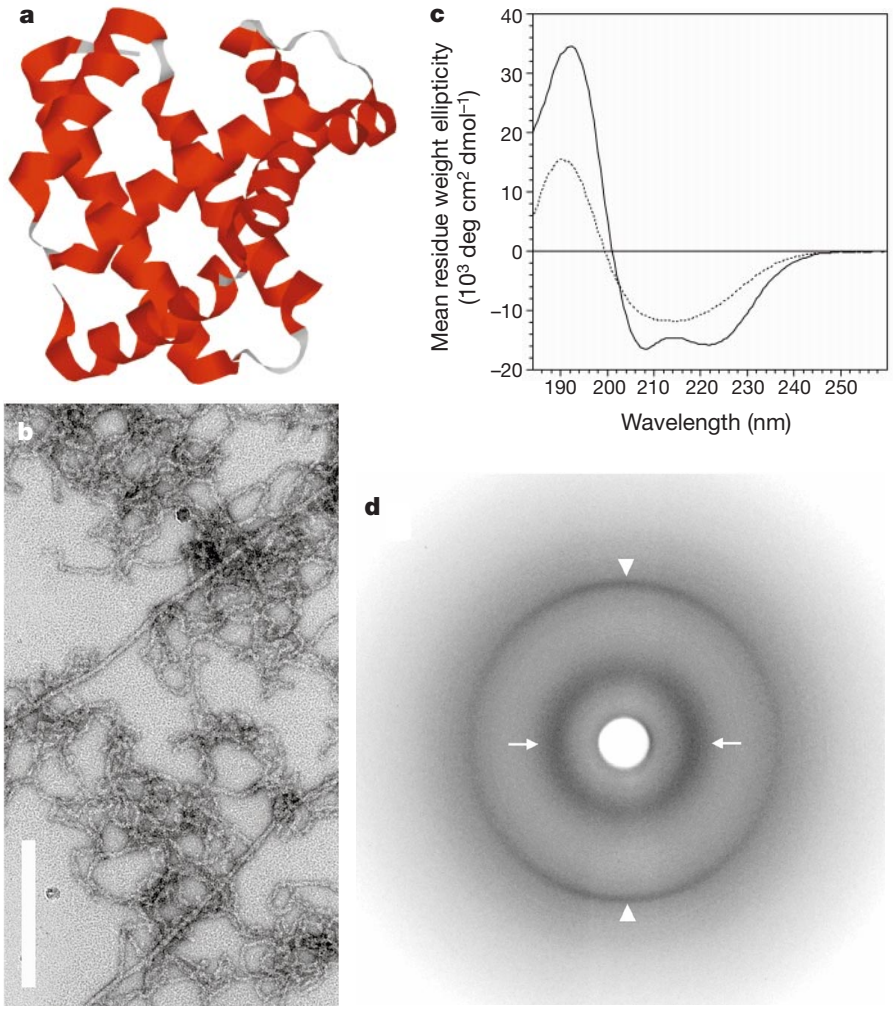

Figure 1 Structure of native and fibrillar myoglobin. **a**, Backbone representation of native myoglobin[11]; α-helical residues are picked out in red. **b,** Electron micrograph of fibrils formed from horse skeletal-muscle myoglobin after haem extraction[12] and stained with uranyl acetate. Scale bar, 300 nm. **c**, Far-ultraviolet circular-dichroism spectra at 22 °C of freshly dissolved apomyoglobin (continuous line) and purified fibrils after incubation for 25 days (dotted line). **d**, X-ray diffraction pattern of fibres obtained using an 18-cm imaging plate detector (MarResearch) with a Rigaku RU200 rotating anode. Arrowheads (on the meridian) indicate the 4.63-Å reflection; arrows indicate the 10.11-Å reflection.

amino-acid sequence and the structure in the native state[1], to be reconciled with the evidence that even a protein such as myoglobin can adopt the fundamentally different but highly organized structure present in amyloid fibrils.

Marcus Fändrich, Matthew A. Fletcher, Christopher M. Dobson
Oxford Centre for Molecular Sciences, New Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QT, UK
e-mail: chris.dobson@chem.ox.ac.uk

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Pattern formation

Spiral cracks without twisting

A fascinating class of patterns, often encountered in nature as meandering cracks on rocks, dried-out fields and tectonic plates, is produced by the fracture of solids[1]. Here we describe the observation and modelling of an unusual type of pattern consisting of spiral cracks in fragments of a thin layer of drying precipitate. We find that this symmetry-breaking cracking mode arises naturally not from twisting forces, but from a propagating stress front induced by the fold-up of the fragments.

Fractured surfaces and lines[2–6] typically show a cellular and hierarchical pattern. Twisting forces produce a spiral fracture, like that often seen in a tibia bone broken in a skiing accident[7]. However, spiral cracks can also be created in other situations, as we show here by drying a fine aqueous suspension of precipitate. During drying, the suspension solidifies and later fragments into isolated parts (Fig. 1a).

