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Journal of Chromatography B, 786 (2003) 187–195
JOURNAL OF CHROMATOGRAPHY B
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Optimisation of expression and purification of the recombinant Yol066 (Rib2) protein from *Saccharomyces cerevisiae*

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Abstract

Yeast protein Yol066 (encoded by YOL066 ORF, also known as Rib2) possesses two distinct sequence domains: C-terminal deaminase domain and N-terminal part related to RNA:pseudouridine (ψ)-synthases. The deaminase domain is implicated in the riboflavine biosynthesis, while the exact function of the RNA:Ψ-synthase domain remains obscure. Here we report the optimisation of growth conditions and purification scheme for recombinant His_6-tagged Yol066 expressed in *E. coli* BL21(DE3) using pET28 plasmid. Production of soluble Yol066 protein is best at low temperature (18 °C) and IPTG concentration (50 μ*M*) and Yol066 purification was achieved using metal-affinity and ion-exchange chromatography. This optimised protocol yields about 10 mg of highly purified recombinant Yol066 from 3 l of *E. coli* culture.
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Keywords: Expression; Purification; *Saccharomyces cerevisiae*; Recombinant Yol066 protein

1. Introduction

Functional identification and characterisation of numerous open reading frames (ORFs) mapped in sequenced genomes represents one of the principal tasks of modern functional genomics. The sequence homology approach is frequently used for such identification, but unambiguous functional assignment is possible only for ~35–40% of unknown genes. In most cases a putative identification has to be followed by an experimental verification of gene and protein functions in vivo and in vitro.

In our work we focused our attention on the yeast ORF YOL066c. This ORF mapped at the left arm of yeast chromosome XV encodes a protein of 67 000 molecular mass which contains two distinct sequence domains. The C-terminal domain has a deaminase-like active site sequence signature and is suggested to perform the deamination reaction in the riboflavin biosynthesis pathway [1–5]. Recent analysis of *E. coli* and yeast riboflavin biosynthesis pathways further reinforced this conclusion (see Ref. [6]). In consequence, the protein, encoded by ORF YOL066c was reported as DRAP-deaminase Rib2. On the other hand, the N-terminal extremity of the same protein possesses several sequence signatures resembling the active site of known RNA:pseudouridine (Ψ)-synthases [7]. One of the closest homologues of the Rib2 (Yol066) N-terminal domain, yeast protein Pus6 (encoded by ORF YGR169c) indeed has a tRNA:Ψ-synthase activity [8]. At least four distinct families of RNA:Ψ-synthases are responsible for the U to Ψ conversion in tRNAs and rRNAs in bacteria and

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doi:10.1016/S1570-0232(02)00742-0

eukaryotes [7,9]. Several prokaryotic members of tRNA- and rRNA:Ψ-synthases families have been expressed in *E. coli* and have been well-characterised biochemically [10–14]. However, the expression in *E. coli* of eukaryotic RNA:Ψ-synthases and, in general, eukaryotic RNA:modification enzymes, is often complicated by problems related to inoptimal codon usage, limited level of expression and insolubility of obtained recombinant proteins. Among eukaryotic RNA:Ψ-synthases only yeast Pus1 (together with its mouse homologue mPus1) and Pus4 proteins were successfully expressed in *E. coli* with a high yield and in a soluble form [15–18]. The other reported attempts of heterologous expression (yeast Pus5 and Pus6 proteins expressed in *E. coli*) were not successful, due to accumulation of the recombinant product in inclusion bodies ([8,19] and unpublished observations). Detailed studies of yeast tRNA:Ψ-synthase Pus1 have already pointed out the importance of cell culture media composition in achieving the highest yield and optimal solubility of the product [15]. Another complication in such heterologous expression–purification procedures was the strong tendency of the protein to aggregate, which limits the final recovery level. Eukaryotic expression systems like methylotrophic yeast *P. pastoris* may be attractive alternatives to *E. coli*, but their practical use is much more laborious at the level of cloning and not as straightforward as prokaryotic expression in *E. coli*. Taking together these considerations, we decided to optimise the expression and purification protocols developed for putative RNA:Ψ-synthase/deaminase Yol066. The final aim was to produce sufficient amounts of protein required for detailed functional and physicochemical characterisation and, ultimately, crystallisation studies.

