ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 6, pp. 686-692. © Pleiades Publishing, Ltd., 2008.
Published in Russian in Biokhimiya, 2008, Vol. 73, No. 6, pp. 854-862.

Molecular Cloning and Analysis of Function of Nucleoside Diphosphate Kinase (NDPK) from the Scallop *Chlamys farreri*

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Received August 29, 2007
Revision received November 22, 2007

Abstract—Nucleoside diphosphate kinase (NDPK) is a key metabolic enzyme that catalyzes the synthesis of non-adenine nucleoside triphosphate (NTP) by transferring the terminal phosphate between nucleoside diphosphate (NDP) and NTP. NDPK regulates a variety of eukaryotic cellular activities including cell proliferation, development, and differentiation. The *ndpk* cDNA was cloned from the hemocytes of the scallop *Chlamys farreri* and designated *Cf-ndpk*. The full-length sequence of *Cf-ndpk* consists of 715 bp encoding a polypeptide of 153 amino acids with a calculated molecular mass of 16927.52 daltons and p*I* of 7.64. The mRNA expression and distribution of *Cf-ndpk* in both bacterially challenged and unchallenged scallops were studied by Northern blotting and *in situ* hybridization. The results showed that *Cf-ndpk* transcripts were present in hemocytes, gill, adductor muscle, mantle, digestive gland, foot, and gonad, and the expression level increased in hemocytes after bacterial challenge. Recombinant Cf-NDPK expressed in *Escherichia coli* could transfer the terminal phosphate between UDP and ATP. The Cf-NDPK protein was present in all tested tissues including foot, adductor muscle, digestive gland, gonad, mantle, gill, and hemolymph. It was up-regulated in hemolymph after bacterial challenge. Taken together, these results suggest that NDPK may play roles in the innate immune response of scallop.

DOI: 10.1134/S0006297908060096

Key words: scallop, *Chlamys farreri*, nucleoside diphosphate kinase (NDPK), expression profile, recombinant expression

Cultivation of the scallop *Chlamys farreri* is very important for aquaculture and accounts for most of the scallop production in China. But in the last ten years, scallops have suffered serious viral and bacterial diseases. *Vibrio anguillarum* is one of the pathogens that cause bacterial diseases. Therefore, understanding the mechanism of innate response of scallop challenged by bacteria may help us to develop a new strategy for controlling diseases. We performed suppression subtractive hybridization (SSH) analysis with normal and challenged scallops to find the immune related genes. The putative *ndpk* gene is one of the relatively abundant genes in the SSH analysis of the challenged scallop. So we choose this gene for further study.

Nucleoside diphosphate kinase (NDPK, EC 2.7.4.6) catalyzes the transfer of phosphate from a donor nucleoside triphosphate (NTP) to an acceptor nucleoside diphosphate (NDP) via an NDPK-phospho-histidine intermediate via a ping-pong mechanism. The enzyme is ubiquitous and plays an important role in the balance between adenine and non-adenine triphosphates [1, 2].

In addition to this housekeeping role, some additional functions of NDPK have been described in animals and plants. In mammals, NDPK is encoded by the *nm23* gene. The first *nm23* gene was discovered as a potential metastasis inhibitor [3]. NDPK-A acts as a metastasis suppressor in certain human tumors, including melanoma and breast carcinoma [4]. Intriguingly, in other human tumors including neuroblastoma, osteosarcoma, and pancreatic carcinoma, NDPK-A appears more effective in promoting metastasis [4]. In humans and zebrafish *Danio rerio*, NM23-H2/NDP kinase B stimulates the affinity of telomerase for its single-stranded telomeric TTAGGG-repeat substrate [5]. NDPK also plays an important role in early embryonic development of Atlantic salmon [6]. In *Drosophila*, NDPK is encoded by the abnormal wing disk (*awd*) gene. NDPK is important for the normal development of *Drosophila*.

Abbreviations: EST) expression sequence tags; IPTG) isopropyl-1-thio-β-D-galactopyranoside; NDPK) nucleoside diphosphate kinase; Cf-NDPK) nucleoside diphosphate kinase from the scallop *Chlamys farreri*; RT-PCR) reverse transcription polymerase chain reaction.

