Microbial Cell Factories

Poster Presentation

Efficient, antibody-mediated allosteric activation of an immobilized, *E. coli* beta-galactosidase recombinant biosensor

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Background

Allosteric biosensors are based on engineered reporter enzymes responsive to analyte binding through detectable changes in the specific activity [1,2]. Since antibodies are efficient allosteric effectors, such devices are especially useful for the diagnosis of infectious diseases. In previous studies, we have introduced an antigenic peptide from the HIV structural protein gp41, spanning amino acids 579 to 613 of the Env precursor [3], into a permissive site of *E. coli* beta-galactosidase, resulting in the chimeric protein NF795gpC. In the presence of immune sera or anti-peptide antibodies, the soluble enzyme is efficiently activated in a fast and homogeneous immunoassay [3,4]. To further develop biosensor devices in solid phases, with wider applicability in field conditions, we have here explored the allosteric properties of NF795gpC when immobilized in an agarose substrate.

Results

The immobilization process was optimised by using a commercial beta-galactosidase and different crosslinked types of agarose, from which we finally considered 4BCL as the optimum for the assay because it permitted the total accommodation of the protein in the agarose porus. We monitored the binding process through activity assays (at pH 9.5) along time in supernatant and suspension, always controlling the stability of the protein [5]. The decrease of activity in the supernatant and not in the suspension indicates the efficient joining of the soluble protein to the support.

After that, we checked the activation capacity of immobilized NF795gpC upon the exposure to an anti-peptide antibody. In figure 1 it is compared the allosteric activation of the immobilized NF795gpC beta-galactosidase and its soluble version. Clearly, the protein maintains its allosteric activation properties even in its immobilized form, although with a differential, and slightly delayed profile regarding the soluble form.

Conclusion

NF795gpC is responsive to the allosteric modification mediated by anti-peptide antibodies even when immobilized in an agarose support, proving that the conformational modifications induced by the adaptive binding and supporting activation do not require the protein in solution. The different activation kinetics observed in soluble and immobilized enzyme versions could be due to either structural constraints to the active site conformational modulation or to a differential accessibility of the antigenic peptide (the allosteric receptor) to activating antibodies. The obtained results are promising regarding the possible use of allosteric biosensors in solid-phase platforms.

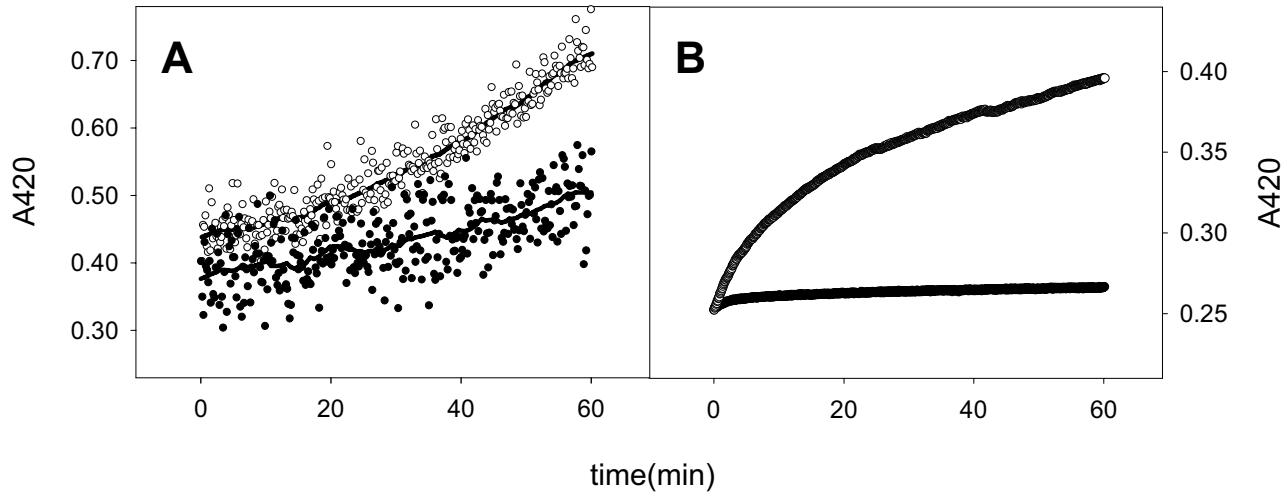

Figure 1
Coloured product of the ONPG hydrolysis formed by immobilized (**A**) and soluble (**B**) beta-galactosidase NF795gpC in presence (❍) and in absence (●) of an anti-peptide antibody.

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