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Kinetics of aggregation and structural properties of proteins in inclusion bodies studied by Fourier transform infrared spectroscopy

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Background

Protein aggregation plays a crucial role in medical sciences and in biotechnology, as it occurs in several diseases and in the expression of recombinant proteins in bacterial cells in the form of inclusion bodies (IBs). Interestingly, it has been suggested that the presence of native-like structures within IBs [1-4] improves the efficiency of refolding protocols that employ mild solubilization methods [5]. This property could also explain the residual enzymatic activity of recombinant proteins in IBs, with possible applications in biocatalysis [6].

As recombinant protein production in bacteria is a central issue in biotechnology, it would be instructive to monitor the kinetics of protein aggregation and the extent of native-like secondary structures within IBs.

Results

We will present a new Fourier transform infrared spectroscopy (FT-IR) approach to study the aggregation of recombinant proteins in *E. coli* in the form of aggregates of increasing complexity. The method enables to monitor the kinetics of aggregate formation within intact cells in a rapid and non invasive way and to obtain structural information on proteins within IBs. We will report results on four recombinant proteins: human growth hormone (h-GH), human interferon-alpha-2b (IFN-alpha-2b) [4,7], *Pseudomonas fragi* lipase [3], and green fluorescent protein – glutathione S-transferase fusion protein (GFP-GST) [8].

Kinetics of aggregate formation was investigated at different production temperatures. The rate of protein aggregation, monitored by the marker band of aggregation in the FT-IR absorption spectrum (Amide I band), was found to increase with the raising of production temperature. Furthermore, the protein expression in its soluble and insoluble fraction was also evaluated by the analysis of the FT-IR spectrum, in excellent agreement with SDS-PAGE analysis.

To obtain structural information on protein aggregates, extracted IBs were also studied in the Amide I absorption region. Two structural features were observed, namely the presence of native-like residual structures and the intermolecular β-sheet interaction of proteins within IBs.

Interestingly, for the same protein the residual native-like structures in IBs were found to change with the level of expression. Therefore, by modulating the culture conditions, the extent of native-like structures in IBs can be optimised with useful applications in biotechnology.

Furthermore, additional structural features were obtained by the comparison of the FT-IR spectra of the native form, IBs and thermal aggregates for the same protein.

Conclusion

This FT-IR analysis offers a simple and rapid method to monitor *in vivo* the development of aggregates formed by

heterologous proteins and the effect of culture condition modification on the process. Furthermore, the method indicates that aggregating proteins modify at different extent their secondary structures from native α-helices and intramolecular β-sheets to intermolecular β-sheets typical of amorphous aggregates and fibrils.

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