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Homozygous mutations of the *awd* gene impair the normal development of the brain, ovaries, wings, and imaginal disks and even cause lethality after the larval stage [2]. NDPK contributes to the activation of G-protein, which plays a crucial role in some signal transduction pathways in eukaryotic cells [7, 8]. NM23-H2/NDPK also has DNA binding and *in vitro* transcriptional activities [9]. Recently, it was reported that NDPK-A acts as a controller of AMP-kinase in airway epithelia [10]. In shrimp, NDPK acts as a defense-related enzyme and plays roles in the anti-viral innate immune reaction [11]. Shin et al. [12] reported that three NDPKs from Chinese cabbage had important physiological roles during development and signal transduction. In rice, the expression of the gene encoding NDPK1 is enhanced upon infection with bacterial pathogens [13].

In contrast, much less is known about the possible role of NDPKs in the scallop *C. farreri*. So, we have cloned the full length of the putative *ndpk* gene and analyzed the expression profiles of the gene by Northern blot and *in situ* hybridization. The tissue distribution was also studied by Western blot using the antiserum against recombinant NDPK. High-performance liquid chromatography (HPLC) was used to analyze its activity by transferring the phosphoryl group between UDP and ATP.

MATERIALS AND METHODS

Collection of animals, tissues, and hemocytes. Scallops *C. farreri* were purchased from Jinan, Shandong Province, China, and cultured in the laboratory in tanks (15-18°C) filled with air-pumped sea water. Scallops were separated into challenged and normal groups. Each scallop of the challenged group was injected into the adductor muscle with 10 μl of *V. anguillarum* suspension (OD_{600} = 0.8, in physiological saline solution) and temporarily cultured in the laboratory.

Hemolymph was taken from the adductor muscle of the two groups using a 5-ml syringe and immediately centrifuged at 800*g* (4°C) for 10 min to separate the hemocytes from the hemolymph. Tissue samples such as gill, adductor muscle, mantle, foot, digestive gland, and gonad were also collected for RNA extraction and other analyses.

Suppression subtractive hybridization (SSH). mRNA was extracted from the hemocytes of 24 h challenged and unchallenged scallops using a QuickPrep micro mRNA Purification Kit (Amersham Biosciences, Great Britain). cDNA was synthesized using a Super SMART-PCR cDNA Synthesis Kit (Invitrogen, USA) according to the manufacturer's instruction. The subtracted cDNA library between bacteria-challenged scallops (tester) and control scallops (driver) was generated using polymerase chain reaction (PCR)-Select cDNA Subtraction Kit (BD Biosciences Clontech, USA) according to the manufacturer's protocol. The subtracted target cDNAs were ligated into a pGEM-T Easy vector using T4 DNA ligase and transformed into *Escherichia coli* DH5α competent cells. The transformed bacteria were plated onto Luria–Bertani (LB) agar plates containing ampicillin, X-gal, and isopropyl-1-thio-β-D-galactopyranoside (IPTG), followed by overnight incubation at 37°C. Randomly selected white clones were sequenced, and the sequences were searched in GenBank with BLASTX. One hundred and fifty clones were sequenced, and among them eight expression sequence tags (ESTs) were nucleoside diphosphate kinase, so we choose *ndpk* for further study.

Cloning the full-length *Cf-ndpk* gene. Expression sequence tags of *ndpk* were obtained from the SSH library constructed using challenged and normal scallop mRNA. RACE (Rapid Amplification of cDNA Ends) was used to obtain the 3′ and 5′ ends of *ndpk* from *C. farreri*. Specific primers F1 and R1 (table) were designed based on the 104 bp sequence obtained from the subtracted cDNA library. The 3′ end fragment was obtained by PCR amplification using specific primer F1 and 3′ anchor R-primer offered by 3′ Full RACE Core Set (TaKaRa, Japan). To obtain the 5′ ends of the *Cf-ndpk* gene, degenerate primer F2 was designed based on the BLAST result of several *ndpk* sequences such as the expressed sequence tag sequence of bay scallop and the complete *ndpk* mRNAs from *Leishmania infantum*, *Schistosoma japonicum*, *Drosophila melanogaster*, and *Hydra vulgaris*. Primers F2 and R1 were used to amplify part of the 5′ ends. Then specific primer R2 was designed. PCR amplification of the 5′ end was carried out using primer R2 and 5′ PCR primer.