The efficiency of recombinant protein expression in bacterial host cells like *E. coli* depends on numerous parameters. Some of them are directly defined by the expression vector used for gene cloning (such as promoter and terminator signals, ribosome binding sequence efficiency and possibility of regulation by repressors, for review see [20,21]). Other important factors are linked to nucleotide and amino acid sequence of the expressed protein (appropriate codon usage, mRNA and protein stability). In most cases these parameters are predefined by the choice of the expression vector and protein sequence to be expressed. Thus, optimisation of protein expression may be performed by varying parameters affecting *E. coli* cell growth (media composition, temperature) and protein solubility (inducer's concentration, induction time). The other area of optimisation relates to developing of a rapid and efficient purification protocol to minimise the losses during purification.

Numerous bacterial expression systems are described in the literature and are commonly used in laboratory practice. Most of them rely on the use of a strong bacterial promoter (such as *lac*, *trp* or synthetic *tac* promoters) and the induction by IPTG (see [20] and Refs. therein). These systems are rather efficient for protein production but their regulation is not as tight as two-step induction systems based on phage T7 late promoters. Protein expression in such host-vector couples (*E. coli* BL21 (DE3) strains and pET-family plasmid) depends on a transcription unit which supplies the T7 RNA polymerase. In addition to the tight regulation and high level of protein expression achieved by pET-plasmid/*E. coli* BL21 (DE3), the availability of numerous versions of pET-derived expression plasmids facilitates the design of expression construct and allows the use of affinity tags for recombinant protein purification [22].

Another limitation in recombinant protein expression is related to differential usage of synonymous codons between *E. coli* and heterologous nucleotide sequences [23,24]. Indeed, arginine codons AGA and AGG are rare codons in *E. coli* while they are frequently used in many eukaryotic organisms [25]. This problem in recombinant protein expression is frequently solved by co-transformation of the producing strain by a plasmid bearing the corresponding rare tRNA gene or even by integration of such tRNA at the genome level [26,27].

Here we report the optimised protocol for expression in *E. coli* and purification of recombinant N-terminally His_6-tagged yeast protein Yol066. The expression was performed using pET28 plasmid as the expression vector and *E. coli* BL21 (DE3)RIL as the host strain. The optimisation was achieved by adaptation of media composition, temperature and inducer's concentration to increase the yield of soluble protein. The purification protocol based on a nickel–nitrilotriacetic acid (Ni^{2+}–NTA) agarose af-

finity column and ion-exchange chromatography steps is also described.

2. Experimental

2.1. Chemicals

Ni^{2+}–NTA agarose was from Qiagen (France), gel filtration molecular mass standards from BioRad (USA), restriction enzymes (*Nhe*I, *Sma*I, *Bam*HI), kanamycin and IPTG from Euromedex (France). All other chemicals were from Merck Biochemicals (Germany). Luria Broth (LB) and minimal M9 media were prepared according to standard procedures [28]. M9 medium was systematically complemented by 2% of casamino acids (Difco, UK).

2.2. Equipment

The following prepacked chromatographic columns and equipment were used: MonoQ HR 5/5, Resource Q 1 ml, Superose 12 HR 10/30, DEAE TSK 5 PW (8×75 mm) and the fast protein liquid chromatography (FPLC) medium-pressure chromatographic system (all from Pharmacia, Sweden). Protein gel electrophoresis was performed using Mini-Protean II and protein bands were quantified using a GelDoc 1000 CCD camera system and Molecular Analyst software package (BioRad, USA).

2.3. Strains and plasmids

Escherichia coli strain BL21-CodonPlus(DE3) RIL (*E. coli* B F^- *ompThsdS*(r_B^- m_B^-) *dcm*$^+$ Tet^r gal λ (DE3) *endA* Hte [*argUileYleuW* Cam^r] (Stratagene, USA) was used for expression of the recombinant Yol066 protein.

