Degradation of FeS proteins.

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Metals play an essential role in biological systems and around one third of the proteins present in a cell are metalloproteins, that is, they contain either a metal as a cofactor or a metal containing cluster. The latter are particularly relevant in terms of protein structural stability and folding since they act as local structural stabilising elements in the folded state, contributing to the maintenance of a given specific structural fold, but are also potential key nucleation points during *in vivo* folding as their binding to the unfolded polypeptide is likely to impose a conformational restriction which lowers the entropy of the unfolded state thus favouring a specific folding pathway.

Proteins containing iron-sulfur clusters (see figure for examples) are widespread in the three domains of life, and they play diverse biological roles, from simple electron transfer processes to catalysis and regulation of transcription. Their ubiquity and involvement in anaerobic metabolic pathways suggests that these proteins are very ancient catalysts.

One approach towards the understanding of the role of the clusters in protein stability consists of the characterisation of their disintegration during protein unfolding, which may elicit the involvement of intermediate cluster structures or highlight the role of particular structural elements. In this respect, we have been studying the stability properties of several thermophilic iron sulfur proteins, including ferredoxins [1,2], rubredoxins and a Rieske Ferredoxin [3], using complementary biochemical, spectroscopic and biophysical methodologies.

This year we have established [4] that during the chemical alkaline degradation of a dicluster, seven iron ferredoxin, shortly after onset of protein unfolding, iron is released monophasically at a rate which is comparable to that of the degradation of the iron-sulfur centres. The ferredoxin degradation pathway comprises an initial step in which the protein is destabilised to an open conformation, which results in an exposure of the iron-sulfur centres. Our data suggests that both iron-sulfur clusters degrade simultaneously, a feature also evidenced by ¹H-NMR studies. More importantly, during this process there is no detectable formation of any intermediate species involving the metal clusters. Thus, a previous hypothesis suggesting that the decomposition of iron sulfur clusters was proceeding via linear three iron sulfur centres has been reassessed.

**References**