Specific primers ExF and ExR were designed to amplify the complete *Cf-ndpk* cDNA and to verify the full-length cDNA from three overlapping fragments. The sequences of primers used in this study are listed in the table.

Sequence analysis. Homology analysis was carried out with BLASTX (http://www.ncbi.nlm.nih.gov/). The deduced amino acid sequence of Cf-NDPK was predicted using ExPASY (http://www.expasy.ch/). The prediction of motifs and signal peptides was performed with SMART (http://smart.embl-heidelberg.de/). Amino acid sequence alignment was performed with ClustalW and GENEDOC software.

Northern blot and RT-PCR (reverse transcription polymerase chain reaction) analysis. Total RNA of the normal group was isolated from tissues including hemocytes, gill, adductor muscle, mantle, foot, digestive gland, and gonad using Unizol reagent (Biostar, China). The recombinant pGEM-T-Easy-*Cf-ndpk* plasmid, with a positively-orientated cDNA insert, was linearized by digestion with *Sac* II and *Pst* I, separately. The two linear plasmids were then transcribed *in vitro* with SP6 and T7 RNA polymerase (DIG RNA Labeling Kit; Roche, USA) into antisense and sense digoxigenin (DIG) RNA probes.

Sequences of primers used in this study

Primer	Sequence (5'–3')	Direction	Position in *ndpk*
SMART F	TACGGCTGCGAGAAGACGACAGAAGGG	forward	
Oligoanchor R	GACCACGCGTATCGATGTCGACT16 (A/C/G)	reverse	
5' PCR primer	TACGGCTGCGAGAAGACGACAGAA	forward	
3' anchor R	GACCACGCGTATCGATGTCGAC	reverse	
F1	CGTGGTGACCTCTGTATAGAC	forward	325-345
R1	TCTCCAAACCAGAGTTTCACC	reverse	399-419
F2	AAACCNGACGG(A/C)GT(C/G/A)CAGCGCGG	forward	46-68
R2	CATTATGTAGACCTGAAAAGCGAACCCTTCTTCCCT	reverse	163-198
ExF	TACTCA GAA TTC ATGTCCGACCCAAAAAATGAAG	forward	7-28
ExR	TACTCA CTC GAG CCAGATCACTGTTCCTTCTTGA	reverse	509-530
actinF	CAATGTTCCCAGGTATCGCAG	forward	
actinR	AGCCAAGATGGAGCCACCGAT	reverse	

Northern blot analysis was performed to detect gene expression in different tissues. Northern blot analysis was performed as described elsewhere in detail [14].

Total RNA of the challenged group was isolated from hemocytes at 2, 6, 12, and 24 h after injection. Then they were reverse transcribed by the first strand cDNAs, which were used as the template for RT-PCR analysis. F1 and R1 were used as primers, and the PCR procedure was followed as one cycle (94°C, 2 min), 30 cycles (94°C, 2 min; 53°C, 45 sec; 72°C, 30 sec), one cycle (72°C, 10 min). Specific primers actin F and actin R were used to amplify *β-actin* gene as the control. RT-PCR was repeated three times. The bands were digitized by Quantity One (Bio-Rad, USA). Statistical analysis was performed by the ratio of *Cf-ndpk* and *β-actin* genes.

***In situ* hybridization.** Hemocytes were collected from the normal scallops as described previously. To resuspend the hemocytes, a five-fold volume of Grace medium (Life Technologies Gibco BRL, USA) used for insect cell culture was added, and the suspension was centrifuged at 200*g* for 10 min at 4°C to precipitate hemocytes. The supernatant was then discarded, and the hemocytes were washed twice using the same volume of Grace medium. The final concentration of hemocytes resuspended in Grace medium was 10^7 cells/ml. Hemocytes were immediately dropped onto gelatin-coated glass slides and air dried for about 1 h. Hemocytes were fixed at room temperature by adding several drops of PBS solution containing 4% paraformaldehyde for 30 min, then washed twice with PBS. Prehybridization and hybridization were performed by the methods provided by the DIG RNA labeling kit (SP6/T7). The anti-sense RNA probe was used for detecting *Cf-ndpk* mRNA, and the sense RNA probe was used as the negative control.

