How do proteins fold?

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Introduction

Proteins are a key component of the human body and how it functions. They are made of amino acids, which are linked together to form long linear chains, like beads on a string. These strings fold in to complex 3 dimensional (3D) shapes. It is the combination of 3D shape and the amino acid sequence that determines the function of different proteins. Changes to either can result in non-functional or mis-folded proteins.

Alzheimer's disease is the commonest cause of dementia among older adults, causing memory loss and problems with thinking, problem solving and language. An accumulation of mis-folded proteins is a hallmark of the disease. We want to better understand the protein folding process with the longer term hope of identifying why it does not work properly in Alzheimer's patient.

We know that proteins that are secreted from cells are synthesised by ribosomes and then go through a region of the cell called the endoplasmic reticulum (ER), where they get folded by the formation of disulphide bonds and glycosylation (see Figure 1).

Disulphide bonds are interactions between specific amino acids called cysteines and they provide stability to the structure of the protein. Glycosylation is the attachment of a sugar onto an amino acid and this gives solubility to the protein (see Figure 2).

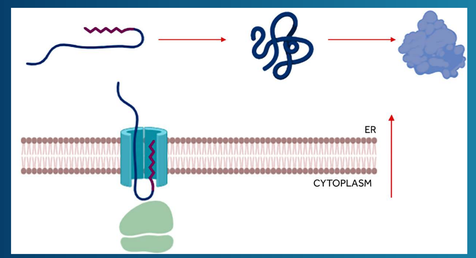

Figure 1 Proteins that are secreted from the cell are synthesised by the ribosome (green) before moving to the endoplasmic reticulum (ER), where the amino acids get modified and the protein starts to fold.

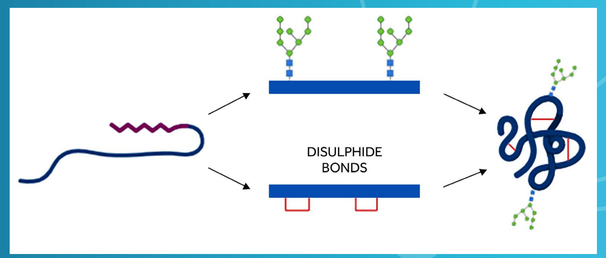

Figure 2 In the endoplasmic reticulum, proteins are modified by the attachment of a sugar (green and pale blue structure) (glycosylation) and formation of disulphide bonds (red) between cysteine residues. This ensures proper folding and function of the protein.

What did we do?

We chose to study a protein called hemopexin, which we know gets glycosylated. To follow the glycosylation of hemopexin we used digitonin to selectively permeabilise the plasma membrane (the membrane which surrounds cells) of cells which we grew in the lab. This left us with only the nucleus (where DNA is found) and the ER. We re-inserted the essential components for protein synthesis, namely mRNA of hemopexin (mRNA is an intermediate molecule that carries the genetic instructions to make proteins which is encoded in the nuclear DNA); the machinery needed to synthesise proteins; and a radioactively labelled amino acid, so we could detect the synthesised protein (see Figure 3).

We broke the cells open, isolated the protein component of the cells and separated the individual proteins by a technique called SDS-PAGE. SDS-PAGE separates the proteins according to their size. Therefore, we could detect which of our proteins were glycosylated or not because the addition of a sugar to the protein makes it heavier than when the protein is not glycosylated (see Figure 4).

As well as carrying out our experiment with the normal (wild type (WT)) protein, we also tested forms of the protein that had been mutated - an amino acid had been changed for a different amino acid at a particular position in the protein sequence. We changed the amino acid asparagine (N) to glutamine (Q) at position 187 (N187Q) and/or position 453 (N453Q) of the protein sequence.

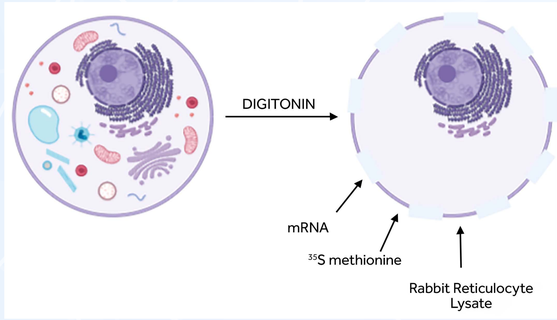

Figure 3 Cell membranes become semi-permeable after digitonin is added. We removed all of the cell components apart from the ER and synthesised radioactively labelled hemopexin.

