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Genetic engineering of *Escherichia coli* to produce a 1:1 complex of the *Anabaena* sp. PCC 7120 nuclease NucA and its inhibitor NuiA

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Abstract

A series of T7-promoter based bicistronic expression vectors was constructed in order to produce the complex of the *Anabaena* sp. PCC 7120 DNA/RNA non-specific nuclease NucA and its inhibitor NuiA. With all constructs, tandem expression of *nucA* and *nuiA* results in aggregation and inclusion body formation of NucA, independent of the order of the genes, the relative expression of the two proteins and the temperature applied during expression. Two constructs in which *nuiA* is the first and *nucA* the second cistron lead to an approximately one order of magnitude higher expression of *nuiA* compared with *nucA*. In these cells inclusion bodies are formed which contain NucA and NuiA in a 1:1 molar ratio. The complex can be solubilized with 6 M urea after disruption of the cells by sonication, renatured by dialysis and purified to homogeneity. 2 mg of the complex are obtained from 1 l *Escherichia coli* culture. As shown by gel filtration and analytical ultracentrifugation, our system leads to a highly pure and homogeneous complex preparation, as required for biophysical and structural studies. Thus, our new method is a superior alternative for the production of the NucA/NuiA complex in which separately produced nuclease and inhibitor are mixed, and 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.

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 variety of prokaryotic and eukaryotic organisms, including humans (Friedhoff et al., 1999a; Meiss et al., 1999). In contrast to the large number of homologues of these nucleases, no homologue of NuiA could be identified in the databanks to date. The core of the catalytic centre of this class of nucleases, consisting of a histidine and an asparagine residue, which was first identified and characterized for *Serratia* nuclease (Friedhoff et al., 1994, 1996; Kolmes et al., 1996; Miller et al., 1994, 1999), was also found to be present in the homing endonuclease I–*Ppo*I, a representative of the Cys–His box family of homing endonucleases (Flick et al., 1998; Friedhoff et al., 1999b). A similar fold characterizes the Colicin E9 DNase (Kühlmann et al., 1999) and T4 endo VII (Raaijmakers et al., 1999). These nucleases are therefore classified as the superfamily of His–Me finger endonucleases (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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The enzymes of the *Serratia* nuclease family fulfil a variety of different cellular functions. Those derived from eukaryotes are involved in mitochondrial DNA replication (Ruiz-Carrillo and Cote, 1993) and DNA repair (Dake et al., 1988), whereas those derived from prokaryotes like *Serratia* nuclease (Benedik and Strych, 1998) and NucA are secreted and probably serve nutritional purposes (Baliaeva et al., 1976). EndA from *Streptococcus pneumoniae* was shown to be necessary for efficient genetic transformation of this bacterium (Puyet et al., 1990).

Because of its stability and high catalytic activity, the *Serratia* nuclease, commercially available as Benzonase® (Merck), is used as a tool in industrial biotechnology for the removal of nucleic acids in the downstream processing of pharmaceutical products. Furthermore, attempts are being made to use a *Serratia* nuclease–*gag* fusion protein for the purposes of gene therapy (Schumann et al., 1997).

NucA was first cloned and expressed in *Escherichia coli* on a low copy number plasmid, pCSAM28, under control of its own promoter (Muro-Pastor et al., 1992). The nuclease could be isolated from the supernatant of *E. coli* cultures of cells bearing this plasmid. When the nuclease gene was cloned into a high copy number plasmid, as from the pUC series, no viable clones were obtained due to the toxicity of intracellularly active nuclease (Muro-Pastor et al., 1992). In contrast to NucA, recombinant NuiA could be overexpressed in *E. coli* and purified in high amounts as a soluble active protein (Muro-Pastor et al., 1997). We have previously established an overexpression system for NucA and NuiA (Meiss et al., 1998). NuiA could be expressed as a soluble N-terminally His_6-tagged protein at levels of 30 mg/l *E. coli* culture using a λP_L-promoter construct, whereas NucA was only obtained at low levels (0.4 mg/l *E. coli* culture) when using the same construct. When the *nucA* ORF was inserted into a high copy number T7-promoter based expression vector, pGMC10H6 (Martin and Keller, 1996), no viable clones were obtained after transformation, even in those *E. coli* strains that lack the T7-RNA polymerase gene 1. However, when the *nuiA* ORF was incorporated into the same plasmid as a second cistron downstream of the *bla* gene under control of the constitutive Tn3*Amp*^{R} promoter, NucA could be obtained from inclusion bodies as an N-terminally His_6-tagged protein at high levels (90 mg/l *E. coli* culture) (Meiss et al., 1998). Recombinant NucA purified by Ni^{2+} affinity chromatography is highly active, exhibiting a specific activity of 2.3×10^6 Kunitz units/mg (Meiss et al., 1998). However, as revealed by gel filtration and ultracentrifugation experiments, this NucA solution contains a major fraction of soluble but inactive aggregates and only a minor fraction of monomeric active nuclease. When this monomeric nuclease fraction was separated from aggregates by gel filtration, the specific activity of the enzyme preparation increased to as much as 8.4×10^6 Kunitz units/mg. The isolated monomeric nuclease tends to form new aggregates and is only stable at 4°C for a short period of time. NucA and NuiA can be mixed to produce the NucA/NuiA complex. However, to obtain the complex in homogeneous form, as required for structural studies, nuclease aggregates and excess NucA or NuiA have to be removed by subsequent gel filtration. To facilitate the production of the NucA/NuiA complex, we have now developed a tandem expression system for NucA and NuiA with which the complex can be isolated from inclusion bodies, renatured and purified to homogeneity by a one-step Ni^{2+} NTA affinity chromatography at levels of approximately 2 mg/l *E. coli* culture without the need for further purification by gel filtration. The NucA/NuiA complex thus obtained is stable and suitable for structural studies.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all chemicals were obtained from Merck. Components for culture media were from GIBCO/BRL. Oligonucleotides used as primers in the PCR were from Interactiva. Restriction endonucleases were from AGS/Hybaid, *Taq* DNA polymerase as well as T4 DNA ligase from Roche Molecular Biochemicals. All enzymes were used according to the respective manufacturer's recommendations.