Recombinant expression and purification of Cf-NDPK (rCf-NDPK). The complete open reading frame of *Cf-ndpk* was amplified using primers ExF and ExR (sequences listed in the table). After digestion with *Eco*R I and *Xho* I, the gene was ligated into the *Eco*R I and *Xho* I sites of the pET-30a(+) vector. The recombinant plasmid was then transformed into competent cells of *E. coli* BL21(DE3) for induced expression.

Overnight culture (3 ml) of pET-30a(+)/*Cf-ndpk* was added to 300 ml of LB broth supplemented with 1.2 ml kanamycin (25 mg/ml). When the OD_{600} reached 0.8, IPTG was added to final concentration of 0.5 mM, and the bacteria were cultured at 37°C for 4 h while shaking at 200 rpm. The bacterial cells were collected by centrifugation at 6000*g* for 10 min, resuspended in 20 ml PBS with 0.2% Triton X-100, and lysed by sonication. The soluble fraction was purified by His Bind resin chromatography (Novagen, USA) following the manufacturer's instructions.

Activity of rCf-NDPK. As NDPK could transfer the phosphoryl group between NDP and NTP, HPLC was used to analyze its activity [15, 16]. The reaction mixture (0.3 ml) contained 5 mM $MgCl_2$, 33 mM Na-Hepes (pH 7.4) and 1 mM UDP and ATP. The purified rCf-NDPK (30 ng) was added to initiate the reaction. The mixture was incubated at 37°C for 5 min, then the reaction was stopped by adding 0.2 ml HCOOH to lower the pH below 3.5. The resulting samples were immediately loaded into the autosampler of the HPLC system. Each sample was analyzed three times.

Production of anti-Cf-NDPK immune serum. The purified rCf-NDPK protein was used to produce polyclonal anti-serum in a 2 kg rabbit. The enzyme (100 μg) was emulsified in complete Freund's adjuvant (Sigma,

USA) and injected subcutaneously into the back of the rabbit every week for the initial three injections. For the next six booster injections, rCf-NDPK protein (100 μg) with physiological saline added to a final volume of 2 ml without adjuvant was injected directly into the parallel ear vein of the rabbit. Blood was collected by cardiac puncture after the final injection. The collected cell-free blood was deposited at room temperature for 6 h, and then serum was collected and kept at –20°C until use.

Western blot analysis. Tissues including gill, adductor muscle, mantle, foot, digestive gland, and gonad of normal scallops were homogenized in buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 3 mM EDTA. The homogenate was centrifuged for 10 min at 12,000*g*. The supernatant was collected and protein was quantified by the method of Bradford [17] using bovine serum albumin as a standard. Soluble protein (200 μg) from each tissue and hemolymph was analyzed by 15% SDS-PAGE following the method of Laemmli [18]. Immunoblot analysis was carried out using the method of a previous report [19]. Western blot analysis was also performed using protein (200 μg) from hemolymph at different times after bacterial challenge.

RESULTS

To study the mechanism of immune response on the pathogenic infection in scallops, we treated them with *V. anguillarum* suspension and extracted mRNA from the hemocytes of normal and challenged scallops. Then the subtracted cDNA library between bacteria-challenged scallops (tester) and control scallops (driver) was generated. Among 150 randomly selected clones sequenced, eight clones were homologous to the sequences of nucleoside diphosphate kinase gene from other species. Thus, we choose the *ndpk* as an actively responding gene for further study.

Cloning a full-length cDNA encoding *Cf-ndpk*. A fragment was obtained from the hemocytes SSH library of *C. farreri*. Sequence analysis showed that the sequence was highly homologous to *ndpk* sequences of other species. The full length of *Cf-ndpk* was obtained by RACE methods.

As shown in Fig. 1 (see color insert), the full-length *Cf-ndpk* cDNA (GenBank accession No. EF213111) consisted of 715 nucleotides with an open reading frame of 462 bp, encoding a protein of 153 amino acids with a calculated molecular mass of 16927.52 daltons and a p*I* of 7.64 (http://www.expasy.ch/tools/pi_tool.html). There were six nucleotides in the 5′ untranslated region and 247 nucleotides in the 3′ untranslated region with a 29 bp polyA tail. The deduced protein had no signal peptide and contained a nucleoside diphosphate kinase domain which can transfer a phosphate from NDP to NTP (http://smart.embl-heidelberg.de/). There was a histidine residue (His120 in Cf-NDPK) which was involved in catalytic activity and conserved in all known NDPK isozymes (http://smart.embl-heidelberg.de/). The tripeptide Arg-Gly-Asp, which was the RGD consensus sequence for the recognition of integrin family members and existed in all previously identified NDPKs except those in bacteria [20], was also present in the sequence (107-109 amino acid residues in Cf-NDPK).

