Microbial Cell Factories

BioMed Central

Poster Presentation

FT-IR spectroscopy for the study of bacterial membrane stress induced by recombinant protein production

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from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):P2 doi:10.1186/1475-2859-5-S1-P2

Background

Microorganisms respond to environmental stresses regulating their membrane fluidity by changing the lipid fatty acid composition. In particular, in bacterial cells the alteration of membrane lipid composition plays an important role in response to heat and toxic stresses [1,2]. It is known that at increasing temperatures the regulation of membrane fluidity occurs through the incorporation of more saturated fatty acids, longer acyl chains and, in some cases, by changing the unsaturated fatty acids from *cis* to *trans* conformation. Among different stresses, production of heterologous proteins is of particular interest for its applications in biotechnology.

Results

In this work we show the potential of FT-IR microspectroscopy to monitor changes in membrane composition during recombinant protein production. In particular, we studied the infrared absorption of *E. coli* strains expressing several proteins with different amount of soluble and aggregated fractions. In addition, as a control experiment, we have studied *E. coli* strains expressing beta galactosidase, as reporter gene, under the chaperone IbpB promoter [3].

In all model systems examined, an increase in the band intensities at 2850 cm^{-1} and at 2925 cm^{-1} was observed in presence of a high level of stress [4]. As these two bands are due to CH_2 stretching vibrations [5], this spectral behaviour may indicate that longer acyl chain and /or more saturated fatty acids are incorporated in cell membranes. The protein expression analysis indicates that the enzymes that lead to the accumulation of Acetyl Co-A (precursor of fatty acids) are strongly accumulated whereas the phospholipide degradation seems being inhibited.

Indeed, when the protein is expressed also in a soluble form – as indicated by the FT-IR protein response in the amide I region and confirmed by the SDS-PAGE analysis – the two CH_2 bands are more intense than in the case of IB formation. This behaviour seems to indicate that the presence of the soluble recombinant protein induces a stress in the cell, suggesting that IBs could limit the toxicity of overexpressed foreign proteins.

Conclusion

This work highlights the potential of FT-IR spectroscopy not only to study protein aggregation in IBs [6-8], but also to monitor the membrane response induced by the heterologous production.

Acknowledgements

This work was supported by INFM (Istituto Nazionale Fisica della Materia) grant to SMD.

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