2.3.1. Preparation of pET28b–Yol066 expression vector

Gene encoding yeast protein Yol066 was amplified by polymerase chain reaction (PCR) using yeast genomic DNA and two DNA oligonucleotides, corresponding to the 5′-extremity (ATG-start codon) and to 3′-UTR, respectively, of the gene. The 5′-oligonucleotide had an *Nhe*I restriction site in frame with starting ATG of YOL066. Amplified blunt-ended PCR product was directly inserted in pUC18 cloning vector cut by *Sma*I restriction endonuclease. The desired orientation of the insert was found by restriction analysis and the complete insert sequence was confirmed by a dideoxy sequencing approach. This intermediate pUC18–Yol066 plasmid was used for further manipulation to construct the pET28–Yol066 expression vector. The pUC18–Yol066 plasmid was cut by *Nhe*I–*Bam*HI restriction enzymes (*Bam*HI site is located downstream to YOL066 stop codon) and the purified fragment was inserted to *Nhe*I/*Bam*HI-digested pET28b expression vector. The correct fragment integration was verified by restriction analysis and partial DNA sequencing.

2.4. Protein expression and cell disruption

E. coli cells bearing pET28b–Yol066 plasmid were grown in rich (LB) and in a minimal (M9) media supplemented by 100 μg/l of kanamycin (Euromedex, France). Preculture in 20 ml of LB medium was inoculated directly from glycerol stock at −80 °C and grown overnight at 37 °C. Then, this preculture was used to inoculate 600 ml LB or M9 media (about 1/40 of the final volume) in order to obtain an initial AU_{600} of 0.1. Cultures were grown at 37 °C with vigorous shaking (200 rpm) to AU_{600} of 0.7 (~2×10^8 cells/ml). Yol066 expression at three different temperatures (18 °C, 28 °C and 37 °C) and three different IPTG concentrations (50 μ*M*, 300 μ*M* and 1000 μ*M*) was studied in 100 ml aliquots. After 3 or 15 h of induction, cells were harvested by centrifugation at 4 °C for 25 min at 4000 *g*. The cells were washed with 5 vol of sterile water and once more pelleted in the same conditions.

Lysis of cells was done in 5 vol of lysis buffer (50 m*M* $NaPO_4$ buffer, pH 7.7, 300 m*M* NaCl, 10 m*M* imidazole) by 1 min sonication (30 cycles of 2 s and 2 s on ice between each pulse). Soluble (S10) and insoluble (P10) fractions were separated by centrifugation at 4 °C for 25 min at 16 000 *g*.

2.5. Monitoring the expression level by SDS–PAGE analysis

Amount of soluble Yol066 protein produced upon expression was estimated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–

PAGE). Electrophoresis was performed in the presence of SDS according to the method of Laemmli [29]. The discontinuous gel consisted of a 5% stacking gel and a 10% separating gel which was run on a vertical electrophoresis unit (Mini-Protean II, BioRad) at 200 V for 45 min. Aliquots of S10 extract (10 μl, about 50 μg of total protein) were loaded on the SDS–PAGE and protein bands were stained with Coomassie Blue G-250. The intensity of protein bands on SDS–PAGE was measured by densitometry using the GelDoc system and Molecular Analyst software package (BioRad). The final yield of Yol066 was calculated in arbitrary units taking into account the amount of soluble Yol066 in S10 and the cell culture density after induction. Although the volume of soluble S10 extract loaded on the gel was the same, the amount of protein varies depending on growth conditions. Therefore, we normalise the intensity of the Yol066 band to the signals of two other constitutive *E. coli* proteins. Cell culture density was monitored photometrically by reading the optical density at 600 nm.

2.6. Data analysis

SYSTAT software v. 10 was used for surface analysis of the experimental data. The quality of the fit to the non-linear model equation was expressed by the coefficient of correlation R^2 and the significance of regression coefficient was tested by a *t*-test.

2.7. Chromatographic procedures

2.7.1. Immobilised metal affinity chromatography (Ni^{2+}–NTA agarose)

For preparative purification of the recombinant Yol066 the *E. coli* culture was grown using the optimised expression conditions (LB medium, 18 °C, 50 μ*M* IPTG, 15 h of induction). Cells harvested from 3 l of culture (about 15 g of cells) were resuspended in 50 ml of the extraction buffer and lysed by sonication as described above. The soluble protein fraction (S10 cell extract) was batch-loaded onto 2 ml of Ni^{2+}–NTA agarose (Qiagen). The resin was previously washed with 3 vol of sterile water and equilibrated with 3 vol of the lysis buffer.

