



Molecular cloning, sequence analysis, prokaryotic expression, and function prediction of foot-specific peroxidase in *Hydra magnipapillata* Chinese strain

H.C. Pan, H.Q. Yang, F.X. Zhao and X.C. Qian

College of Life Science, Anhui Normal University, Wuhu, Anhui, China

Corresponding author: H.C. Pan
E-mail: panhongchun@126.com

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ABSTRACT. The cDNA sequence of foot-specific peroxidase PPOD1 from the Chinese strain of *Hydra magnipapillata* was cloned by reverse transcription-polymerase chain reaction. The cDNA sequence contained a coding region with an 873-bp open reading frame, a 31-bp 5'-untranslated region, and a 36-bp 3'-untranslated region. The structure prediction results showed that PPOD1 contains 10.34% of α -helix, 38.62% of extended strand, 12.41% of β -turn, and 38.62% of random coil. The structural core was α -helix at the N terminus. The GenBank protein blast server showed that PPOD1 contains 2 fascin-like domains. In addition, high-level PPOD1 activity was only present in the ectodermal epithelial cells located on the edge of the adhesive face of the basal disc, and that these cells extended lamellipodia and filopodia when the basal disc was tightly attached to a glass slide. The fascin-like domains of *Hydra* PPOD1 might contribute to the bundling of the actin filament of these cells, and hence, the formation of filopodia. In conclusion, these cells might play an important role in strengthening the adsorbability of the

basal disc to substrates.

Key words: *Hydra magnipapillata*; Foot-specific peroxidase; Filopodia; cDNA sequence Fascin

INTRODUCTION

Animals in the genus *Hydra* have a simple body plan, which is basically a tube with a head at the apical end, and a foot, or basal disc, at the other end (Siebert et al., 2005). These animals are generally sedentary or sessile, and attach to various substrates (such as plants or rocks) in the water with the basal disc, in which ectodermal mucous cells secrete a sticky fluid. This sticky fluid is responsible for the adhesive properties of the basal disc, with the *Hydra* extending its body to maximum length, and then slowly extending its tentacles. Once fully extended, the tentacles are slowly maneuvered around, until they come in contact with a suitable prey animal. On contact, nematocysts on the tentacles fire into the prey, and within 2 min, the tentacles surround the prey and transfer it into the opened mouth aperture (Xu et al., 2010). In brief, the basal disc plays an important role as an adhesive structure in the life-history activities of *Hydra*.

The ectoderm of the basal disc is formed from specifically differentiated epithelial cells, termed the foot mucous cells, which are characterized by the presence of acidic mucopolysaccharide material. Moreover, foot mucous cells have been shown to harbor peroxidase activity, which serves as an excellent marker for these cells (Hoffmeister and Schaller et al., 1985). The putative peroxidase is only present in high concentrations in the ectodermal foot mucous cells, and may be easily assayed by histochemical methods using H₂O₂ and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) or diaminobenzidine (DAB) (Ghaskadbi et al., 2005). During foot regeneration, the new production of large amounts of this enzyme coincides with the period the animal needs the ability to adhere to a substrate (Xu et al., 2010). Therefore, the appearance of this peroxidase activity provides a suitable marker for foot mucous cell differentiation in *Hydra*. By measuring enzyme concentrations, a rapid and reliable test to quantify the differentiation of foot-specific cells, and the process of foot regeneration, has been established (Hoffmeister, 1996; Harafuji et al., 2001). In addition, the cDNA sequence that encodes foot-specific peroxidase has been cloned based on information obtained from the amino acid sequences of the tryptic peptides of purified peroxidase, and was named the PPOD1 gene (Hoffmeister et al., 2002; Thomsen and Bosch, 2006).

The finding that a high-level peroxidase activity occurs in the foot mucous cells of the basal disc of *Hydra* has provided a valuable tool for the study of foot-specific differentiation processes. However, the physiological role of the strong peroxidase activity in foot mucous cells has yet to be elucidated. In the present study, we cloned the cDNA of PPOD1 of *Hydra magnipapillata* Chinese strain based on genomic data (Chapman et al., 2010), and assessed its physiological function by using a combination of bioinformatic analysis and the morphological characteristics of the ectodermal epithelial cells located on the edge of the adhesive face of the basal disc.

