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Amyloidosis

In this paper I will define and describe the class of diseases known as amyloidosis. I will present evidence suggesting that amyloid fibrils from many forms of amyloidosis share strikingly similar structural characteristics, independent of the specific nature of their constituent proteins. I will point out the problems with these claims and show the reader the weakness of the evidence. Finally, I will propose one experiment that will confirm or destroy these claims about the structure of amyloid fibers, and I will propose another experiment that might give us clues as to the causes of these diseases.

Section 1

Amyloidosis is a disease in which a kind of glycoprotein called amyloid P is deposited intercellularly (often along with other precursor proteins) to form amyloid fibrils [1]. The amyloid fibrils are often identified by their characteristic tinctorial properties, i.e. they exhibit a green birefringence when stained with a dye called Congo Red [2]. Depending on the specific form of amyloidosis, amyloid deposits can occur throughout the body or at certain targeted organs. Localized amyloidosis diseases make up the most commonly heard of forms of amyloidosis. At a particular locale, the brain, forms of cerebral amyloidosis occur, including cerebral amyloid angiopathy, Alzheimer's disease, and transmissible encephalopathies.

Cerebral amyloid angiopathy is characterized by amyloid deposition in and around blood vessels of the brain, causing serious and fatal hemorrhaging. It is found in 36% of dead people between the ages of 60 and 97 years and 58% of dead people over the age of 90 years. Alzheimer's disease is diagnosed *unequivocally* only when three symptoms are observed microscopically: (1) cerebral amyloid angiopathy, (2) neuritic amyloid plaques, (3) neurofibrillary tangles. A neuritic amyloid plaque is an abnormal cluster of dead and dying brain cells, nerve cells, and protein (including amyloid). Neurofibrillary tangles are dense arrays of paired helical amyloid filaments located in various parts of the brain [3].

Transmissible encephalopathies exist in animals and human beings. The well known transmissible spongiform encephalopathies of man are Cruetzfeld-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS) and kuru. All are characterized by amyloid plaques in the brain. Scrapie is the sheep and goat form of CJD, and bovine spongiform encephalopathy is the cow form of CJD known as mad-cow disease. In all of these diseases, Prion proteins are a major component of amyloid fibrils. It has been proposed that within this class of amyloidosis, the amyloid fibrils are nothing more than arrays of the infective agent itself, prion protein [1].

Section 2

In a 1997 JMB paper, Sunde *et al.* presented x-ray diffraction data of six different *ex vivo* amyloid fibril samples and two different synthetically prepared fibril samples [2].

The samples differed from one another in that the amyloid fibrils came from diseased patients with different forms of amyloidosis. This means that the amyloid fibrils isolated from the patients, as well as the synthetic samples, were made of various different proteins. They showed that all of these samples gave very similar diffraction patterns, and that the basic structure of the fibrils were, therefore, the same.

The diffraction patterns are identified as cross-β reflections (figure 1). Their characteristic features are two mutually perpendicular arcs of intensity, one corresponding to an approximate inter β–sheet spacing of 10 Å, and the other corresponding to an inter β–strand spacing of about 4.7 Å (figure 2). Such diffraction patterns had been observed before, but ref. 2 was the first comparison of many different forms of amyloidosis [4,5,6].

The authors of ref. 2 went on further to model the structure of amyloid fibrils as a helix made of multiple β–sheets (figure 3). Their motivation for this was twofold. Firstly, they observed one-dimensional crystallinity in their diffraction patterns; perfect helices are one dimensional crystals. All eight of their samples exhibited many meridional reflections (oriented along the fiber axis), the highest angle reflections corresponding to 'spacings' as small as 2Å. They also found a common helical pitch (axial length per turn) of 115 Å. Their second motivation for such a model came from a series of papers published in 1951 and 1953 by Pauling and Cohen [7,8]. In refs. 7 and 8, the authors consider rotations of components of polypeptides around their bond axes, as well as bond axis bending, and propose models for physically plausible secondary structures. Among these are the "pleated sheet" and the "rippled pleated sheet" (figure 4). In ref. 7, the authors predict the pitch of the possible sheet structures, and mention that this twisting of polypeptide sheets is a well-known phenomenon.