Adsorption was done for 30 min at 4 °C with constant mixing on a motorised wheel. After incubation the resin was transferred into a small column (10×100 mm) or left in batch for following wash and elution. The resin was washed with 5 vol of wash buffer (50 m*M* $NaPO_4$, pH 7.7, 300 m*M* NaCl, 20 m*M* imidazole). Elution of bound protein was performed using 3 ml of elution buffer containing imidazole at different concentrations (50 m*M* $NaPO_4$, pH 7.7, 300 m*M* NaCl, 100, 150, 200 or 250 m*M* imidazole).

Before performing the second purification step, concentrated protein fractions were pooled and subjected to overnight dialysis against 25 m*M* Tris–HCl, pH 7.5, 100 m*M* NaCl, 100 m*M* imidazole and 1 m*M* 2-mercaptoethanol to remove the excess of NaCl present in the elution buffer.

2.7.2. Ion-exchange chromatography

Three different chromatographic supports were tested for further purification of the recombinant Yol066 protein. Two columns had strong tertiary aminoethyl groups (MonoQ and Resource Q) and the third one was a weak anion-exchanger DEAE TSK. Dialysed protein fraction from Ni^{2+}–NTA column (200 μl, about 1 mg of Yol066 protein) was loaded onto a column equilibrated with buffer A (25 m*M* Tris–HCl, pH 7.5, 1 m*M* 2-mercaptoethanol) and chromatographied at a flow-rate of 1 ml/min using the Pharmacia FPLC system. The column was washed with 3 column vol of the same buffer, bound protein fraction was eluted with a linear NaCl gradient (0 –1 *M*) in 30 ml of buffer A (80 ml for DEAE–TSK column). The fractions containing Yol066 protein were pooled and dialysed against 25 m*M* Tris–HCl, pH 7.5, 100 m*M* NaCl, 2 m*M* DTT, 50% of glycerol and stored at −20 °C. The presence of Yol066 protein in eluted protein fractions was controlled by SDS–PAGE. Protein concentration in samples was determined by the Bradford dye binding method [30] using bovine serum albumin as the protein standard.

2.7.3. Gel filtration on a Superose 12 column

Native molecular mass determination was performed by gel filtration using a Superose 12 FPLC column. The column was equilibrated with buffer containing 25 m*M* Tris–HCl, pH 7.5, 150 m*M* NaCl and 1 m*M* 2-mercaptoethanol and elution was per-

formed at a flow-rate of 0.2 ml/min after injection of 1 mg of Ni^{2+}–NTA purified Yol066. For calibration, the following molecular mass standards (BioRad) were used: vitamin B12 (1350), horse myoglobin (17 500), chicken ovalbumin (44 000), beef α-globulin (158 000), and thyroglobulin (670 000). The void and total volumes of the column, 6.9 ml and 18.2 ml, respectively, were determined with blue dextran dye and potassium dichromate to enable calculation of the distribution coefficient K_e.

3. Results and discussion

3.1. Optimisation of expression

3.1.1. Strategy of optimisation

Preliminary tests of the Yol066 expression in *E. coli* strain BL21-CodonPlus(DE3) RIL indicated that the recombinant protein is well expressed during IPTG induction, but the majority (up to 60%) of the product is present in an insoluble form in cell extract (data not shown). Thus we first attempted the optimisation of protein production to gain in yield and solubility. The following parameters were chosen for optimisation: media composition (rich versus minimal media), inducer's (IPTG) concentration, temperature and expression time. Besides the choice of the expression system itself, these parameters generally have the most important impact on the yield of soluble protein upon heterologous expression. Earlier observations suggested that medium composition and growth temperature are important factors since they affect the ratio of soluble and insoluble enzyme forms upon expression (see for examples Refs. [31,32]). Most likely the influence of medium composition and temperature is related to modulation of the chaperones activity or some other accessory proteins involved in protein folding. The inducer's concentration and induction time also play a crucial role in protein production since low concentration may not be sufficient for full induction, whereas high concentration of the inducer may affect the growth of the cell, especially in the case of production of toxic proteins [33]. Moreover, excessive induction time may conduct to pronounced degradation of the expressed recombinant protein.