Similarity analysis of NDPK. Sequence comparison (Fig. 2, see color insert) revealed that the amino acid sequence of the putative NDPK from scallop shared identities with NDPK sequences from leishmania *Leishmania infantum* (50.84%), sponge *Suberites domuncula* (48.60%), hydra *Hydra vulgaris* (47.49%), schistosome *Schistosoma japonicum* (49.16%), fruit fly *Drosophila melanogaster* (53.07%), rainbow trout *Oncorhynchus mykiss* (51.96%), zebrafish *Danio rerio* (53.07%), African clawed toad *Xenopus laevis* (51.40%), domestic pigeon *Columba livia* (53.63%), house mouse *Mus musculus* (54.75%), and human *Homo sapiens* (56.98%).

Expression profiles of *Cf-ndpk*. Northern blot analysis (Fig. 3a, see color insert) indicates that *Cf-ndpk* mRNA is constitutively expressed in all tested tissues. The time-course analysis of *Cf-ndpk* mRNA in hemocytes indicated that the mRNA expression was up-regulated after bacterial challenge (Fig. 3b).

The distribution of *Cf-ndpk* mRNA in hemocytes was also detected by *in situ* hybridization. As shown in Fig. 4, there were strong signals in parts of the hemocytes. The result was consistent with that of Northern hybridization analysis.

Recombinant expression and purification of Cf-NDPK. Recombinant protein was highly expressed in *E. coli* BL21(DE3)-pET-30a(+)/*Cf-ndpk* after induction with 0.5 mM IPTG. The expected molecular mass of rCf-NDPK was about 22.5 kD including 16.9 kD Cf-NDPK and a 5.6 kD N-terminal expressed tag sequence (Fig. 5). The expression level of Cf-NDPK was 19.13% of the total cell protein.

Tissue distribution of Cf-NDPK. Western blot analysis was carried out to analyze tissue distribution and expression profile of Cf-NDPK at the protein level. As shown in Fig. 6a, Cf-NDPK was detected in all tissues. The expression level of the protein in hemolymph increased after bacterial challenge.

Activity of recombinant Cf-NDPK. The phosphotransferase activity of rCf-NDPK was confirmed by reverse-phase chromatography of phosphate donor and acceptor nucleotides. NDPK could catalyze the reversible transfer of a phosphoryl group from a nucleoside triphosphate to a nucleoside diphosphate:

$$N_1TP + N_2DP \rightarrow N_1DP + N_2TP.$$

In this study, UDP and ATP were used to perform an HPLC-based assay to analyze the activity of rCf-NDPK.