MATERIAL AND METHODS

Animals used and preparation of excised foot pieces from the whole *Hydra* body

H. magnipapillata Chinese strain was collected from Wuhu, Anhui, China, and cultured

in a medium containing 1 mM CaCl₂, 0.1 mM KCl, 0.1 mM MgCl₂, and 0.5 mM NaH₂PO₄, pH 7.6. The temperature of the medium was maintained at 20°C ± 0.5. The animals were fed daily between 9 and 10 am with the nauplii of *Artemia salina*, and were transferred to fresh culture medium 6 h after each feeding. For the preparation of excised foot pieces, the foot was cut just above the end of the peduncles, and the pieces were frozen at -70°C before RNA isolation.

RNA isolation

Total RNAs were isolated from about 100 mg excised foot pieces using the Total Tissue/Cell RNA Extraction Kits (Sangon Biotech Co., Ltd., Shanghai, China), following manufacturer protocols. The total RNAs extracted were dissolved in diethyl pyrocarbonate (DEPC) water, and stored at -70°C.

Primer design, RT-PCR, cloning of RT-PCR products, and sequencing

The polymerase chain reaction (PCR) primers were designed by Primer Premier 5.0, based on the putative cDNA sequence of PPOD1 from the *Hydra* Genome Project (GenBank accession No. NW_002092004) (Chapman et al., 2010). The specific primers of the cDNA sequences were as follows: PPOD1 forward: 5'-TATTCATTTGTTTAATATAAC-3'; and PPOD1 reverse: 5'-TGATTAGAATATAAACTTATCAC-3'.

Total RNAs were used to synthesize the first-stranded cDNAs using a reverse transcription kit with Oligo dT as the primers (Promega, USA). Twenty microliters of the first-strand cDNA synthesis reaction system contained 1 mg total RNAs, 5 mM MgCl₂, 1 mM dNTPs, 0.5 µg Oligo dT₁₅, 10 U/µL RNase inhibitor, and 15 U AMV reverse transcriptase. The mixture was incubated at 42°C for 60 min.

The first-strand cDNA synthesized was used as a template. The total reaction volume for DNA amplification was 25 µL. The reaction mixture contained 1.5 mM MgCl₂, 200 µM of each of dATP, dGTP, dCTP, and dTTP, 0.3 µM of each primer, and 5 U Taq plus DNA polymerase. DNA amplification was performed using an MJ Research thermocycler, Model PTC-200 (Watertown, MA, USA). The program was set to 4 min at 94°C, followed by 30 cycles of 1 min at 94°C, 0.5 min at 48°C, and 1.5 min at 72°C, and then a final extension for 10 min at 72°C. After amplification, the PCR products were separated by electrophoresis on 1.5% agarose gel with 1X TAE (Tris-acetate-EDTA) buffer that had been stained with ethidium bromide, and was visualized under UV light. The expected fragments of the PCR products were harvested and purified from gel using a DNA harvesting kit (Takara Co., Ltd., Dalian, China), and then ligated into a pMD19-T vector at 16°C for 24 h. The recombinant molecules were transformed into *Escherichia coli* competent cells (DH5α), and then spread on an LB-plate containing 50 µg/mL ampicillin, 200 mg/mL isopropyl-beta-D-thiogalactopyranoside (IPTG), and 20 mg/mL X-gal. Plasmid DNA was isolated and digested by *Pst*I and *Sca*II, to verify the insert size. Plasmid DNA was sequenced by Sangon Biotech Co., Ltd. (Shanghai, China).

Construction of the expression vector and prokaryotic expression of recombinant PPOD1

The PCR fragment encoding partial PPOD1 amino acid sequence (residues 82-186)

was amplified from the PPOD1 cDNA clone with the forward primer, 5'-AGCTTGGATCCAA CGGGAAGTTTGTTCAGTGC-3' (*Bam*HI) and the reverse primer, 5'-ACGTAGCTAGCAATT AATGATTGCTTTCCAG-3' (*Nhe*I). PCR was performed at 94°C for 3 min; 30 cycles of 30 s at 94°C, 45 s at 52°C, 1 min at 72°C, and 10 min at 72°C. The amplified PCR product was cut and ligated into the corresponding site of the pET-GST vector (Stratagene, USA). The recombinant plasmid was then transferred into the host cells *E. coli* BL21 (DE3). The expression of recombinant protein was induced using 1.0 mM IPTG at 37°C. The induced cells were pelleted at 12,000 rpm at 4°C for 2 min. The samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after the pellets were treated with SDS loading buffer.