2.2. Plasmids and bacterial strains

Plasmid pGMC10H6 (Martin and Keller, 1996), a derivative of pET3d, was a kind gift of G. Martin (Basel). Plasmid pBB, a derivative of pGMC10H6, is described by Meiss et al. (1998). *E. coli* strain XL1BlueMRF$'$ {Δ(*mcrA*)183 Δ(*mcrBC–hsdSMR–mrr*) *173 endA*1 *supE*44 *thi*-1 *recA*1 *gyrA*96 *relA*1 *lac*[F$'$ *proAB lacI*^{q}*ZΔM15*Tn*10*(Tet^R)]} (Stratagene) was used as general host in the cloning procedures. *E. coli* BL21Gold(DE3) [F$^-$ *ompT hsdS*(r_B–m_B–)*dcm*$^+$ Tet^R *gal*(DE3)*endA Hte*] (Stratagene) was used for the expression of genes under control of the T7ϕ10-promoter. Bacteria were routinely cultured in LB broth at 37°C. Ampicillin (150 μg/ml) and tetracycline (10 μg/ml) were added to the culture medium when appropriate.

2.3. Expression and purification of NucA and NuiA

Recombinant NucA and NuiA were produced as His_6-tagged proteins in *E. coli* as described in detail before (Meiss et al., 1998).