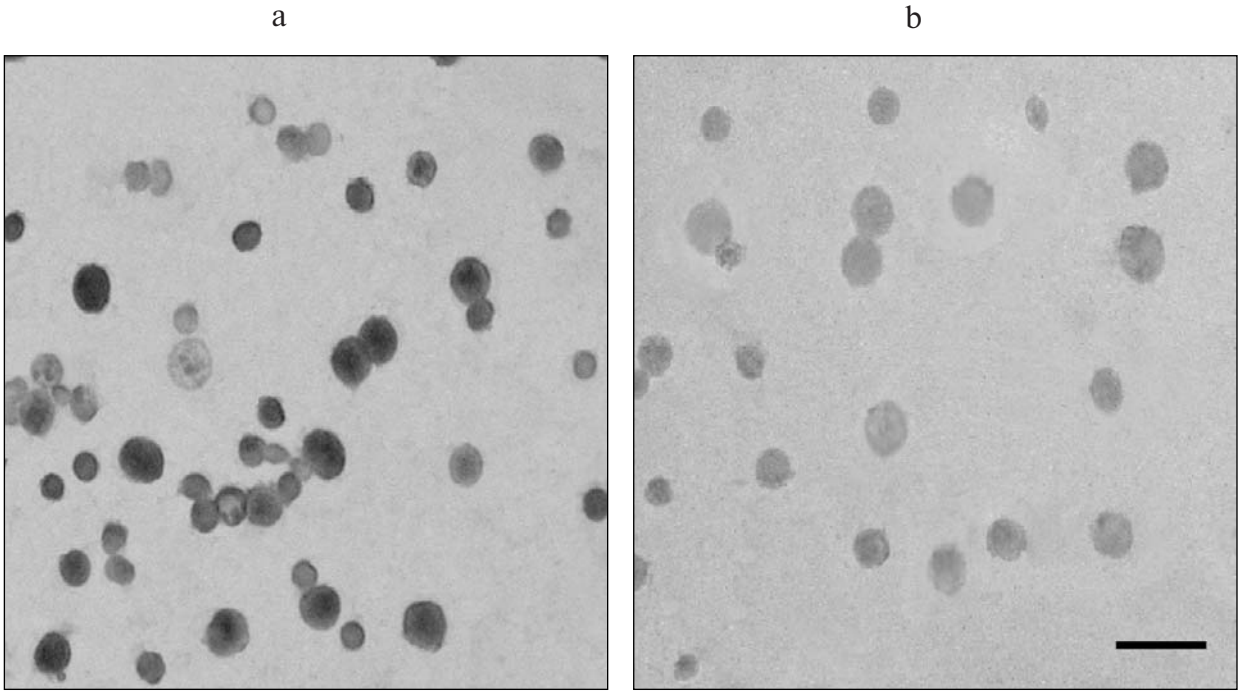

Fig. 4. Detection of *C. farreri* NDPK mRNA in scallop hemocytes by *in situ* hybridization. a) Hemocytes from normal scallops analyzed using an antisense Dig-RNA probe. b) Hemocytes from normal scallops analyzed using a sense Dig-RNA probe (control). Bar, 10 μm.

Figure 7a shows the chromatogram of a control mixture of UDP, ADP, and ATP showing that the UDP acceptor and ATP donor peaks are well resolved by this chromatographic method. In the presence of rCf-NDPK (Fig. 7b), the levels of donor ATP and acceptor UDP both decreased and the product UTP and ADP peaks clearly increased compared with the control (no enzyme), which was incubated for the same time and at the same temperature with the experimental group (with enzyme). This result demonstrated that the observed activity was the result of a phosphotransferase reaction. The peak areas of both UDP and UTP were used to calculate the enzyme activity of rCf-NDPK. The definition of one activity unit (U) of rNDPK was the amount of enzyme that could transfer 1 μmol substrate per minute under the best conditions. Activity was calculated by the following formula:

$$\text{Activity (U/mg)} = [0.3A_p/(A_p + A_s)]/5 \text{ min} \times 30 \text{ ng [15]},$$

where A_p and A_s represent the peak area of UTP and UDP. The enzyme activity here was 700 U/mg rCf-NDPK.

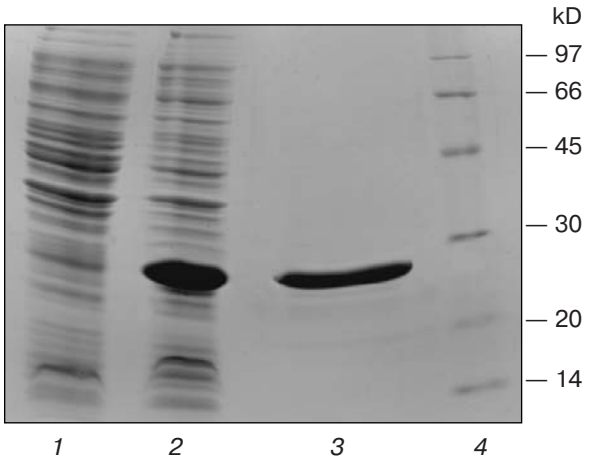

Fig. 5. SDS-PAGE analysis of recombinant *C. farreri* NDPK expression in *E. coli* BL21(DE3) and purified rNDPK. Lanes: *1*) total protein in *E. coli* transformed with pET-30a(+)/*Cf-ndpk*, not induced; *2*) total protein in *E. coli* transformed with pET-30a(+)/*Cf-ndpk*, induced with 0.5 mM IPTG; *3*) purified rNDPK; *4*) protein standard.

DISCUSSION

We cloned and characterized a new member of the *NDPK* gene family, *Cf-ndpk*, from the scallop *C. farreri*. To our knowledge this is the first report of the gene in scallop.

