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Names and affiliations of applicants (* indicates experimentalists):

Prof. Degano Massimo

Dr. Muzzolini Laura

Dipartimento di Biocristallografia Sincrotone Trieste in Area Science Park
S.S. 14, Km 163,5 – 34012 Basovizza (TS), Italy

Report: Two protein systems have been studied: DPS-like proteins and the adenosine monophosphate nucleosidase

CRYSTAL STRUCTURE OF TWO DPS-LIKE PROTEINS FROM *BACILLUS ANTHRACIS*

Elena Papinutto [a)], William G. Dundon [b)], Nea Pitulis [a)],
Roberto Battistutta [a)], Cesare Montecucco [b)] and Giuseppe Zanotti [a)]

[a)] Dept. of Organic Chemistry and Biopolymer Research Center, U. of Padova, Via Marzolo 1, 35131 Padova, Italy
[b)] Dept. of Biomedical Sciences and Biomembrane Research Center, U. of Padova, Via Colombo 10, 35131 Padova, Italy

A class of molecules with architecture and properties similar to those of ferritins has been recently discovered in bacteria: these proteins are made up by 12 identical subunits, with a fold similar to that of ferritins. They arrange to form a dodecameric shell with 32 symmetry and a large cavity in the center. Despite their similarity with ferritins and the fact that some members of this family are able to incorporate iron *in vitro*, their function is still an open question. At least eight members of the family have been till now identified, and for some of them a specific function has been recognized: *E. coli* and *Bacillus subtilis* Dps are DNA binding proteins that protect DNA from oxidative damage, *L. innocua* dodecameric ferritin is a mini-ferritin, but for others completely different functions have been hypothesized: Flp from *L. monocytogenes* is supposed to be a

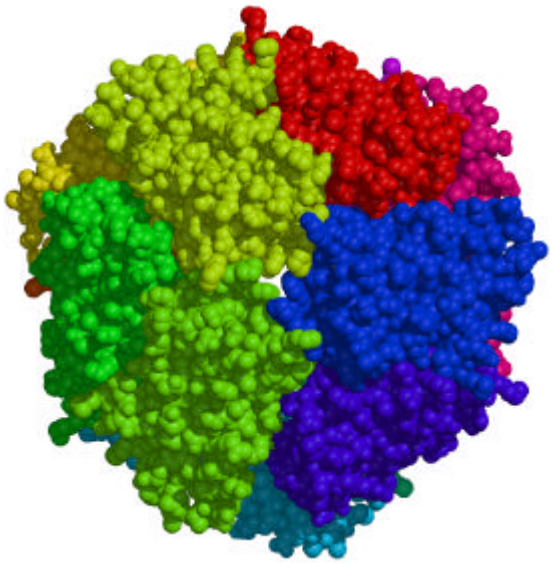
cold shock protein, *H. ducreyi* FtpA a structural protein of fine tangled pili. In *Bacillus Antracis* two different genes with amino acid sequences similar to those of the other members of this family have been identified by us. The two proteins, termed BA-Dlp-1 and BA-Dlp2, have been characterized.

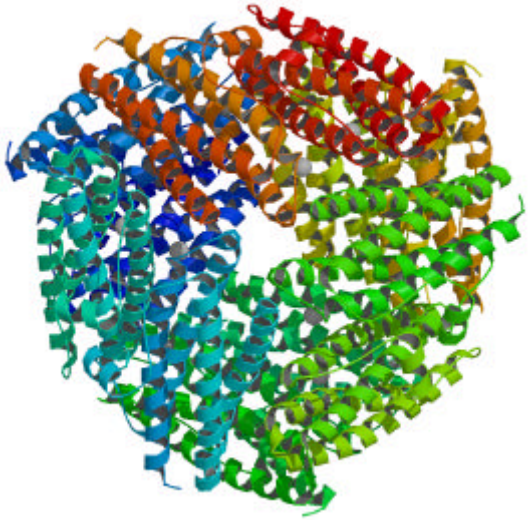
BA-Dlp-1 and 2, like other members of the family whose structure is known, are dodecamers, about 90 Å in diameter, with 32 symmetry (Fig. 1). In both cases, one of the three-fold axes of the molecule is coincident with a crystallographic one. The arrangement of the twelve monomers gives rise to a nearly spherical shell, with an internal cavity where the iron is eventually stored. It must be pointed out that in the two proteins presented here no iron was observed in the internal cavity, which is likely filled with unordered solvent molecules. This must not necessarily reflect a physiological behavior since, after the heterologous expression and purification, the proteins were crystallized as they were, without any specific treatment to add or extract iron.

The Fe^{2+} binding site of BA-Dlp-1 and BA-Dlp-2 is similar to those of HP-NAP and of *L. innocua* ferritin. The environment of the cation is virtually identical in all these proteins, corresponding to a tetrahedral coordination made up by two oxygen atoms, one of an Asp and the other of a Glu residue, one nitrogen of a His and a solvent molecule. The two acidic residues and the His belong to two different monomers. The fourth coordination position in all cases is occupied by a solvent molecule, that in our case is clearly an O of a water molecule, as confirmed by its thermal parameter and by the inter-atomic distance.