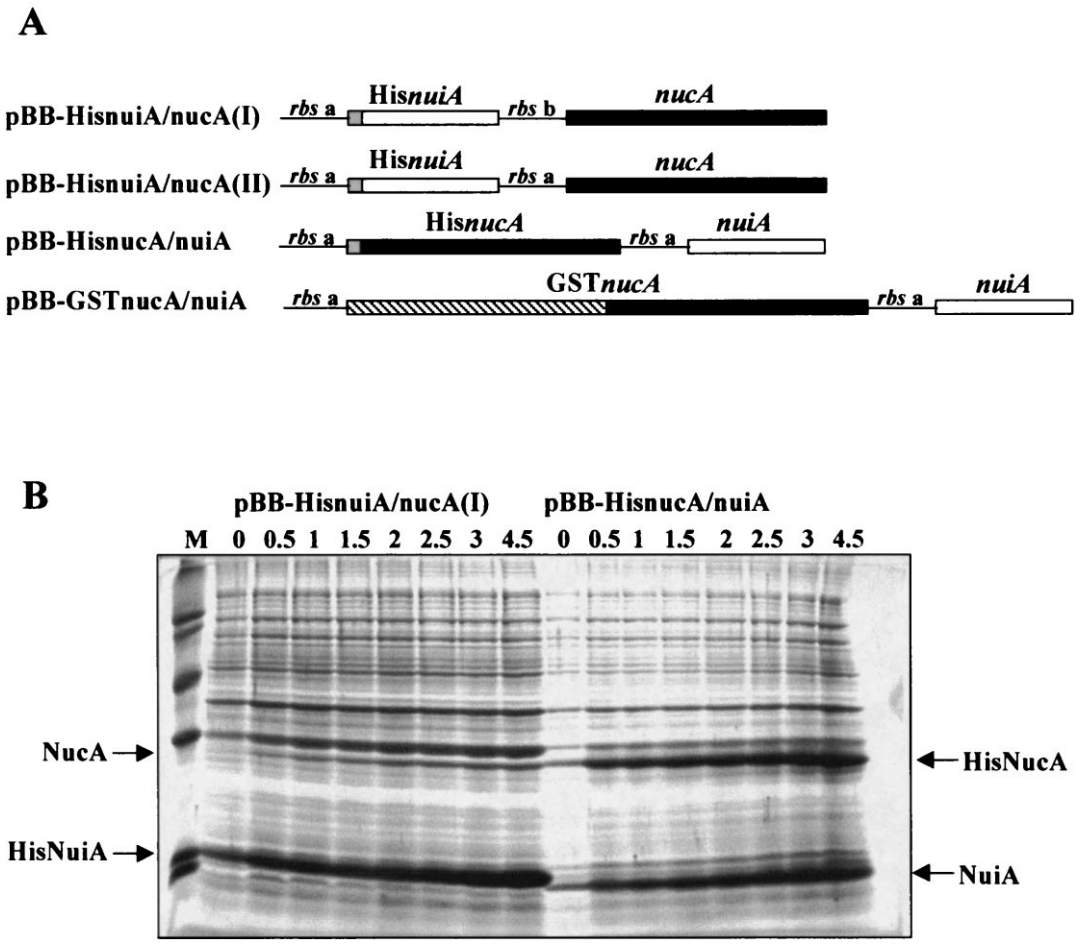

Fig. 1. Kinetics of the coexpression of NucA and NuiA using different bicistronic expression vectors. (**A**) Four vector constructs with different relative locations of the *nucA* and *nuiA* genes, ribosomal binding sites and coding for different affinity tags were used: rbs *a* (AAGAAGGAGATATACCATGG); rbs *b* (AGGAATTAAAGCATGC); His_6 tag (MAHHHHHHM); GST, glutathione-S-transferase. (**B**) SDS-PAGE of whole cell lysates of *E. coli* BL21Gold(DE3) cells transformed with the indicated expression vectors prior to induction (0) and at different time intervals (0.5–4.5 h) after induction with IPTG (1 mM final concentration).

2.4. Construction of bicistronic expression vectors for tandem expression of the nucA and nuiA genes

A series of bicistronic expression vectors (Fig. 1A) was produced, based on a derivative of pBB-DFF45 (Meiss, unpublished results) carrying the gene for the DNA fragmentation factor 45 kDa subunit (Liu et al., 1997). For this purpose, the *nuiA* gene was inserted into the *Nde*I and *Bgl*II sites of pBB-DFF45 after amplification by PCR using primers introducing the respective restriction sites. The resulting plasmid, pBB-HisnuiA, was then used to insert the *nucA* gene downstream of *nuiA* between the *Bgl*II and *Nhe*I sites of the vector, creating a second ribosomal binding site upstream of *nucA* introduced together with the *Bgl*II site and an additional *Sph*I site by the forward primer used. In pBB-HisnuiA/nucA(I), the newly introduced ribosomal binding site resembles that of the *tc*R gene, whereas in pBB-HisnuiA/nucA(II) it resembles that from *nuiA* provided by the vector (see Fig. 1 caption). In pBB-rbsnuiA, the *nuiA* gene was inserted into the *Bgl*II and *Nhe*I sites of pBB-DFF45 such that an *Sph*I site and a ribosomal binding site were created upstream of *nuiA* resembling that provided by the vector. This plasmid was then used to insert the *nucA* gene into the *Nde*I and *Bgl*II sites of pBB-rbsnuiA, resulting in pBB-HisnucA/nuiA. To obtain pBB-GSTnucA/nuiA the GST ORF was amplified by PCR from pGEX2T (Pharmacia) and inserted into the *Nco*I and *Nde*I sites of pBB-HisnucA/nuiA, replacing the coding region for the His_6 affinity tag. To obtain untagged NucA the coding region for the His_6 tag of plasmid pBBI-nucA (Meiss et al., 1998) was eliminated by inverse PCR.

2.5. Expression and purification of the NucA/NuiA complex

For the production of the NucA/NuiA complex by tandem overexpression of the *nucA* and *nuiA* genes, *E. coli* BL21Gold(DE3) cells were transformed with the

respective plasmids. Proteins produced with a His_6 affinity tag at the N-terminus were purified by Ni^{2+} NTA affinity chromatography. A 500 ml culture of transformed *E. coli* BL21Gold(DE3) cells was grown until an OD^{600} of 0.5 was reached and expression induced by the addition of IPTG to a final concentration of 1 mM. After 2 h of induction, cells were harvested and resuspended in 10 ml STE buffer (10 mM Tris–HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA). After centrifugation, the pellet was resuspended in buffer *A* (10 mM Tris–HCl, pH 8.2) and sonicated using a Branson sonifier. The suspension was centrifuged and the presence of NucA and NuiA in the supernatant as well as in the solubilized pellet checked by SDS-PAGE. Solutions containing native proteins were applied directly to 1 ml of a Ni^{2+} NTA resin, whereas denatured proteins from the solubilized pellets, using buffer *A* containing 6 M urea, were first dialysed against buffer *A* and then applied to the column. The column was washed with 20 ml buffer *A* containing 20 mM imidazole and then with buffer *A* containing 500 mM NaCl. Elution was performed using buffer *A* supplemented with 200 mM imidazole, and eluted proteins were dialysed against buffer *A* containing 20% glycerol and 0.01% lubrol. The variant carrying a GST-*tag* at the N-terminus was purified on 1 ml glutathione-sepharose columns using 50 ml 10 mM Tris–HCl, pH 8.2, 100 mM NaCl as the washing buffer and 10 ml 10 mM Tris–HCl, pH 8.2, containing 0.062 g reduced glutathione as the elution buffer. The concentrations of the enzymes were determined by UV spectroscopy using extinction coefficients calculated according to Pace et al. (1995): 51 910 $M^{-1}\,cm^{-1}$ for NucA and 16 960 $M^{-1}\,cm^{-1}$ for NuiA.

