

Abstract

A series of T7-promoter based bicistronic expression vectors was constructed in order to produce the complex of NucA and NuiA from *Anabaena* sp. PCC 7120 DNA/RNA non-specific nuclease NucA and its inhibitor NuiA. With all constructs, tandem expression of the two genes 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 the first construct. Inclusion bodies are formed which contain NucA and NuiA in a 1:1 molar ratio. The complex can be solubilized after disruption of the cells by sonication, renatured by dialysis and purified to homogeneity. 2 mg of the complex can be obtained from 1 l *Escherichia coli* culture. As shown by gel filtration and analytical ultracentrifugation, our system leads to a homogeneous and homogeneous complex preparation, as required for biophysical and structural studies. Thus, our new method is an alternative for the production of the NucA/NuiA complex in which separately produced nuclease and inhibitor are mixed, an excess of one or the other component, as well as aggregates of NucA, have to be removed from the preparation. © 2000 Elsevier Science B.V. All rights reserved.

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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 variety of prokaryotic and eukaryotic organisms, including humans (Muro-Pastor et al., 1999a; Meiss et al., 1999). In contrast to the large number of homologues of these nucleases, only one homologue of NuiA could be identified in the database. The core of the catalytic centre of this complex is consisting of a histidine and an aspartate residue, which was first identified and characterized in the nuclease (Friedhoff et al., 1994, 1996, 1999; Miller et al., 1994, 1999), was also present in the homing endonuclease I–II, a representative of the Cys–His box family of homing endonucleases (Flick et al., 1998; Friedhoff et al., 1999). A similar fold characterizes the ColE1 endonuclease (Kühlmann et al., 1999) and T4 endonuclease V (Flick et al., 1999). These nucleases are therefore members of the superfamily of His–Me finger nucleases (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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