Getting out of shape

Christopher M. Dobson

Two greatly debilitating illnesses — Alzheimer's disease, which is largely associated with ageing, and variant Creutzfeldt–Jakob disease (vCJD), which is linked with the recent bovine spongiform encephalopathy (BSE) epidemic in Britain — have had a huge impact on public consciousness. Despite their very different origins, these diseases are closely related to each other and also to the 20 or so other 'amyloidoses' — so called because they involve the aberrant deposition of proteins in the form of amyloid fibrils or plaques. Other diseases in this group, which are less publicized but no less devastating to those affected, are Parkinson's disease, type II (late-onset) diabetes and rare conditions such as familial insomnia.

Amyloidoses thus include some of the most feared and costly diseases in the Western world. Alzheimer's may very soon be the most prevalent and socially disruptive illness in the ageing populations of all developed countries. It is remarkable, therefore, that most of these diseases were virtually unknown until relatively recently. Indeed, the first detailed description of an amyloid pathology was made less than 100 years ago, when Alzheimer identified the form of dementia that bears his name. As with many amyloidoses, Alzheimer's disease is usually sporadic, although there are less common hereditary forms that can afflict relatively young people — Alzheimer's most famous patient was in her early 50s.

The first form of a transmissible human amyloid disease was recognized only in the 1950s, when the fatal condition kuru, which was afflicting people in Papua New Guinea, was found to be the consequence of ritualistic cannibalism. Shortly afterwards, a similar 'transmissible spongiform encephalopathy' (TSE) emerged, this time in Europe and the United States, when a form of CJD struck individuals who as children had been treated with growth hormone extracted from human cadavers. And, of course, the consequences of the BSE catastrophe in the 1980s threaten many more people. Although a widespread epidemic of vCJD as a result of eating infected beef seems unlikely, about 100 people have already died of this truly dreadful illness. As other animal species besides cows and sheep are now known to be infected with similar conditions, including deer and elk in parts of North America, further tragedies may be waiting to unfold.

As mentioned above, all of these diseases, whether sporadic, familial or transmissible, are associated with deposits in tissue of proteins that are normally soluble. The deposits, depending on the disease, can be in the brain, in skeletal tissue or in other organs. The amount of protein involved ranges from scarcely detectable quantities to kilograms. Affected tissues are often found to be riddled with thread-like fibrils, sometimes assembled into plaques, with a single predominant protein component that is characteristic of each disease (for example, the prion protein in TSEs). This observation led to the idea that the structures of the soluble forms of the proteins involved have an unusual ability to convert to an alternative conformation, which can then assemble into the thread-like structures. A major puzzle was that although the proteins involved have different structures in their soluble forms, the fibrils look remarkably similar, suggesting that the molecular structures in the aggregated forms are essentially the same.

While investigations of the emerging amyloid diseases were taking place in medical schools, research into the nature of protein structures was developing in chemistry and physics departments. In the 1930s it was discovered that many proteins can exist in two forms: a globular, soluble state and a fibrous form, which is often produced at extreme temperatures or pH values. As the globular forms were usually those with biological activity, virtually all attention was focused on them. Indeed, the observation of alternative forms of proteins (except in the case of the prion protein) was not linked to amyloid diseases until very recently, following accidental discoveries that 'ordinary' proteins can form fibrils *in vitro* that are indistinguishable from those extracted from patients. Moreover, with sufficient patience and cunning, conditions could be found in which seemingly any protein could form amyloid fibrils. This observation suggested that the ability to form such fibrils is 'generic' to proteins, although the propensity to form such structures under given circumstances can vary greatly from one protein to another.

Why does this alternative form of protein structure exist? And why have 'new' diseases associated with it apparently emerged only recently? Both of these questions can be addressed by considering the nature of biological evolution. We each produce about 50,000 different proteins, but this number is only a minute fraction of the countless ways in which the 20 different amino acids can be linked together to give polypeptides of the length that is typical in living organisms (about 300 residues). Natural proteins are therefore a very special group of polypeptides with remarkable properties that have emerged over several billion years of evolution. One factor in increasing the competitive ability of an organism is the efficiency of its information transfer, which is largely achieved by rapid molecular diffusion. Cells are very densely packed with molecules that can communicate rapidly with each other. The fact that proteins do not normally interact inappropriately or even precipitate in such crowded environments is a triumph of evolutionary design.

The astonishing complexity and variety of natural proteins is determined by the unique way in which the various side chains of the constituent amino acids pack together. In amyloid fibrils, however, the structure of the polypeptide chain is dominated by hydrogen bonding between the atoms of the main chain that results in the formation of extended β-sheets, rather than by the specific interactions of the side chains that dictate the structures of globular proteins. In fact, the generic fibrillar forms of proteins can be regarded as the intrinsic 'polymer' structure

Protein-misfolding diseases

Molecular evolution has not always kept pace with our aspirations for a longer and better life.

T. PHILLIPS, COURTESY OF THE NATIONAL PORTRAIT GALLERY, LONDON

The novelist and philosopher Iris Murdoch, one of the most famous victims of Alzheimer's.

of a polypeptide chain. After all, synthetic polymers such as nylon (a polypeptide without side chains) often form fibrillar structures. However, evolutionary processes have selected sequences of amino acids with the remarkable ability to form monomeric structures in which the main chain is folded in a unique way within the mass of close-packed side chains, preventing it from interacting with other molecules.