2.6. Size-exclusion chromatography

Size-exclusion chromatography on a Superdex-75 HR10-30 column (Pharmacia) equilibrated with 10 mM Na phosphate, pH 7.5, 500 mM NaCl was used to examine complex formation between NucA and NuiA. The flow rate was 0.5 ml/min. Elution was monitored at 220 nm.

2.7. Analytical ultracentrifugation

Ultracentrifugation was performed in a Beckman XL-A analytical ultracentrifuge equipped with absorption scanner optics using the An-60 four-place rotor with double sector charcoal filled epon centre pieces. Sedimentation velocity was measured at 50 000 rpm using 400 µl of solutions containing NucA, NuiA and the complex, respectively, at an A^{280} of 0.4 in a buffer consisting of 20 mM Tris–HCl, pH 8.2, 0.01% (w/v) lubrol. Scans were taken at appropriate time intervals and the sedimentation constant was determined by simulating the position and shape of the moving boundary using numeric integration of Lamm's differential equation (Lamm, 1929).

2.8. Circular dichroism spectroscopy

CD spectra were recorded on a Jasco J-710 dichrograph between 250 and 185 nm at 16°C in a cylindrical cuvette of 0.05 cm path length at protein concentrations of 3.5 µM in a buffer consisting of 20 mM Tris–HCl, pH 8.2, 0.01% (w/v) lubrol. Concentrations of the proteins were determined by UV spectroscopy before recording the CD spectra (see above).

3. Results

3.1. Kinetics of NucA and NuiA expression from different bicistronic vectors

In order to optimise the coexpression of NucA and NuiA we have generated different bicistronic constructs (Fig. 1A). NucA and NuiA could be coexpressed using these constructs (Fig. 1B). When the order of the genes was such that *nuiA* was the first and *nucA* the second cistron, induction of expression led to production of an approximately one order of magnitude higher amount of the inhibitor than of the nuclease (Fig. 1B). This was independent of the nature of the engineered ribosomal binding site used for *nucA*, as no significant difference in the expression level of *nucA* was observed whether pBB-HisnuiA/nucA(I) or (II) was used, respectively (data not shown). However, when *nucA* was the first and *nuiA* the second cistron, equal amounts of both proteins were produced (Fig. 1B). It must be emphasized here that the higher expression level of *nucA* when it is first in the bicistronic arrangement is probably based on polarity effects due to its location, rather than on the presence or absence of a His tag, as constructs expressing *nucA* alone do not differ in their expression levels when non-tagged or His-tagged NucA, respectively, is produced (Gast, unpublished results).

3.2. Solubility and intracellular localization of NucA and NuiA

To investigate whether NucA and NuiA produced from bicistronic constructs, as generated in this work, are predominantly found in the soluble fraction or in inclusion bodies, cells were disrupted by sonication and after centrifugation, fractions from the supernatant as well as from urea extracts of the insoluble material were subjected to SDS-PAGE (Fig. 2A). In all cases of coexpression from the constructs investigated here, NucA was found to be insoluble, even when NuiA was produced in higher amounts and independent of the pres-

