**Kinetic and calorimetric study of an *E. coli* large-oligomer protein**

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**Highlights**

* Protein aggregation has been recently described as a native and regulated process.
* The *E. coli* sirtuin CobB was purified as a dimer and as a large-oligomer.
* The oligomer showed a higher thermostability than the dimer.
* The dimer and large-oligomer showed similar enzymatic and substrate-binding parameters.

**1. Introduction**

*Escherichia coli* (*E. coli*) is a Gram-negative bacterium widely employed in biology and biotechnology as a model microorganism to produce high-value compounds such as proteins or drugs with a great commercial interest (1,2). Recombinant protein overexpression in *E. coli* still is the most used strategy for protein production. However, many difficulties can difficult protein expression and purification, such as low expression, protein toxicity, difficult purification steps or inclusion bodies formation (3). To solve these drawbacks, new purification strategies are being developed (4). Protein inclusion bodies are insoluble protein aggregates which are formed when protein aggregates are large enough. However, proteins can form soluble large-aggregates in response to diverse conditions, such as heat shock, other environmental stress conditions or a fast and high expression of recombinant proteins (5). Moreover, protein aggregation has been recently described as a native and regulated process in bacteria, yeast and mammalian cells (6).

**2. Methods**

In this study, the overexpression and purification of a soluble large-oligomer *E. coli* CobB protein is presented. Native electrophoresis, HPLC size-exclusion chromatography, enzymatic characterization, Differential Scanning Calorimetry (DSC) and Isothermal Titration Calorimetry (ITC) were carried out to evaluate and compare the two protein conformations.

**3. Results and discussion**

The results showed that the *E. coli* sirtuin CobB, highly conserved since prokaryotes to humans, was purified in two different conformations: a dimer and a large-oligomer. The two protein conformations were time-stable and an equilibrium between them was not observed. Moreover, the large-oligomer conformation showed an enzymatic activity and a binding constant for Acs (Acetyl-CoA synthetase) protein similar to the dimer. Finally, the oligomer showed a higher thermostability, with a melting temperature almost 15 degrees higher.

**4. Conclusions**

The results of this work emphasize the importance of protein large-oligomer conformations for their use in biotechnology as a strategy to carry out processes with extreme conditions (7).

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