Comparison of NDPK from the scallop *C. farreri* with those from other animals. NDPKs from different animals are highly conserved, and the conservation extended from the first glutamate residue (Glu7 in Cf-NDPK), which

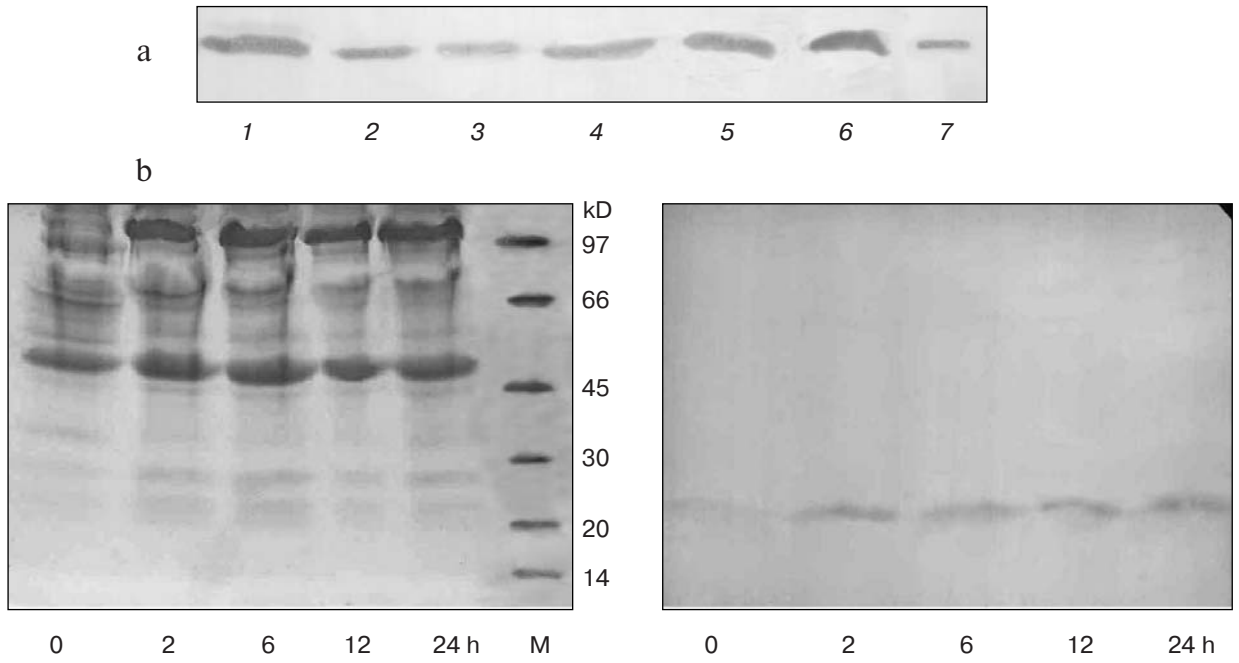

Fig. 6. a) Western blot analysis of soluble protein (200 μg) extracted from different tissues of normal scallops. Lanes: *1-7*) foot, adductor muscle, digestive gland, gonad, mantle, gill, and hemolymph, respectively. b) Left: SDS-PAGE analysis of total protein (200 μg) after bacterial challenge at 2, 6, 12, and 24 h in hemolymph. Right: the same samples were analyzed by Western blot.