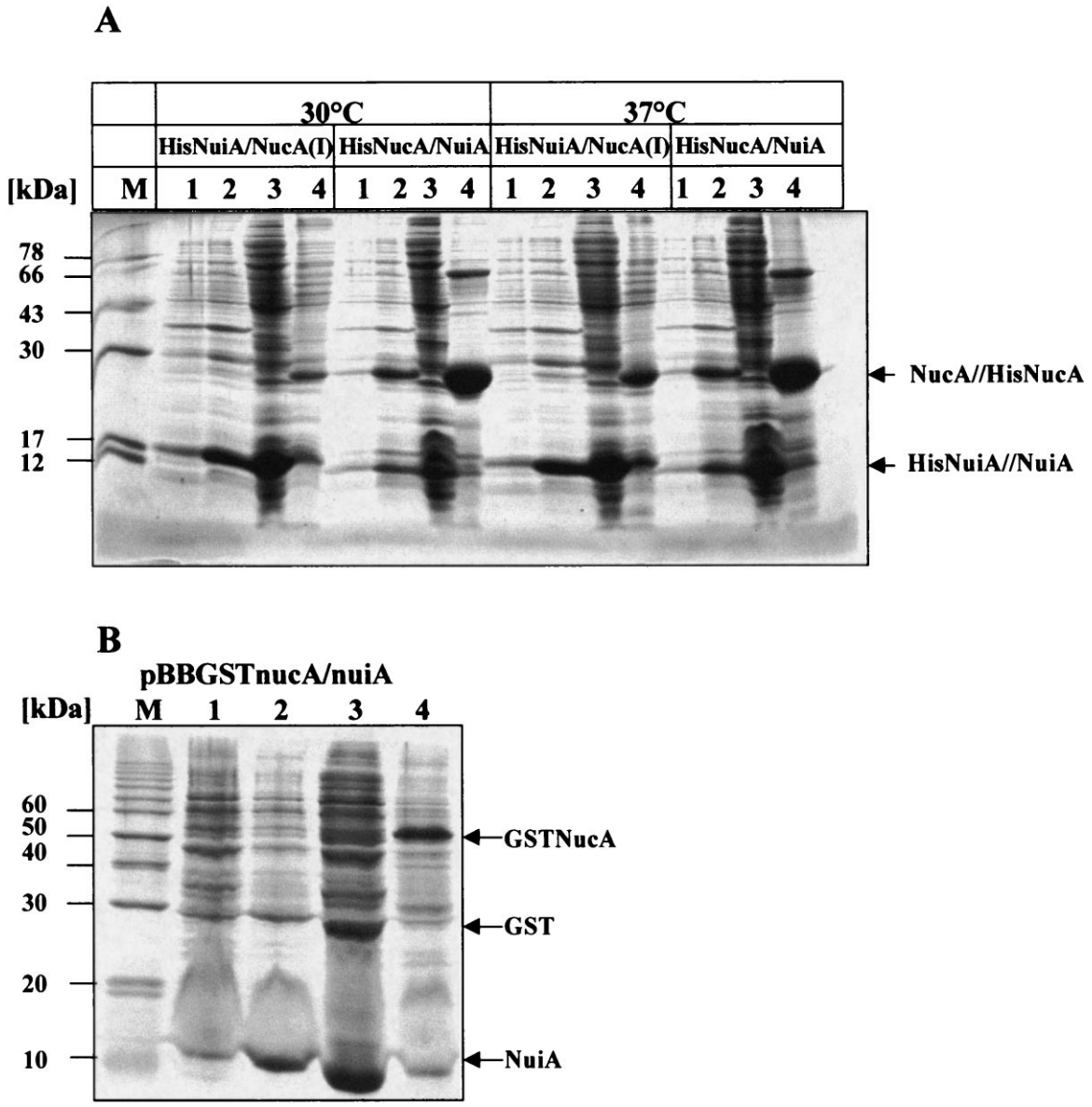

Fig. 2. SDS-PAGE to analyse inclusion body formation of recombinant NucA and NuiA coexpressed using different bicistronic expression vectors. (**A**) Comparison of inclusion body formation with NucA and NuiA, arising from overexpression of pBB-HisnuiA/nucA(I) and pBB-HisnucA/nuiA at 30 and 37°C, respectively. (**B**) Inclusion body formation of GSTNucA overexpressed from pBB-GSTnucA/nuiA. M, marker; 1, whole cell lysate prior to induction; 2, whole cell lysate after induction; 3, soluble protein fraction; 4, insoluble protein extracted from inclusion bodies.

ence or absence of a His_6 tag at the N-terminus of the proteins. In contrast, the major fraction of NuiA was produced as a soluble protein. The formation of inclusion bodies from NucA was not affected by the temperature applied during induction of gene expression, as NucA was found to be insoluble regardless of whether *E. coli* cultures were grown at 37 or 30°C during expression (Fig. 2A). The inclusion bodies formed during expression from pBB-HisnuiA/nucA(I) and (II) also contained NuiA, which is normally produced as a soluble protein, even when expressed in high amounts at elevated temperatures (42°C in the λP_L-promoter expression system) (Meiss et al., 1998).

3.3. *Influence of GST on the solubility of NucA*

In an attempt to improve the solubility of NucA, a GST tag was fused to the N-terminus of the nuclease (pBB-GSTnucA/nuiA). GST fusion proteins have often been shown to be more soluble than the target proteins themselves, and can easily be purified by affinity chromatography. However, the GST–NucA fusion protein produced upon overexpression in *E. coli* was found to be insoluble and to form inclusion bodies. Furthermore, SDS-PAGE analysis showed that, besides the production of insoluble fusion protein, also high amounts of soluble GST was produced (Fig. 2B).

3.4. Purification of the NucA/NuiA complex

To purify the NucA/NuiA complex *E. coli* cells were transformed with plasmid pBB-HisnuiA/nucA(II), which provides two identical ribosomal binding sites for each cistron. The insoluble material obtained after lysis of *E. coli* cells containing pBB-HisnuiA/nucA(II) was used to extract His_6-NuiA and untagged NucA under denaturing conditions using 6 M urea. After dialysis the proteins were loaded onto a Ni^{2+} NTA column and copurified by Ni^{2+} affinity chromatography as described in the Materials and methods section (Fig. 3).