Assay of the region-specific expression of PPOD1

The *Hydra* samples were stained with 2, 2-azino-di (3-ethyl-benzthiazoline-sulfonic acid-6) ammonium salt (ABTS, Sigma-Aldrich Group, St. Louis, MO, USA) to detect foot-specific peroxidase (Hoffmeister and Schaller, 1985). The *Hydra* samples were incubated in 3 mL of freshly prepared staining solution containing 65.5 mM citric acid, 34.5 mM trisodium citrate, 0.1% ABTS, and 0.003% hydrogen peroxide for 20 min at room temperature. The reaction was terminated by the addition of 0.4 mM sodium azide. After microscopic observation, the *Hydra* was preserved in sodium azide until being photographed.

Data analysis

Homology research of protein sequence was performed using the GenBank Blast server, and respective homologous DNA and protein sequences were aligned by CLUSTAL. The SOPMA server (<http://www.ibcp.fr/predict.html?lang=fr>) was used for secondary structure prediction, the SignalP 4.0 server (<http://www.cbs.dtu.dk/services/SignalP/>) was used to predict the presence and location of signal peptide cleavage sites in the amino acid sequences, and the PHD server (<http://www.ibcp.fr/predict.html?lang=fr>) was used for transmembrane helix prediction. Homology structure modeling (protein 3D structure prediction) was prepared using the SWISS-MODEL server (<http://swiss-model.expasy.org/>), Geno3D server (http://geno3d-pbil.ibcp.fr/cgi-bin/geno3d_automat.pl?page=/GENO3D/html), and CPHmodels 3.2 server (<http://www.cbs.dtu.dk/services/CPHmodels/>). In addition, the neighbor-joining (NJ) method was applied to infer relationships among taxa based on a pairwise matrix of the distance from Kimura's 2-parameter model, using MEGA 5.1.

RESULTS

Isolation of PPOD1 by RT-PCR

About 1000 bp of the cDNA fragment was amplified from the *Hydra* samples using the primers PPOD1 forward and PPOD1 reverse (Figure 1). The length of the cloned cDNA was 940 bp (Figure 2).

The PPOD1 cDNA sequence was submitted to the GenBank (accession No. JF951860).

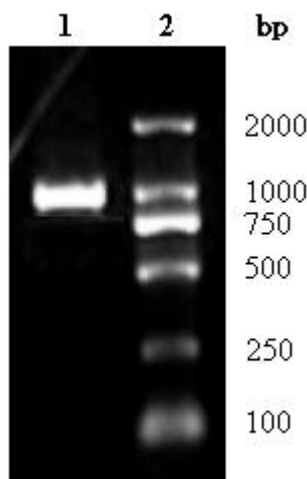


Figure 1. Results of PPOD1 cDNA amplified by RT-PCR. *Lane 1* = RT-PCR products of PPOD1 cDNA; *lane 2* = 2000-bp DNA ladder.