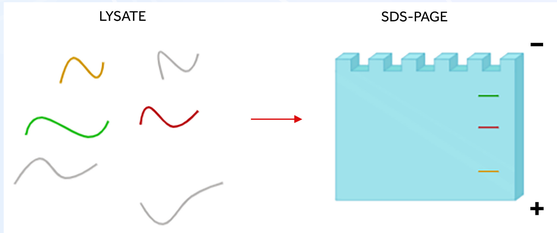

Figure 4 SDS-PAGE separates the proteins according to their size. The smaller the protein (yellow protein and band) the closer to the +ve end of the SDS-PAGE and the larger the protein (green protein and band) the closer to the -ve end of the SDS-PAGE. In our experiment, this technique allowed us to recognise whether hemopexin was glycosylated or not because we only detected radioactively labelled hemopexin, which was larger when glycosylated.

What did we find?

Our SDS-PAGE results showed that when we mutated our hemopexin by changing asparagine to glutamine the amount of glycosylation was reduced (the mass of the protein was less, the strong band on the gel was lower). When 2 of the asparagine sites were mutated in the same protein it became even smaller. The WT protein shows two bands with different molecular masses; the faint lower band is due to the lack of one sugar molecule, this is called hypo-glycosylation.
We found that the asparagine at position 187 (N187) is responsible for hypo-glycosylation in the hemopexin because the lower band disappears when the protein was mutated at the N187 site. This was not seen when the N453 site alone was mutated (see Figure 5).

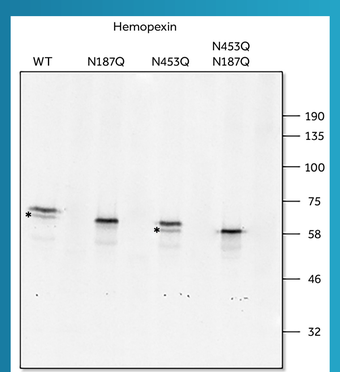

Figure 5 Our SDS-PAGE results showed as well as fully glycosylated hemopexin, some hemopexin with less sugar is synthesised (hypo-glycosylation). The N187Q mutated proteins showed that asparagine 187 was responsible for this reduced glycosylation as the lower fainter hypoglycosylated protein band (*) disappeared in the N18Q and N187Q/ N453Q mutated protein.

What does this mean?

The amino acid residue responsible for the sugar attachment in hemopexin is asparagine (N). The structure of hemopexin shows that next to the sugar-attachment site N187, there is a cysteine (C188) which forms a short disulphide bond with C200 (see Figure 6). We predict that the disulphide bond is impeding the glycosylation of N187. The mutant N187Q (either alone or in combination with N453Q) is the only sample that does not show hypo-glycosylation. We can conclude that there is a competition between disulphide bond formation and N-glycosylation in the normal (WT) protein. Our findings have increased our understanding of the complexities of protein folding and we hope will ultimately help us identify why proteins mis-fold in Alzheimer's disease.

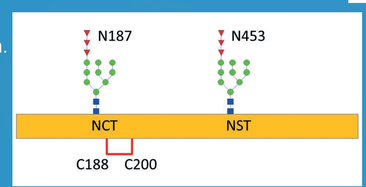

Figure 6 Representation of the hemopexin protein showing the position of a short disulphide bond which forms very close to the asparagine 187 glycosylation site. The disulphide bond blocks attachment of sugar to the asparagine.

Who am I?

I am a fourth year student at the University of Glasgow. I study Molecular and Cellular Biology and I have worked both in biochemistry and cell biology. My career plan is to carry on in academic research and do a PhD. My main interest is to study fundamental biology pathways to better understand the diseases that arise from them; and try to develop targeted drugs.

Giorgia was supervised during her Vacation Scholarship project, *"Identifying membrane proteins involved in maintaining redox homeostasis in the early secretory pathway"*, by Professor Neil Bulleid at the University of Glasgow.