BioUpdate Foundation

Disulphide Bridges
An Evolutionary Black Hole

Cysteine. An essential amino acid, important for the formation for disulphide bridges, a key component of maintaining the structure of folded proteins. Or is it?

Despite being interested in protein structure and evolution, I never really though much about cysteine, accepting what I had been taught about disulphide bridges. Then in the early 1980's, a scientist called Tom Creighton, who was working further down the corridor at the Laboratory of Molecular Biology, did some interesting work with Bovine Pancreatic Trypsin Inhibitor (BPTI). BPTI is a small protein, 58 amino acids with 6 cysteines and 3 disulphide bridges. What Creighton found was that as denatured BPTI refolded, it made the wrong disulphide bridges during the folding process, and this said that, even with a small protein, the energetics of protein folding were greater than any stabilisation effect of disulphide bridges. Disulphide bridges could not be important in maintaining protein structure, any disulphide bridge which could form, as a result of spatial proximity, would form. Do cys-S-S-cys bridges make the lowest energy potential well deeper? Presumably if they did, that would apply equally to all intermediate potential wells and make the folding pathway more difficult.

I am not sure many other people share the view that disulphide bridges are not structurally important, and it certainly has been a useful topic for encouraging discussion and debate, over several years. More recently, however, I have thought about it again and that has made me wonder, what is the truth about cysteine, and about disulphide bridges in particular?

Back in the 1980's I should have taken more note of the work that was being undertaken, on the floor below, to understand what made thermostable proteins thermostable. One thing the answer is not, is more disulphide bridges. Thermostability of proteins seems to be linked to salt bridges and additional side chain to side chain hydrogen bonds (for an open access review see Kumar *et al*, Protein Eng. (2000) 13 (3): 179-191 doi: 10.1093/protein/13.3.179). In fact cysteine occurs less frequently in thermostable proteins than in other proteins. Another thought that struck me was that cysteine residues in proteins always occur in multiples of 2. With the single exception of thiol proteinases, where a cysteine residue is part of the catalytic mechanism, I can't think of any protein which has an unpaired cysteine with a free SH group. Even the c type cytochromes, which bind haem via cysteine typically do so via a dual cysteine Cys-X-X-Cys-His motif. There are several single base mutations which would change a serine (cysteine's OH analogue) into a cysteine, and, surely, this must have happened during the evolution of proteins?

If disulphide bridges result from the reactivity of cysteine thiols, and not from any structural consideration, and if unpaired cysteine residues aren't, in general, found in proteins, then perhaps

cysteine is just too reactive to allow a functional, stable structure. That would suggest that serine to cysteine mutations, or even cysteine to serine single mutations, where the cysteine was part of a disulphide bridge, are an evolutionary dead end. That raises the question of how did disulphide bridges get there in the first place and more intriguingly why haven't they been selected out.

In answer to the first question, the disulphide bridge may be a lingering echo of a primordial past. One can imagine early protopeptides with only one cysteine, and these would naturally form dimers. A clue, perhaps, to their early survival. Then by some chance fusion of two protogenes, the disulphide bridge was born. Random fusions would have created single cysteine protoproteins as well, but as we have discussed before only a tiny fraction of all possible protein sequences exist (see our blog "Most proteins don't exist"). The only cysteine bearing proteins which have survived are those having cysteine residues occurring in multiples of two; a single cysteine being a one way ticket to oblivion. It is tempting to speculate that thiol proteinases evolved much later when the chemical reactivity could be harnessed and exploited to advantage. Did thiol proteinases arise by a single mutation in an ancestral serine proteinase gene?

It is the answer to the second question that is most fascinating – there is no mechanism by which a disulphide bond can be lost during evolution! Well it is possible, but it would need a very unlikely simultaneous double mutation at exactly the right points in the gene sequence. A single mutation affecting only one cysteine residue, would leave an unpaired cysteine. This would leave us in the same position as a serine to cysteine mutation, a dangerously reactive unpaired cysteine, a non functional protein and an evolutionary dead end.

The number of disulphide bridges in a protein are, therefore, fixed throughout evolutionary time. Unless there is a rare double mutation, either losing or gaining a single cysteine results in a reactive rogue thiol and a protein with no future. Disulphide bridges are somewhat akin to a Black Hole, there is no escape.

PostScript: This is, of course, hypothesis, but a hypothesis which can be tested. Genetic engineering allows us to construct both cysteine free and unpaired cysteine proteins. Now there is a challenge!

Tony Auffret
April 2015