For optimal exploration of parameter's importance we decided to apply the incomplete factorial and response surface design. This approach is used when two or more factors are varied simultaneously and allows reducing very considerably the total number of experiments in comparison to full factorial design. Four parameters which might affect Yol066 yield (media composition, inducer's concentration, temperature and induction time) were chosen and tested at two or three levels using the matrix generated by the interactive software SAmBA [34]. This software helps to set up an optimal experimental design with a minimal number of tests required to explore all possible combinations of parameters. Out of 36 possible combinations 12 representative sets of parameters were tested. Selected parameter sets and results of total and soluble protein expression are presented in Table 1. The total and soluble protein expression was estimated by quantification of protein SDS gel electrophoresis pattern of whole *E. coli* cells and soluble S10 extract (Fig. 1, see also Experimental section). The yield of soluble protein was calculated in arbitrary units taking into account the cell culture density after expression and amount of Yol066 in S10 supernatant.

3.1.2. Global expression vs. solubility

The results presented in Fig. 1 and Table 1, show that the intracellular concentration of soluble Yol066 depends mainly on induction temperature and IPTG concentration. Slowing down the expression rate (low temperature and IPTG concentration, 18 °C and 50 μ*M* IPTG, respectively) favours the accumulation of the soluble product, while the use of high IPTG concentration (1 m*M*) and high temperatures (37 °C) diminishes the yield considerably. This is most probably related to saturation of endogenous *E. coli* chaperones during the massive expression of the foreign polypeptide. On the other hand, the limited solubility of the expressed product may be in part compensated by the accumulation of cell biomass. Taken together, these two factors determine optimal expression conditions. As shown in Table 1, the maximal expression of soluble Yol066 is achieved during long (15 h) induction at 18 °C, however, optimal concentration of IPTG varied in function of media used. The induction in minimal medium required relatively high IPTG concentration (1000

Table 1
Optimisation of Yol066 expression conditions

Experiment	Medium type	Growth temperature (°C)	IPTG concentration (μM)	Induction time (h)	Solubility criterion (Relative units)	Cellular density (AU_{600}/ml)	Final Yield (Arbitrary units)
1	M9	18	1000	15	2.90	1.45	4.21
2	M9	28	300	15	1.95	2.01	3.91
3	M9	18	300	3	1.87	0.92	1.72
4	M9	37	50	3	0.96	1.82	1.75
5	M9	28	50	15	1.76	2.31	4.06
6	M9	37	1000	3	0.72	1.68	1.21
7	LB	18	50	15	3.09	1.56	4.80
8	LB	37	300	15	1.22	2.34	2.85
9	LB	28	50	3	1.64	1.90	3.12
10	LB	28	1000	3	2.10	1.90	4.01
11	LB	37	1000	15	1.13	2.30	2.60
12	LB	18	300	3	1.72	1.37	2.35

μ*M*), while only low concentration (50 μ*M*) was sufficient for successful soluble expression in LB medium. The optimised expression protocol increased the yield of soluble Yol066 by at least four times (comparing to ''standard'' expression conditions, 1000 μ*M* of IPTG and induction at 37 °C for 3 h).