The nature of the cation is on the contrary an open question: it has been shown in fact that 24-mers ferritins can bind divalent cations other than Fe^{2+}, like Cd^{2+}, Zn^{2+} and Ca^{2+}, or even trivalent, like Tb^{2+} (Lawson et al., Nature 1991, 349, 541-544). Moreover, a tetrahedral coordination is not the most common for the iron ion. In Dlp-2, an anomalous set of data at a wavelength of 1.73 Å was measured, in order to demonstrate unequivocally the presence of the iron. The anomalous difference map, calculated with the phases of the refined model, shows that only a small peak (2 sigma) is present in correspondence of the metal site. On the contrary, all the maxima higher than 3.5 sigma contour levels correspond to all the S atoms of methionines (there are no cysteines in BA2), an indication of the good quality of the phases and consequently of the calculated map. Since the position refine quite well with a Fe^{2+} (B = 7.77), the cation present in Dlp-2 crystals is more likely an ion with an electron content similar to iron and with a definite preference for tetrahedral coordination, like for example Zn^{2+}. It must be noticed that the Dlp we have crystallized were expressed in *E. coli* and this could explain the presence of a different cation in the metal site, that could reflect the different conditions of expression more than a difference in the biological behavior.

Atomic coordinates of both proteins have been deposited at the PDB with accession number codes 1JI5 and 1JIG.





Ribbon diagram (top) and space-filling model (bottom) of Dlp-2 dodecameric ferritin from *Bacillus anthracis.*

Methods

Data were measured at two wavelength: the first 0.9 A at the beamline ID14-3, the second of 1.73 A at beamline ID29. The latter was used in order to detect the presence of the iron ions bound to the protein, one per subunit. The structure has been solved and refined, as shown by the following tables.

Table I. Statistics on data collection and refinement of BA-Dlp-2 from Bacillus anthracis.

	BA-Dlp -2 (λ = 0.93 Å)	BA-Dlp-2 * (λ = 1.73 Å)
Resolution (Å)	50-1.46 (1.54-1.46)	50-2.29 (2.43-2.29)
Independent reflections	105192 (13823)	25424 (5209)
Multiplicity	3.0 (1.8)	4.5 (2.1)
Completeness (%)	98.2 (88.2)	78.6 (54.9)
$\langle I/\sigma(I)\rangle$	12.7 (9.0)	10.3 (7.1)
Rmerge	0.032 (0.068)	0.056 (0.079)

*Statistics of this column refer to merged Bijvoet pairs.

	BA-DLP-2
Protein atoms	4656
Solvent molecules - Fe atoms	328
$R_{cryst.}$ (%)	18.6 (22.4)
R_{free} (%)	20.5 (23.1)
R.m.s. on bonds distances (Å)	0.007
R.m.s. on bond angles (°)	1.1

Data collection at beamline ID29 on E. coli AMP nucleosidase.

We used three shifts at beamline ID29 for diffraction experiments on the AMP nucleosidase from E. coli, an allosteric enzyme composed of six identical subunits of 52kDa each.

The tetragonal crystals diffract up to 2.7Å, which is by far the best diffraction ever observed from these crystals, resulting in a significant improvement to the previous 3.5Å. We collected a native data set for the ternary complex between AMP nucleosidase, its allosteric activator ATP, and the competitive transition state-like inhibitor formycin monophosphate. This crystal initially diffracted to 2.7Å resolution, but decayed rapidly due to radiation damage preventing us from collecting a complete dataset at this resolution. The characteristics of the ID29 beamline were undoubtedly crucial for the improvement observed. First of all, the high brilliance allowed observation of reflections at high resolution. Secondly, the availability of high photon fluxes coupled to small beam sizes (as low as 0.05x0.05 mm) made possible collection of data from crystals where multiple lattices were present.

We were also successful in collecting a three-wavelength MAD data set at the bromine edge, taking advantage of the presence of 300mM NaBr in the crystallisation medium. Unfortunately this crystal also decayed in the beam, thus reducing the useful resolution limit for the subsequent analysis to 4.5Å. The analysis of the collected data is in progress, in the attempt to identify bromide binding sites using direct as well as Patterson search methods.

Data set	Resolution limit (Å)	Completeness (%)	Multiplicity	$\langle I/\sigma(I) \rangle$	R_{sym} (%)
Br-peak	4.0	97.6	6.8	4.9	9.4
Br-infl.	4.0	97.7	5.2	6.8	9.0
Br-rem.	4.5	98.7	6.7	5.4	9.9

In the meantime we successfully cocrystallized the protein in the presence of 8-Bromo ATP, which binds to the allosteric site. These crystals diffract to better than 3.0Å and are suitable for MAD experiments. Hence, we plan to collect further data at the bromine edge to allow the solution of the phase problem using the MAD technique. The search for heavy atom derivatives is in progress, and we identified potassium tetrachloroplatinate as a likely candidate for SIRAS measurements.

In summary, we achieved better resolution for the native crystals taking advantage of the high brilliance of ID29. We feel that a complete dataset could be collected with a slightly attenuated beam, or in

the presence of radical scavengers in the cryoprotectant. Moreover, a high resolution native could be obtained by merging of partial datasets collected from different crystals. We also collected a MAD data set, which might provide partial phasing information, but is at the moment not sufficient for solution of the structure. We have established cocrystallization conditions with a brominated ligand, ensuring the presence of at least three anomalous4 scatterers in the asymmetric unit. We also identified a heavy atom derivative for MIRAS studies. Hence, the progress of the structural studies on AMP nucleosidase from E. coli rely heavily on the access at the ID29 beamline, since its brilliance and beam characteristics are unique, and are indispensable for the collection of data of high quality and resolution required for the solution of the protein structure.

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