Section 3

When looking at the x-ray scattering patterns presented in refs. 4 and 5, one might ask if observing two mutually perpendicular arcs of intensity is sufficient for concluding that the scattering source has the cross-β structure. One can conceive of many structures that give rise to such a simple scattering pattern (figure 5). Furthermore, the 4.7 Å measurement (supposed to correspond to the β–strand spacing in the sheets) disagrees with Pauling's prediction [7,8] by about 100%. Finally, since the sheet spacing (10 Å measurement in ref. 2) is supposed to be heavily dependent on side-chain groups, no general prediction can be made other than that the measurement should fall within "acceptable limits for amino acid compositions found in globular proteins [2]."

It should seem obvious that if one wants to prove that a scattering source is made of sheets (i.e. planes) or cylinders or helices, one has to show that the scattering pattern obtained is truly representative of such objects. Using Bragg's law to back out 'spacings' from 2θ measurements is insufficient. The x-ray evidence for cross-β structure is weak and ambiguous.

It is just as bad when considering the helical superstructure model [2]. Granted, a helix was to be expected. However, their pitch of 115 Å disagrees horribly with the predictions they site [8,9]. Additionally, their observation of one dimensional crystallinity is insufficient for claiming that the scattering source is a helix. The amyloid fibrils were already known to be chains. A chain is a one dimensional crystal. If one wishes to show that their chain is helical, they must look at the reflections *off of the*

meridian and *off of the equator*. The authors of ref. 4 did not do this. For all I care, they wouldn't even have to analyze their data. They could just show a picture that gave the characteristic helical diffraction pattern!

Section 4

To make the argument for the β–sheet spacing more convincing, they should have gone one step further. They should have made a series of synthetic samples totally equivalent to one another except in one respect: the side groups. Since sheet spacing is sensitive to side group species, they should have tested this variable exhaustively. In this case, one could at least say that if the 4.7 Å reflection remained while the alleged side group peak moved, then the experiments really do support this cross-β structural picture. Furthermore, if we have more reason to believe the cross-β structure, we have more reason to believe the helix model, since β–sheets tend to twist.

My criticism stands about the quality of their diffraction data. The problem is that these 'wet' samples are tricky, and getting well oriented samples is difficult if one doesn't want to damage them or artificially induce structure in them. Despite my criticism, the fact that all of the aforementioned amyloid fiber samples have strikingly similar diffraction patterns does support the conclusions of ref. 2 that amyloid fiber structure is independent of the specific nature of their constituent proteins.

A similar phenomenon occurs in other biological systems. DNA, f-actin, and linear bacteriophages all form tightly packed bundles under similar conditions. Polyelectrolyte attraction is a well established and exhaustively studied topic in polymer science and has direct application to biology [9]. As an example, consider f-actin. F-actin is the filamentous form of the structural protein, actin. F-actin is helical in shape and highly charged. By tuning the concentration of counterions in a solution of f-actin, one can replicate, in a test tube, every phase of actin aggregation observed in cells. These observations (still to be published) teach us of a possible mechanism by which the cell regulates its structure.

I propose that a series of experiments be carried out on synthetically produced amyloid fibrils in which the hierarchical structure of the fibrils is probed by changing biologically relevant parameters such as pH and counterion concentration. The polypeptide chains would have to be made with appropriate side-groups which were ionic under biologically relevant pH ranges. (Whether naturally occurring amyloid precursors are charged is a very important question, the answer to which I do not know.) This kind of experiment seems particularly appropriate considering the generic nature of amyloid fibers. If, as for the case of f-actin, we could reproduce the known amyloid structures by merely tuning counterion concentration or pH, then we might have found a very generic clue as to the causes or the cure of amyloidosis.

References

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