3.2. Purification method

3.2.1. Optimisation of Ni–NTA agarose chromatography

The presence of His_6-tag at the N-terminal extremity of the recombinant Yol066 protein greatly facilitates the development of appropriate purifica-

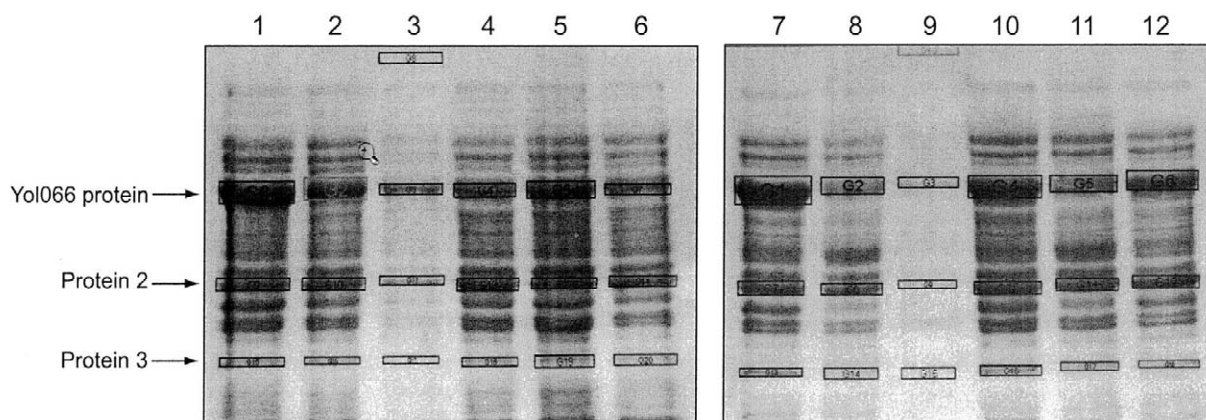

Fig. 1. Expression of soluble Yol066 in minimal (M9, left panel) and rich (LB, right panel) media. Expression was analysed by SDS–PAGE according to Laemmli. Total protein (~50 μg) from soluble fraction (S10) was loaded onto a 8% polyacrylamide gel (PAAG) and Coomassie Blue stained after separation. Lanes are labelled according to experiment number from Table 1. The intensity of protein bands was measured using the GelDoc 1000 image analysis System (BioRad). After deduction of background values, the intensity of signal for Yol066 protein was normalised to two other protein bands corresponding to constitutive *E. coli* proteins.

tion procedure. The first purification step for isolation of His_6-tagged proteins usually consists of column or batch adsorption on Ni^{2+}–NTA agarose followed by elution at a high imidazole concentration. While the whole purification procedure is quite straightforward, the yield and the purity of the eluted protein fraction depends on the ratio (total protein)/(matrix), and also adsorption, wash and elution conditions. In order to limit nonspecific protein adsorption on Ni^{2+}–NTA agarose, 300 m*M* of NaCl and 10 m*M* of imidazole were included in the lysis buffer used for direct protein load on the resin. Wash buffer contained 20 m*M* of imidazole, which was a higher limit for Yol066 adsorption on the matrix. Elution of Yol066 was achieved by 250 m*M* of imidazole in the same buffer. Lower concentrations led to incomplete elution of the Yol066 from Ni^{2+}–NTA and consequent losses of protein during purification (data not shown). Typical results of protein purification from *E. coli* cell extract are presented in Fig. 2. Small amounts of Yol066 still remain in the unbound fraction (lane 2) and are present in the wash fractions (lanes 3 and 4). This incomplete adsorption is most probably explained by the saturation of matrix binding sites by Yol066 protein present in the extract. As shown on Fig. 2 (lanes 5 and 6), the simple adsorption–step elution procedure obtains almost homogeneous Yol066 protein in a single purification step.

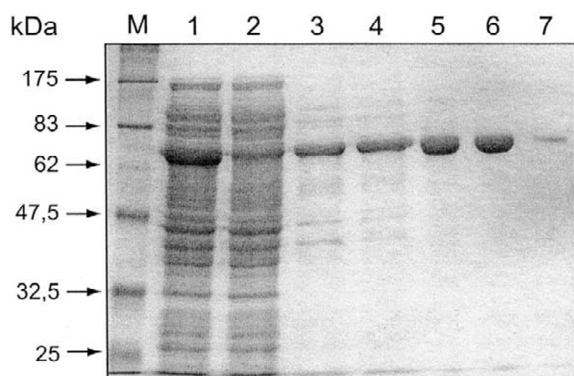

Fig. 2. Purification of Yol066 protein on Ni^{2+}–NTA agarose. Analysis was done by SDS–PAGE as described in the Experimental section. The following samples were loaded onto the gel: crude *E. coli* cell extract (10 μl, lane 1), unbound protein fraction (10 μl, lane 2), two successive wash fractions (10 μl, lanes 3 and 4) and three elution fractions (1 μl, lanes 5, 6 and 7). Sizes of molecular mass markers (*M*) are indicated on the left.