3.5. Gel filtration analysis of the NucA/NuiA complex

The NucA/NuiA complex, and for comparison NucA and NuiA, prepared as described previously (Meiss et al., 1998), were subjected to gel filtration as described in the Materials and methods section. The NucA preparation contained high molecular weight aggregates and monomeric nuclease, while the inhibitor preparation showed a single peak in the chromatogram, representing NuiA. In the NucA/NuiA complex preparation, the complex was the only species with an apparent molecular weight of approximately 42 kDa, as determined from a plot of V_0/V_e vs. log M_r of standard proteins. In contrast to the NucA preparation, no aggregates of NucA were found in the complex preparation (Fig. 4).

3.6. Ultracentrifugation analysis of the NucA/NuiA complex

Sedimentation velocity runs were performed in the analytical ultracentrifuge in order to determine the sedimentation coefficients for the NucA/NuiA complex and for comparison also for NuiA from the slope of a ln(radius) vs. time plot (Fig. 5). Sedimentation coefficients of 1.79 and 3.49 S were found for NuiA and the NucA/NuiA complex, respectively. No aggregates were present in these preparations, in contrast to typical NucA preparations (data not shown).

3.7. Circular dichroism spectroscopic analysis of the NucA/NuiA complex

In order to structurally characterize the complex produced by the purification procedure described above, CD spectra of the purified complex as well as NucA and NuiA produced as single proteins were recorded (Fig. 6). In contrast to the results obtained in an earlier study, in which the NucA/NuiA complex was produced by mixing equimolar amounts of purified NucA and NuiA, the CD spectrum of the complex copurified as described here shows a significant difference compared with the sum of the CD spectra of NucA and NuiA. This is indicative of a structural rearrangement which leads to a change in secondary structure composition of one or both proteins upon formation of the NucA/NuiA complex.

4. Discussion

In order to study the biochemical and biophysical properties of a protein it is of great advantage to have an overproducer for the protein to be studied. In the case of highly active nucleases it has often been difficult

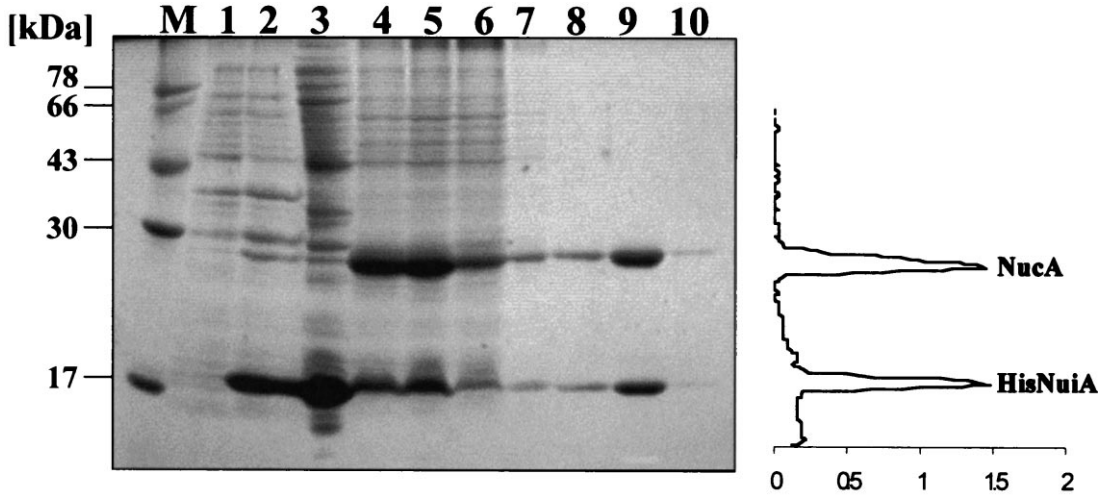

Fig. 3. SDS-PAGE to monitor the coexpression and purification of the NucA/NuiA complex. M, marker; 1, whole cell lysate of *E. coli* BL21Gold(DE3) cells transformed with pBB-HisnuiA/nucA(II), prior to induction; 2, after induction; 3, soluble protein fraction obtained after cell disruption; 4, proteins extracted from inclusion bodies; 5, proteins renatured from 4 by dialysis; 6, flow-through of a Ni^{2+} NTA affinity column; 7, wash; 8, 9, 10, eluates. The diagram on the right shows a scan of lane 9 of the Coommassie-stained SDS-PA gel containing a major part of the copurified NucA/NuiA complex.