Surprisingly, for very fine precipitates in a solidified layer of thickness between 0.2 and 0.5 mm, regular spiral as well as circular cracking pathways show up inside the fragments (Fig. 1b). Depending on the grain size, precipitate type and layer thickness, the size of the spirals varies widely from several hundred micrometres to a few millimetres. To the naked eye, they look like small dots, but their detail is revealed under a microscope (Fig. 1c). These spiral cracks do not occur in one particular material — we were able to generate them in three different precipitates, from nickel phosphate $Ni_3(PO_4)_2$, ferric ferrocyanide $Fe_4[Fe(CN)_6)]_3$ and ferric hydroxide $Fe(OH)_3$.

Careful *in situ* observation suggested a mechanism for the formation of these cracks. The spirals and circle-shaped structures form only after the fragmentation process is over. Owing to the humidity gradient across the thickness, the fragments gradually fold up and detach from the substrate, generating large tensile stresses in the radial direction, at and normal to the front of detachment. The extent of the attached area shrinks as the ring-shaped front advances inwards as a result of ongoing desiccation.

When the stress at the front exceeds the material strength, a crack is nucleated. As the nucleation is seldom symmetrical with respect to the boundaries, the crack tends to propagate along the front in only one direction, where more stresses can be released. By the time the crack growth completes a cycle, the front has already advanced, leading eventually to an inward spiral crack. As the stresses are concentrated at the layer–substrate interface, the spiral is confined there, with a typical penetration of 20–60% of the thickness.

The fact that the patterns are largely spiral suggests that crack propagation is favoured over nucleation, otherwise we would see more cylindrical concentric cracks. Although in a few instances we did observe this type of pattern, the majority are spiral in structure.

To test the proposed mechanism, we implemented it in a mesoscopic computer model[8] that describes fracture on a frictional substrate. In this model, the grains in the layer are represented by blocks on a triangular lattice, interconnected among neighbours by springs. The system is pre-strained, and then relieved quasi-statically in a physical way. The relaxation is dictated by the competition between stick-and-slip and bond-breaking.

Focusing on the post-fragmentation process, we imposed a circular, inwardly propagating stress field to mimic the advancing detachment front. In a rather narrow parameter region, the spiral cracks were successfully reproduced (Fig. 1d). More tightly bound spirals can be obtained for smaller penetration depth, in agreement with experiment and prediction based on screening effects in the stress field. Other evidence has also been reported for the formation of similar spiral crack patterns under specific conditions[9].

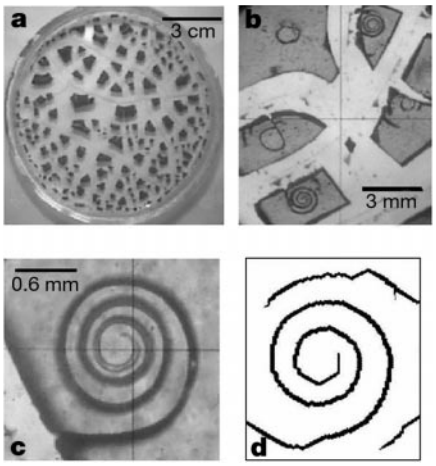

Figure 1 Spiral cracks revealed at small scales. **a,** Typical fragmentation pattern of nickel phosphate precipitate after desiccation in a Petri dish. **b,** Spiral and circular structures obtained inside the fragments. **c,** Close-up of a spiral crack. **d,** Computer-simulated spiral crack obtained using a mesoscopic spring-block model.

K.-t. Leung*, L. Józsa†, M. Ravasz‡, Z. Néda‡§
**Institute of Physics, Academia Sinica, Taipei, Taiwan 11529, Republic of China*
Departments of †Chemistry and ‡Physics, Babes-Bolyai University, Cluj, RO-3400, Romania
§Department of Physics, University of Notre Dame, Notre Dame, Indiana 46556, USA
e-mail: zneda@nd.edu

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Polynesian origins

Slow boat to Melanesia?

The origin of the Polynesian islanders and of the Austronesian languages that they speak has been debated for more than 200 years. Diamond has presented the predominantly held modern viewpoint, described as the 'express train to Polynesia' model, which proposes that the ancestors of the Polynesians were early farmers who dispersed south from a homeland in South China/Taiwan, through Island Southeast Asia (replacing an indigenous 'Australoid' hunter-gatherer population), and then on east, out into the Pacific — all within the past 6,000 years[1]. However, evidence is accumulating from several genetic markers that Polynesian lineages have a much deeper ancestry within tropical Island Southeast Asia than this hypothesis would suggest. The new evidence implies that the Polynesians originated not in China/Taiwan, but in eastern Indonesia, somewhere between Wallace's line and the island of New Guinea.