3.2.2. Ion-exchange chromatography

Anion-exchange chromatography is very frequently used for protein purification, since proteins with the isoelectric point (p*I*) below 7 are most common [35]. Since the calculated p*I* of His_6-tagged Yol066 is ~6.5, we used various ion-exchange matrices at the second step of purification. We tested both strong and weak anion-exchange resins (MonoQ, Resource Q and DEAE–TSK, respectively). Since the elution from Ni^{2+}–NTA column was performed at a relatively high ionic strength (300 m*M* NaCl), the eluted protein fraction was dialysed against low-salt Tris–HCl buffer (see Experimental section). Dialysis buffer contained 100 m*M* NaCl and 100 m*M* imidazole since we observed the extensive precipitation of the purified Yol066 during dialysis against low-ionic strength buffer (data not shown). Dialysed protein fraction was loaded onto the ion-exchange column after clarification by centrifugation. The best results were obtained by the Resource Q column (see Fig. 3). However, Yol066 protein elutes from the column as double peak and both protein fractions (fractions 7–8 and 10–11, Fig. 3B) contain the protein with the same molecular mass. Thus the heterogeneity observed during Resource Q chromatography is not related to protein degradation, but rather reflects charge differences or existence of Yol066 protein isoforms.

3.3. Storage conditions

Purified Yol066 protein was stored at −20 °C in the buffer containing 25 m*M* Tris–HCl, pH 8.0, 100 m*M* NaCl, 100 m*M* of imidazole and 50% of glycerol. Storage at +4 °C with or without glycerol led to precipitation and inactivation of the purified protein. As in the case of dialysis, the use of low ionic strength buffers for storage also favours extensive precipitation of the purified Yol066.

Optimised expression and purification protocol for recombinant Yol066 obtains about 10 mg of pure protein from 3 l of *E. coli* cell culture and thus opens