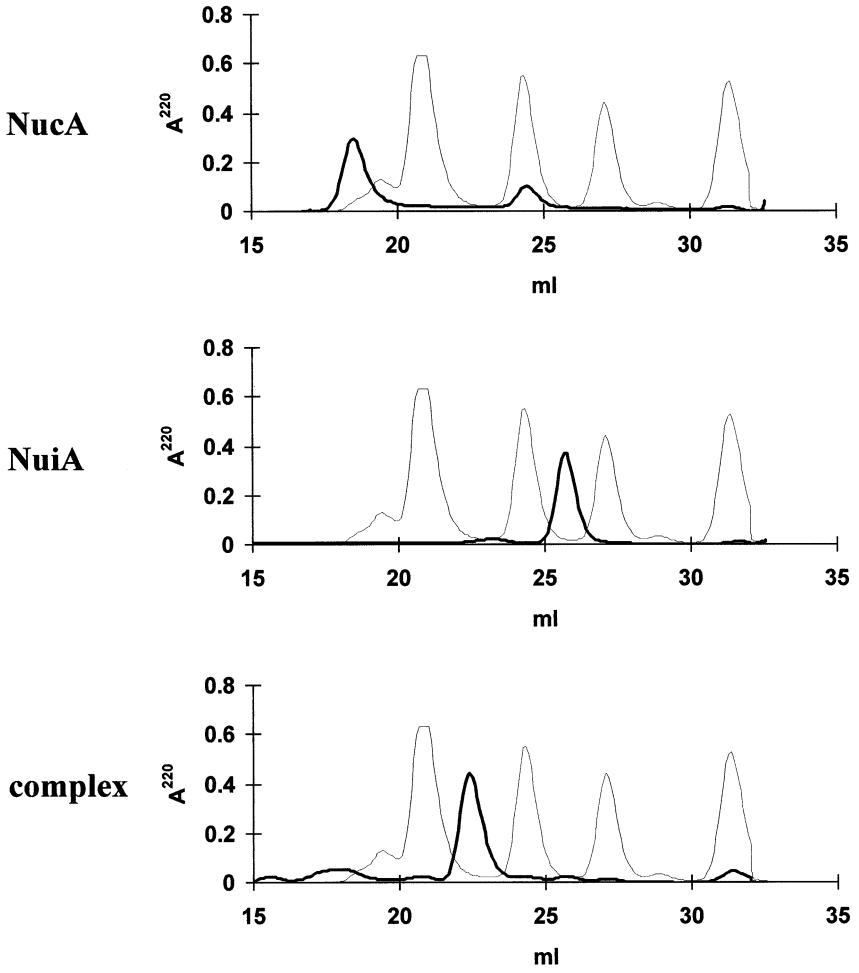

Fig. 4. Gel filtration of the NucA/NuiA complex in comparison with NucA and NuiA. The following proteins were used as standard (faint lines): bovine serum albumine (M_r=66 kDa), carbonic anhydrase (M_r=29 kDa), cytochrome c (M_r=12 kDa) and aprotinin (M_r=6 kDa). The numbers at the peaks indicate the respective molecular weight of the eluting protein (kDa). While the NucA preparation contains high molecular weight aggregates in addition to monomeric NucA, the NuiA and NucA/NuiA complex preparation contain only one molecular species (bold lines).

to express the enzyme in large amounts, as these proteins, if intracellularly active, cause severe damage to the genetic material which is followed by cell death. It is obvious, therefore, that the heterologous expression, especially of non-specific nucleases, must be tightly controlled intracellularly in order to avoid damage to the cell's genetic material. One way of doing this is to coexpress a specific inhibitor. With barnase, an RNase from *Bacillus amyloliquefaciens*, coexpression of the inhibitor barstar allowed for the production of the nuclease in very large amounts (Hartley, 1988). Similarly, for overexpression of the *E. coli* colicin E9 DNase (Kleanthous et al., 1998), the naturally occurring bicistronic arrangement of E9 and its inhibitor Im9 (immunoprotein 9), was put under control of a T7-promoter. With this construct the E9/Im9 complex could be expressed in large amounts (Garinot-Schneider et al., 1996). Our new procedure for the production of the NucA/NuiA complex also uses bicistronic constructs leading to 2 mg complex per litre *E. coli* culture.

The purification scheme established consists of the coexpression of NucA and NuiA from pBB-HisnuiA/nucA(I) or (II), respectively, coextraction of the proteins from inclusion bodies as well as their corenaturation followed by Ni^{2+} NTA affinity chromatography. This procedure leads to a pure homogeneous recombinant complex as analysed by gel filtration and analytical ultracentrifugation experiments. It is remarkable that NucA and NuiA, when produced from pBB-HisnuiA/nucA, are both deposited in inclusion bodies, although NuiA is soluble when expressed alone (Meiss et al., 1998). This indicates that the NucA/NuiA complex is formed very rapidly after translation, then aggregates and precipitates. It is also remarkable that this process is reversible and that the NucA/NuiA complex can be extracted by treatment with denaturing agents

