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Abstract

A series of T7-promoter based bicistronic expression vectors was constructed in order to produce the comple sp. PCC 7120 DNA/RNA non-specific nuclease NucA and its inhibitor NuiA. With all constructs, tandem e and *nuiA* results in aggregation and inclusion body formation of NucA, independent of the order of the expression of the two proteins and the temperature applied during expression. Two constructs in which *nuiA* is the second cistron lead to an approximately one order of magnitude higher expression of *nuiA* compared with inclusion bodies are formed which contain NucA and NuiA in a 1:1 molar ratio. The complex can be solubili after disruption of the cells by sonication, renatured by dialysis and purified to homogeneity. 2 mg of the cor from 1 l *Escherichia coli* culture. As shown by gel filtration and analytical ultracentrifugation, our system lead and homogeneous complex preparation, as required for biophysical and structural studies. Thus, our new me alternative for the production of the NucA/NuiA complex in which separately produced nuclease and inhibit an excess of one or the other component, as well as aggregates of NucA, have to be removed from the pr

Keywords: Complex formation; Copurification; Tandem expression

1. Introduction

The *nucA* and *nuiA* genes from *Anabaena* sp. PCC 7120 code for a sugar non-specific nuclease and its inhibitor (Muro-Pastor et al., 1992, 1994, 1997). NucA belongs to the *Serratia* nuclease family of nucleases, that are well conserved among a variet and eukaryotic organisms, including hu et al., 1999a; Meiss et al., 1999). In con number of homologues of these nuclease of NuiA could be identified in the da The core of the catalytic centre of this c consisting of a histidine and an asp which was first identified and character nuclease (Friedhoff et al., 1994, 1996 1996; Miller et al., 1994, 1999), was a present in the homing endonuclease I– tative of the Cys–His box family of ho ases (Flick et al., 1998; Friedhoff et similar fold characterizes the Coli (Kühlmann et al., 1999) and T4 endo V et al., 1999). These nucleases are there the superfamily of His–Me finger (http://scop.mrc-lmb.cam.ac.uk/scop).

Abbreviations: *bla*, β-lactamase gene; CD, circular dichroism; DFF45, DNA fragmentation factor 45 kDa subunit; GST, glutathione-S-transferase; NTA, nitrilo-triacetic-acid; *nucA*, gene coding for *Anabaena* sp. PCC 7120 DNA/RNA non-specific nuclease NucA; *nuiA*, gene coding for the inhibitor of NucA, NuiA; ORF, open reading frame; λP_L, phage λ leftward directed promoter; *tcR*, tetracycline resistance conferring gene; Tn3*Amp*^{R}, transposon 3 ampicillin resistance conferring gene.

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