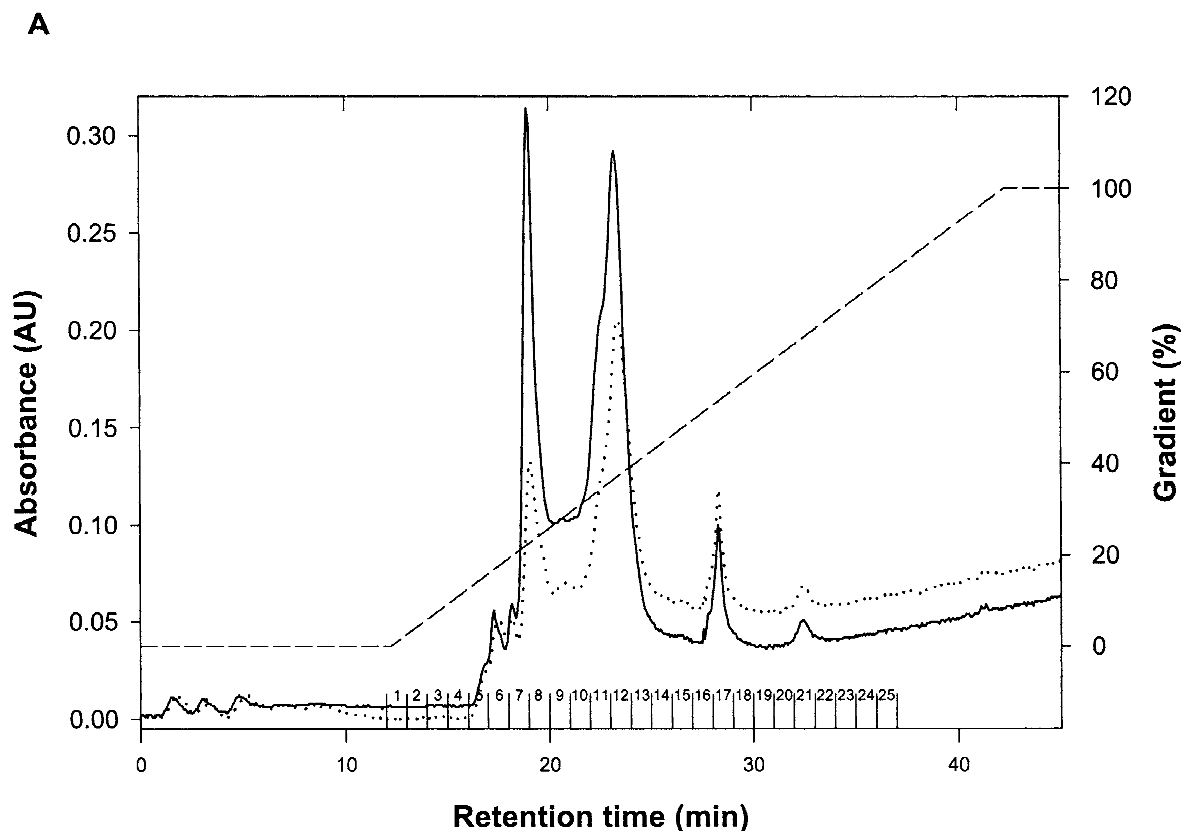

Fig. 3. Chromatography of Ni^{2+}–NTA purified Yol066 on a Resource Q anion-exchange column (A). An elution profile was recorded using optical density at 280 (solid line) and 254 nm (dotted line). Elution was performed by NaCl gradient from 0 to 1 *M* in buffer A (dashed line). Fraction analysis of Yol066 purification by SDS–PAGE (B). Yol066 fraction from Ni^{2+}–NTA column (Ni) and Resource Q fractions from 6 to 12 were loaded onto 8% PAAG and gel was Coomassie Blue stained. Sizes of molecular mass markers (*M*) are indicated on the left.

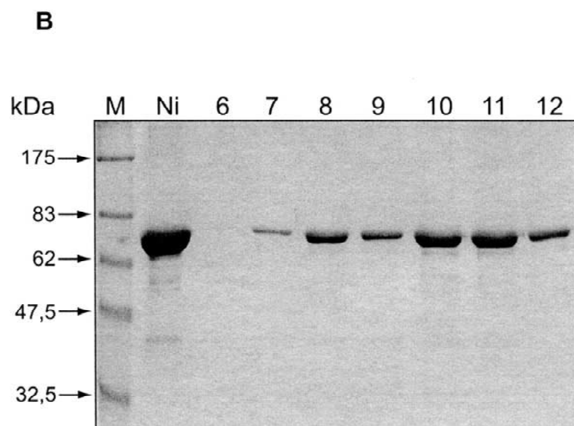

Fig. 3. (*continued*)

the possibility of detailed functional and physico-chemical characterisation.

3.4. Characterisation of recombinant Yol066 by analytical gel filtration

Native molecular mass of Yol066 protein was determined by gel filtration on a Superose 12 molecular sieve column. Protein elutes from the column as a single peak with an apparent molecular mass of 74 000 (data not shown) which corresponds well to a monomeric form of the protein (calculated molecular mass 69 489, including His_6-tag). These data demonstrate that in contrast to some other described yeast RNA:Ψ-synthases (Pus1 and Pus4, for example) [15,18], putative RNA:Ψ-synthase Yol066 protein

does not aggregate in solution even in the absence of RNA.

4. Nomenclature

Ψ	Pseudouridine
PCR	Polymerase chain reaction
IPTG	Isopropyl-β-D-thiogalactopyranoside
PAAG	Polyacrylamide gel
PAGE	Polyacrylamide gel electrophoresis

Acknowledgements

This work was supported by laboratory funds from the CNRS and ''Région Lorraine''. I. Ansmant is a pre-doctoral fellow supported by a fellowship from the ''Ministère de la Recherche et de l'Enseignement Supérieur''. We thank C. Branlant (MAEM, Nancy) for fruitful discussions and comments on the manuscript and S. Azza (MAEM, Nancy) for help in conducting chromatographic experiments.

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