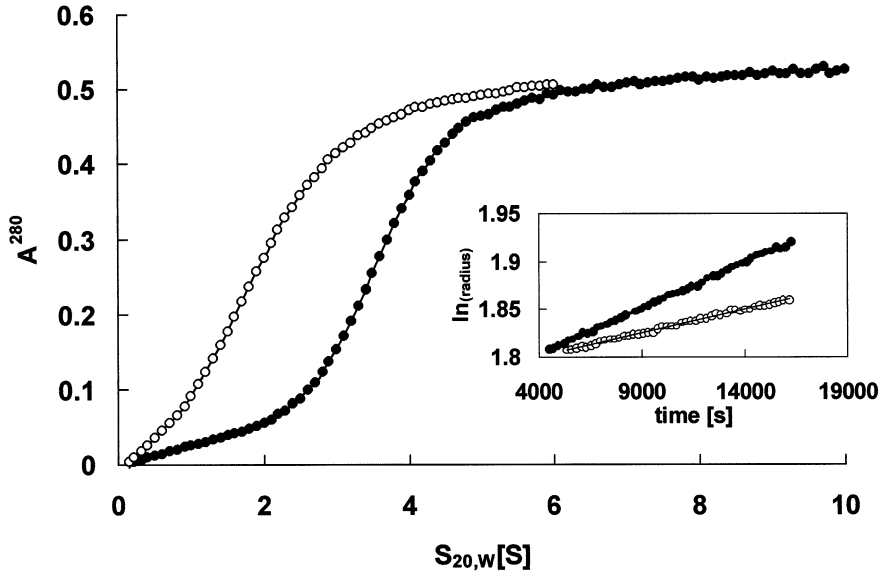

Fig. 5. Analytical ultracentrifugation of the NucA/NuiA complex. Purified NucA/NuiA complex (●) as well as NuiA (○) was sedimented at 50 000 rpm in an An-60 four-place rotor. $S_{20,w}$ value distribution and ln(radius) vs. time plot (insert), used to calculate the sedimentation coefficient, are shown. NuiA and the NucA/NuiA complex sediment as a single species.

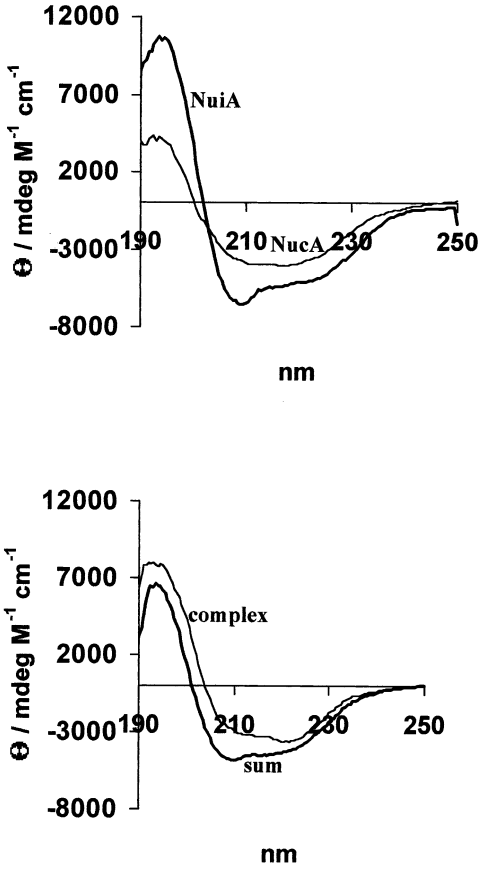

Fig. 6. CD spectra of the NucA/NuiA complex in comparison with free NucA and NuiA. The sum of the spectra of separately purified and individually analysed NucA and NuiA differs from the spectrum of the NucA/NuiA complex.

like urea, and renatured by dialysis. It seems that in the process of renaturation of the coextracted proteins free NucA does not accumulate, as no soluble aggregates of NucA can be detected after dialysis, which are always present when NucA is overproduced alone (Meiss et al., 1998), and also, when the isolated proteins are separately denatured, mixed together and renatured by dialysis (data not shown). Coextraction of NucA and NuiA from inclusion bodies and corenaturation, therefore, seems to prevent aberrant folding and aggregation of NucA. Thus, the production of pure complex using pBB-HisnucA/nuiA constructs is superior to the alternative procedure of mixing separately produced NucA and NuiA, which would require additional purification steps. Pure complex is required for biophysical studies, for example a CD spectroscopic analysis as presented here, which showed that the complex differs in secondary structure composition from the sum of its components, NucA and NuiA.

The success of our procedure for the purification of the NucA/NuiA complex relies on the unexpected 'coprecipitation' of NuiA and NucA into inclusion bodies upon overexpression. The tendency of NucA to aggregate and form inclusion bodies cannot be lowered by the presence of excess NuiA, or the fusion of a highly soluble protein, such as GST, to the N-terminus of the nuclease. Although NucA could not be obtained as a soluble protein in our new tandem expression systems, the fact that it binds in a 1:1 stoichiometry to the inhibitor NuiA and together with NuiA forms inclusion bodies from which both proteins can be extracted, renatured and purified as an intact 1:1 complex is an

important step towards the structural analysis of the NucA/NuiA complex.

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