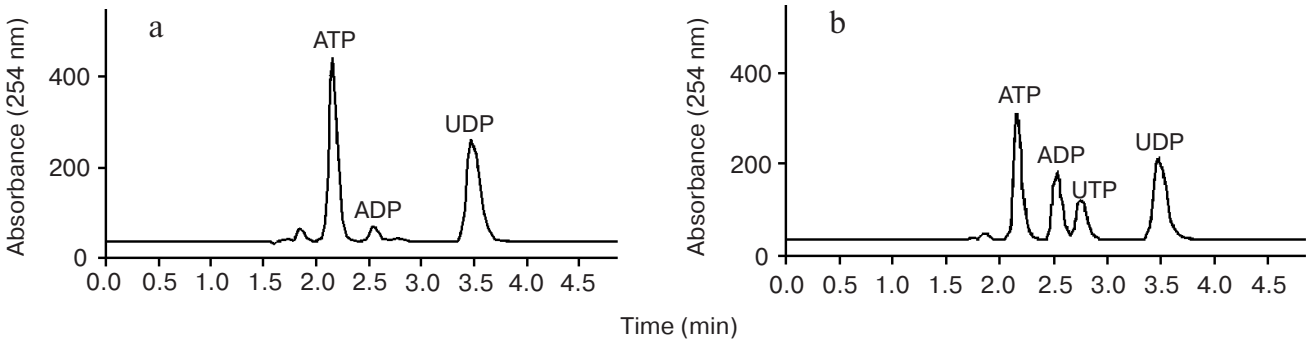

Fig. 7. HPLC-based assay to analyze the phosphoryl transferase activity of recombinant NDPK. a) Control reaction containing ATP, UDP, and ADP mixture without enzyme; b) the reaction mixture containing 30 ng rNDPK.

was highly homologous in all members of the analyzed sequences, to the tyrosine-glutamate (YE) motif (C-terminal residues) at the end of most isoforms [12] (see Fig. 2). The proline (Pro98 in Cf-NDPK), whose mutation to serine together with a null mutation of the *prune* eye color gene could lead to death during early development in *Drosophila* [2, 21], was also completely conserved in all sequences. The catalytic site of the enzyme was the NXXHGSD motif [12], which was crucial for NDPK substrate binding and highly conserved from lower to higher animals, including Cf-NDPK. Histidine (His120 in Cf-NDPK), which formed the phospho-histidine intermediate in the catalytic reaction, was also conserved in all selected sequences.

Expression and possible function of Cf-NDPK. The distribution of NDPK was ubiquitous, and its expression can vary in different tissues [22]. *Cf-ndpk* mRNA expression profiles were analyzed by Northern blot using total RNA from different tissues of normal scallops. Results showed that the mRNA of *Cf-ndpk* was constitutively expressed in all tissues of the scallop.

NDPK belongs to a multifunctional protein family. Besides its housekeeping role, which maintained the intracellular levels of all NTPs except ATP, NDPK was also crucial for the control of cell proliferation, transcriptional regulation, and protein phosphotransferase activity in animals [23]. Hartsough [24] reported that NM-23 could phosphorylate the KSR (kinase suppressor of Ras),

which is a scaffold protein for the MAPK cascade, and then suppress tumor metastasis. It was reported that NDPK plays an important role in defense against bacterial pathogens in rice [13]. NDPK is also involved in the immune response of shrimp [11]. Morais Guedes et al. [25] reported that NDPK was found to be one of the differentially expressed hemolymph proteins in *Drosophila* larvae after bacterial immune challenge. *Cf-ndpk* was first obtained from an SSH library of hemocytes challenged by bacteria, so we wondered whether it was involved in innate immunity of scallop. After bacterial challenge, its mRNA expression level in hemocytes was up-regulated. Western blot was used to examine the tissue distribution and expression profile of Cf-NDPK at the protein level. NDPK was constitutively expressed in foot, adductor muscle, digestive gland, gonad, mantle, gill, and hemolymph. The expression level of NDPK increased in hemolymph after bacterial challenge. This meant that Cf-NDPK protein might be regulated by bacterial infection. NDPK could transfer the phosphate of ATP directly to GDP that was bound to Ras-related GTPases, and it resulted the activation of GTPases which transformed from GDP-bound (inactive) to GTP-bound (active) states [26, 27]. Ras-related GTPases (Rho and Rac) have been found to be relevant to phagocytosis and to be involved in innate immunity [28, 29]. So, NDPK may be indirectly related to the innate immune reaction.

The recombinant Cf-NDPK catalyzed the transfer of the γ-phosphate from a nucleoside triphosphate to a nucleoside diphosphate *in vitro*, which suggested that Cf-NDPK may play an important role in the balance between adenine and non-adenine triphosphates *in vivo*. Therefore, Cf-NDPK may play important housekeeping roles in all tissues.

In this paper, a new *ndpk* gene was obtained from scallop *C. farreri*. In addition to the housekeeping roles, Cf-NDPK may also be involved in the defense response against bacterial infection.

This work was supported by grants from the National Natural Science Foundation of China (No. 30770282) and the National High Technology Research and Development Program of China (863 Program) (No. 2006AA100311 and 2007AA09Z425).

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