Protein folding and misfolding mechanism and principles

Every function in the living body depends on proteins, and before proteins can carry out any function they must be able to assemble or fold themselves into their three-dimensional structure. Proteins are linear chains of amino acids that adopt unique three dimensional structures (`native states') which allow them to carry out intricate biological functions. All of the information needed to specify a protein's three-dimensional structure is contained within its amino-acid sequence. In suitable conditions, most small proteins will spontaneously fold into their native states during or after synthesis (1).

The majority of proteins are folded in the cytoplasm of the cell others can be folded in specific compartment such as the mitochondria or the endoplasmic reticulum after the newly sequenced amino acids are released by the ribosome(2); this is referred to as the primary structure of the protein. The amino acids in a polypeptide chain, linked through peptide bonds that form the covalent backbone of the proteins. To prevent any inappropriate interactions or aggregation of exposed hydrophobic surfaces with other molecules in the cell and to prevent misfolding or unfolding in the face of certain stresses, such as changes in the cellular environment due to ageing or temperature fluctuation, genetic mutation, molecular chaperones interact with the unfolded protein and direct their substrates into productive folding, transport or degradation pathways.

Molecular chaperones do not themselves increase the rate of individual steps in protein folding; rather, they increase the efficiency of the overall process by reducing the probability of competing reactions, particularly aggregation. Some chaperones help rescue misfolded proteins and even aggregated proteins giving them a second chance to fold correctly. (2)

The secondary structure of a protein occurs mainly as alpha helices and beta strands, this structure hugely depends on the primary structure. The α-helix is a common secondary structure encountered in proteins of the globular class (3). After a polypeptide has acquired most of its correct secondary structure, with the alpha helices and beta-sheets, it has a loose tertiary structure than its native state and is referred to as its molten globular state.

The tertiary structure is the final specific shape that a protein assumes. This final shape is determined by a variety of bonding interactions between the side chains on the amino acids. These bonding interactions are generally stronger than the hydrogen bonds between amide groups and carbonyl group in alpha helices and beta sheets, holding the helical structure. As a result, bonding interactions between side chains causes a number of folds, bends, and loops in the protein chain. Different fragments of the same chain may become bonded together.

Protein Misfolding

An obvious consequence of protein misfolding is aggregation, loss of function, and gain of toxic function (1). Structure of a protein and its ability to carry out its correct function are very tightly linked such that small structural defects can lead to a number of protein folding diseases. Diseases include genetic diseases such as cystic fibrosis and sickle cell anaemia, which are caused by single residue deletion and mutation respectively, rendering the protein incapable of its normal function.

Unfolded or misfolded proteins accumulate in the ER. Abnormal or misfolded proteins may deposit in tissues and interfere with normal functions. The

deposits can be intracellular, extracellular, or both, and there is accumulating evidence that the aggregates may either directly or indirectly cause the pathologic changes such as amyloidosis.

Misfolded proteins are usually degraded by the cell as there is no use for them, they are firstly labelled by ubiquitin, and then degraded into amino acids so they can be reused, this energy requiring process in eukaryotes occurs via the large proteolytic complex, the 26S proteosome.(2)

Amyloid Formation

Amyloid formation can be found as a complex mixture that can include natively folded, partially folded and highly unfolded protein species, any one of which could initiate the aggregation process (4).

Amyloid fibrils are highly organized aggregates formed by peptides and proteins with a wide variety of structures and functions (1). Fibrils are rope-like structures made up of proteins sometimes known as fibres, but there may be toxic phases during their formation which can damage cells and cause disease. Latest research suggests the length of amyloid fibrils found in diseases such as Alzheimer and Parkinson appears to play a role in the degree of their toxicity (3).

Amyloid fibrils are said to be protease-resistant and insoluble they are composed of β-sheets. The cross-beta structure and texture is a robust, stable structure in which the protein chains are held together securely by repetitive hydrogen-bonding that extends the length of the fibrils. (6)

Amyloid fibres that are associated with neurodegenerative diseases are considered the product of a protein misfolding event. Deposits are usually extracellular and fibrils are associated with diverse group of human diseases that includes Alzheimer’s disease, Creutzfeldt-Jakob disease and type II diabetes (2).

As well as having a distinct X-ray fibre diffraction pattern, amyloid deposits change from pink to red this is because of their unique ability to bind the dye Congo red, which helps aid their identification. (5)

Amyloidosis of wild type β2 Microglobulin

Alzheimer’s disease and Creutzfeldt-Jakob disease are the best-known examples of a group of diseases known as the amyloidosis. They are characterized by the extracellular deposition of toxic, insoluble amyloid fibrils. (1)

β2-microglobulin is a low molecular weight protein (11. 8 kDa) that forms the light chain of the major histocompatibility antigens (1) it typically consists of 99 amino acid residues. In its native soluble form, the human protein β2-microglobulin (β2m) has a classical immunoglobulin fold (2). Structurally β2M is a mainly β-sheet protein, containing a sandwich of two sheets, one with four β-strands and one with three β-strands. The two β-sheets are linked by a single disulfide bond. (3)

β2M fibrils contain many other constituents in addition to β2M itself, including glycosaminoglycans, apolipoprotein E, β2-macroglobulin and other protease inhibitors, and serum amyloid P. While some of these components

may serve to limit or regulate fibril growth, some of them are probably essential for fibril growth in vivo. (4)

Aggregation of amyloid fibres in vivo, lead to a pathological disorder recognized as amyloidosis. (5)This disease involves the conversion of normally soluble proteins or peptides into insoluble fibrillar arrays, although the clinical manifestations of each disease are specific to the identity of the aggregating protein. (6) Dialysis-related amyloidosis (DRA), involves the aggregation of full-length, wild-type, human β2-microglobulin (β2m) into amyloid fibrils.(7) In dialysis patients β2 microglobulin builds up in the blood and deposits in the joints as amyloid.

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Amyloid formation

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