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-31                               g t t a a c t g c a a g g a g t t t g t g t g t c t a a a
 1 a t g a a g c t a t g c a t a a c a g t g t t c a t g a t g g t t a t c t g c g t g a t t g c a a a g a a a a c g a
 1 M K L C I T U F M M U I C U I A K E K R
 61 g a t a c a c c t g c a t g g a c a a a a g c t t a t a c a g t a g c t c t c a a a t c g t t t a c c a a c g g a a a a
 21 D T P A W T K A Y T U A L K S F T N G K
121 t t t g t a t g t g c t g a a a a t g t a g g a a g t c a a c c a t t g a t t g c c a a t a g a g a t g c t a t t g g t
 41 F U C A E N U G S Q P L I A N R D A I G
181 t t a t g g g a a a c t t t t g a a a t t c g t t t c a c t a a c g c a c a a a c a t t t g c a t t g a a a t c a c a t
 61 L W E T F E I R F T N A Q T F A L K S H
241 g c a a a c g g g a a g t t t g t c a g t g c a a g t a a t g g t g g a a a t a a c t t g c t t a t t g c c a a c a a a
 81 A N G K F U S A S N G G N N L L I A N K
301 g a c c a g g c t g c a g t a t g g g a g a c t t t c a c c t t a g t t c c a a a t t a t g g a a c t t t g g a t t t
101 D Q A A U W E T F T L U P N Y G T F G F
361 a a a t c g a a t g g t a a t g c a a a a t t t g t g g c t g c t g a a g a a g c a g g a a g t a a a t c a c t t g c a
121 K S N G N A K F U A A E E A G S K S L A
421 g c t a a c c g c a a c g c t c t t g a c g t t t g g g a a a t g t c a g c t t g g t c t a c g t g t g g c c g t c a
141 A N R N A L D U W E M F S L U Y U W P S
481 g t t c a t a a a g t g g c a a t a a a a g c a t t g g t c a a t g g c t t a t a c g t a t g t g t g a a a t g c t
161 U H K U A I K A L U N G L Y U C A E N A
541 g g a a g c a a t c a t t a a t t g c t a a c a g a g g c c a a a t a g g c c c t t g g g a a a c a t t t g a a a t t
181 G K Q S L I A N R G Q I G P W E T F E I
601 c g c t t t a c t g a c c a c c a a a c a t t t a c t c t t a a t c a c t t g c g a a c g g c a a g t t a g t t t g t
201 R F T D H Q T F T L K S L A N G K L U C
661 g c a g a a a a t a a t g g c c a a t c t c c t t a t t g c t a a t a g a g a c c a a a t t g g a c c g t g g g a g
221 A E N N G Q S P L I A N R D Q I G P W E
721 a c t t t t a c g c t c g t t c a a a a t a a g g a a g g t g t t g c t t t t a a a t c a c a c g c t a a t g g a a a a
241 T F T L U Q N K E G U A F K S H A N G K
781 t t a g t g a c a g c a g a a a a c g c a g g a c a t g a t a a c c t a a t t g c c a a c c g c g a t a a t c c t g a t
261 L U T A E N A G H D N L I A N R D N P D
841 a t t t g g g a a a g a t t c g t t t t a t c t a c t t g t g a t a a a c t t t a a t a t t a a g t t g a t t g t a
281 I W E R F U F I Y L *
901 a g c t a a a t c

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Figure 2. Nucleotide and deduced amino acid sequence of PPOD1 in *Hydra magnipapillata*; the sequence contains a single open reading frame which encodes a protein with 290 amino acids.

The cDNA sequence contained a coding sequence region with a 873-bp open reading frame (Figure 2), a 31-bp 5'-untranslated region, and a 36-bp 3'-untranslated region. The deduced PPOD1 amino acid sequence of *H. magnipapillata* was 94.8 and 92.4% similar to those of *H. vulgaris* (GenBank accession No. AAZ31364) and *H. oligactis* (GenBank accession No. AAZ31365), respectively (Figure 3).