The existence of such structures has enabled proteins to develop a vast array of biological functions. But their ability to revert to the 'primordial' structure lingers, because the main chain of the polypeptide is preserved as a common feature of all natural proteins. To maintain proteins in their uniquely folded states, they must be kept in a carefully controlled environment. Under some circumstances, even partial unfolding will expose significant regions of the polypeptide chain to the outside world, allowing the protein to aggregate and convert into amyloid fibrils. Once formed, the strong hydrogen bonding between molecules can make this process effectively irreversible. This ability of proteins to change conformation is the essence of the amyloidoses; in these diseases, the proteins have converted into the 'primordial' amyloid structure rather than remaining in their evolved, globular states.

Although the manifestation of such a structural change differs between amyloid pathologies, it is a common factor in all of them. But why, in these diseases, does the normally tightly regulated environment within an organism fail to maintain a particular protein in its soluble state? One way is if a genetic mutation were to produce a variant that is less stable and less cooperative than the wild-type protein, and will therefore tend to unfold and aggregate more readily. This mechanism can explain many familial amyloidoses; indeed, the age of onset of some of these diseases correlates closely with the extent to which the familial mutation destabilizes the native protein. In other conditions, such as some dementias, mutations might increase the propensity of an incompletely folded protein to aggregate.

A unique characteristic of the prion group of amyloid diseases is their transmissibility between individuals (and in some cases, with much lower probability, between species). Transmission probably results from ingestion, from an external source, of prion proteins that have already aggregated. As with crystallization, the formation of amyloid fibrils is 'seeded' by pre-formed aggregates, a phenomenon that might also be responsible for the rapid progression of sporadic diseases such as Alzheimer's once the symptoms become evident. Such a seeding mechanism could explain transmission of TSEs by injection of contaminated growth hormone, by cannibalism or by eating infected beef.

In other cases, the physiological environment may not always be sufficiently well controlled to ensure that protein unfolding and aggregation are avoided. The loss of such control may be a characteristic of old age, and hence could explain why many amyloidoses are associated with ageing. It is interesting that for simple organisms such as bacteria, the lifespan of an individual is less than that of its molecules, as the latter are passed on through cell division. Perhaps to evolve long lifespans it is important to replace molecules before damage or aggregation can reap its toll — the lifespan of our proteins is rarely more than a few weeks. Yet, remarkably, some proteins in specialized organs, notably the eye lenses, last a lifetime. This astonishing fact shows that evolution can produce tremendously robust proteins for a particular function, although the occurrence of cataracts (which are themselves protein aggregates that are large enough to scatter light) shows that even the eye's lens proteins are not immortal.

The ability of proteins to change conformation is the essence of the amyloidoses — in these diseases, the proteins have converted into the 'primordial' structure rather than remaining in their evolved states.

So why have these diseases apparently become so much more prevalent in recent years? In my view, this must be at least partly due to societies changing more rapidly than molecular evolution can respond. The proteins that have emerged under evolutionary pressure are normally robust enough to resist reversion to aggregated states, and 'chaperone' proteins help still further to protect against such changes. In old age, however, we see the imperfections of these mechanisms. Proteins can be engineered to be more resistant to aggregation than our natural ones. But it is unlikely that there has been evolutionary pressure to do better than is necessary to pass on our genes and to protect our offspring until they reach maturity; indeed, perhaps evolutionary pressure opposes old age. The recent much longer average lifespans of people in the developed world mean that we are now entering uncharted evolutionary territory.

The rapidity of change in our societies relative to the rate of biological evolution is also revealed in conditions that are not so directly associated with old age. Modern medical practices provide many examples of events not previously experienced in evolutionary biology. In the context of this article, it has been found that the release of a protein, β2-microglobulin, during blood dialysis almost invariably results in amyloid deposits in skeletal tissue after several years. Fortunately, now that this problem has been recognized, new procedures and improvements to the design of dialysis membranes should alleviate this problem. Likewise, evolution has been powerless to deal with the effects of injecting contaminated natural growth hormone — now thankfully avoided by use of recombinant protein.

Such an argument may also explain the origins of kuru and vCJD. Cannibalism is an activity that has presumably been eliminated largely as a result of social pressures acting during evolution. BSE has almost undoubtedly resulted from the highly unnatural practice of feeding young cows on the remains of old ones, with the disease then being transmitted to humans as vCJD. Both kuru and BSE have virtually disappeared as a result of effective action taken once their origins were understood.

What can we learn from this complex story? The discovery of the underlying origin of amyloidoses is tremendously important in view of the increasing impact of these diseases. It allows us the opportunity to develop new strategies for drug therapy, improved medical procedures and higher food standards. But the most fundamental message is that evolution acts slowly, and we are an impatient species. The properties of biological molecules have developed slowly in response to the pressures experienced by their host species. We are now in effective control of both our own evolution and that of many other organisms on the planet. We must therefore strive to understand completely the underlying principles of 'molecular evolution' to take advantage of the tremendous opportunities of this 'post-evolutionary' era. In this way, not only can we avoid accidentally unleashing new scourges on our descendants, but we can take advantage of our new-found knowledge to extend and enhance the quality of all our lives. ■

Christopher M. Dobson is in the Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK.

FURTHER READING

Dobson, C. M., Ellis, R. J. & Fersht, A. R. (eds) *Phil. Trans. R. Soc. Lond. B* **335,** 129–227 (2001).
Solomon, B., Taraboulos, A. & Katchalski-Katzir, E. (eds) *Conformational Diseases: A Compendium* (Karger, Tunbridge Wells, UK, 2001).
Csermely, P. *Trends Genom.* **17,** 701–704 (2001).
Ellis, R. J. & Pinheiro, T. J. T. *Nature* **416,** 483–484 (2002).
Iverson, L. *Nature* **417,** 231–233 (2002).
Ferguson, N. M. *et al. Nature* **415,** 420–423 (2002).