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ACCEPTED MANUSCRIPT

PROBING BULKY LIGAND ENTRY IN ENGINEERED ARCHAEAL FERRITINS.

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ABSTRACT

Background

A set of engineered ferritin mutants from *Archaeoglobus fulgidus* (Af-Ft) and *Pyrococcus furiosus* (Pf-Ft) bearing cysteine thiols in selected topological positions inside or outside the ferritin shell have been obtained. The two apo-proteins were taken as model systems for ferritin internal cavity accessibility in that Af-Ft is characterized by the presence of a 45 Å wide aperture on the protein surface whereas Pf-Ft displays canonical (threefold) channels.

Methods

Thiol reactivity has been probed in kinetic experiments in order to assess the protein matrix permeation properties towards the bulky thiol reactive DTNB (5,5'-dithiobis-2-nitrobenzoic acid) molecule.

Results

Reaction of DTNB with thiols was observed in all ferritin mutants, including those bearing free cysteine thiols inside the ferritin cavity. As expected, a ferritin mutant from Pf-Ft, in which the cysteine thiol is on the outer surface displays the fastest binding kinetics. In turn, also the Pf-Ft mutant in which the cysteine thiol is placed within the internal cavity, is still capable of full stoichiometric DTNB binding albeit with an almost 200-fold slower rate. The behaviour of Af-Ft bearing a cysteine thiol in a topologically equivalent position in the internal cavity was intermediate among the two Pf-Ft mutants.

Conclusions and General Significance

The data thus obtained indicate clearly that the protein matrix in archaea ferritins does not provide a significant barrier against bulky, negatively charged ligands such as DTNB, a finding of relevance in view of the multiple biotechnological applications of these ferritins that envisage ligand encapsulation within the internal cavity.

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