<i>Hydra vulgaris</i>	MKLCITVFHIVTCVI AKEKRDTPVWTKAYTVAIKSFTNGKFVCAENVGSQPLIANRDAIG	60
<i>Hydra magnipapillata</i>	MKLCITVFMMVICVI AKEKRDTPAWTKAYTVALKSFTNGKFVCAENVGSQPLIANRDAIG	60
<i>Hydra oligactis</i>	MKLCITVFHIFTCVF AEEKRGIPGWNKAYTVAIKSHINGKFVCAENSGNQPLIANRGAIG	60
<i>Hydra vulgaris</i>	LWETFEIRFTNAKTF ALKSYANGKFVSAGNGGNNLLIANKDQATVWETFTLVPNYATFGF	120
<i>Hydra magnipapillata</i>	LWETFEIRFTNAQTF ALKSHANGKFVSASNGGNNLLIANKDQAAVWETFTLVPNYGTFGF	120
<i>Hydra oligactis</i>	LWETFEIRFTNAKTF TLKSHANGKFVSAGNGGNNLLIANRDHAAVWETFTLVPRHEAVGF	120
<i>Hydra vulgaris</i>	KSNGNAKFVTAEEAGSKSLAANRNVLDWWEFSLVIWVPSVHKVAIKALVNGLYVCAENA	180
<i>Hydra magnipapillata</i>	KSNGNAKFVAEEAGSKSLAANRNALDWEFSLVYVWVPSVHKVAIKALVNGLYVCAENA	180
<i>Hydra oligactis</i>	KSNGNAKLVTAEEAGNKPLAANRVVLDWWEFSLVYVWVPSVHKVAIKALVNGLFVCAENA	180
<i>Hydra vulgaris</i>	GKQSLIANRGKIGPWETFEIRFTDPQTFTLKSVANGKLVCAENNGRSPLIANRDQIGPWE	240
<i>Hydra magnipapillata</i>	GKQSLIANRGQIGPWETFEIRFTDHTFTLKSLANGKLVCAENNGQSPLIANRDQIGPWE	240
<i>Hydra oligactis</i>	GQQSLIANRGQIGPWETFEIRFTDPQTFTLKSLANGKLVCTENNGQSPLIANRNQIGPWE	240
<i>Hydra vulgaris</i>	TFTLVQNKEGVAFKSHANGKLVTAENAGRDNLIANRDNPDIWERFAFIYL	290
<i>Hydra magnipapillata</i>	TFTLVQNKEGVAFKSHANGKLVTAENAGHDNLIANRDNPDIWERFVFIYL	290
<i>Hydra oligactis</i>	TFTLVQNKEGVAFKSHANGKLVTAENAGHSNLIANRDIPDIWERFVFIYL	290

Figure 3. Alignment of deduced amino acid sequences of PPOD1 from *Hydra magnipapillata*, *Hydra vulgaris* and *Hydra oligactis*. These protein sequences were aligned using the Clustal program. Identical amino acids are shown on a gray background.

Sequence analysis of PPOD1

The SOMPA analysis of the secondary structure prediction showed that PPOD1 contained 10.34% of α -helix, 38.62% of extended strand, 12.41% of β -turn, and 38.62% of random coil (Figure 4). PHD analysis indicated that the N-terminal sequence (residues 1-12, MKLCITVFMMVI) was the transmembrane helix structure. SOPMA also showed that the N-terminal sequence had a helix structure (Figure 4). In addition, SignalP 4.0 analysis predicted an N-terminal signal peptide sequence (residues 1-70, MKLCITVFMMVICVIAKEKRDTPAWTKAYTVALKSFTNGKFVCAENVGSQPLIANRDAIGLWETFEIRFT) (Figure 5).

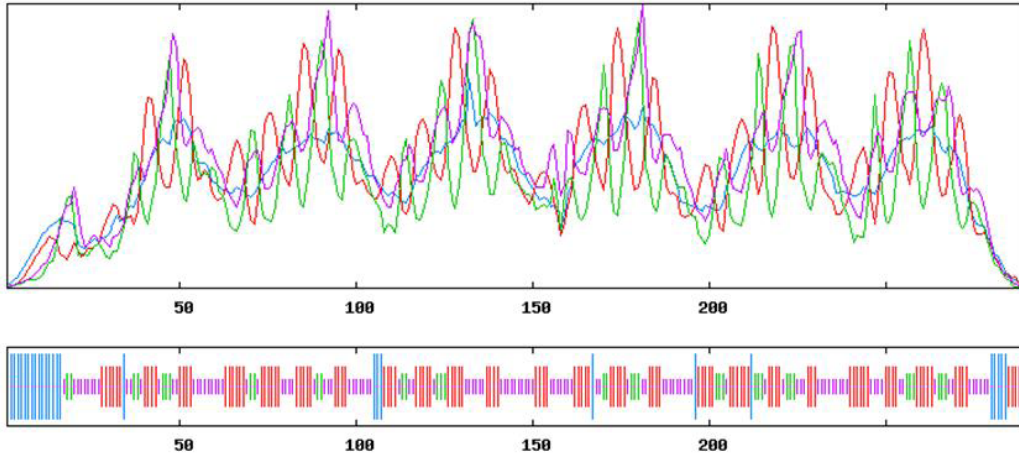


Figure 4. SOPMA result for PPOD1 of *Hydra magnipapillata*. PPOD1 protein contains 10.34% of α -helix (blue lane), 38.62% of extended strand (red lane), 12.41% of β -turn (green lane), and 38.62% of random coil (purple lane).

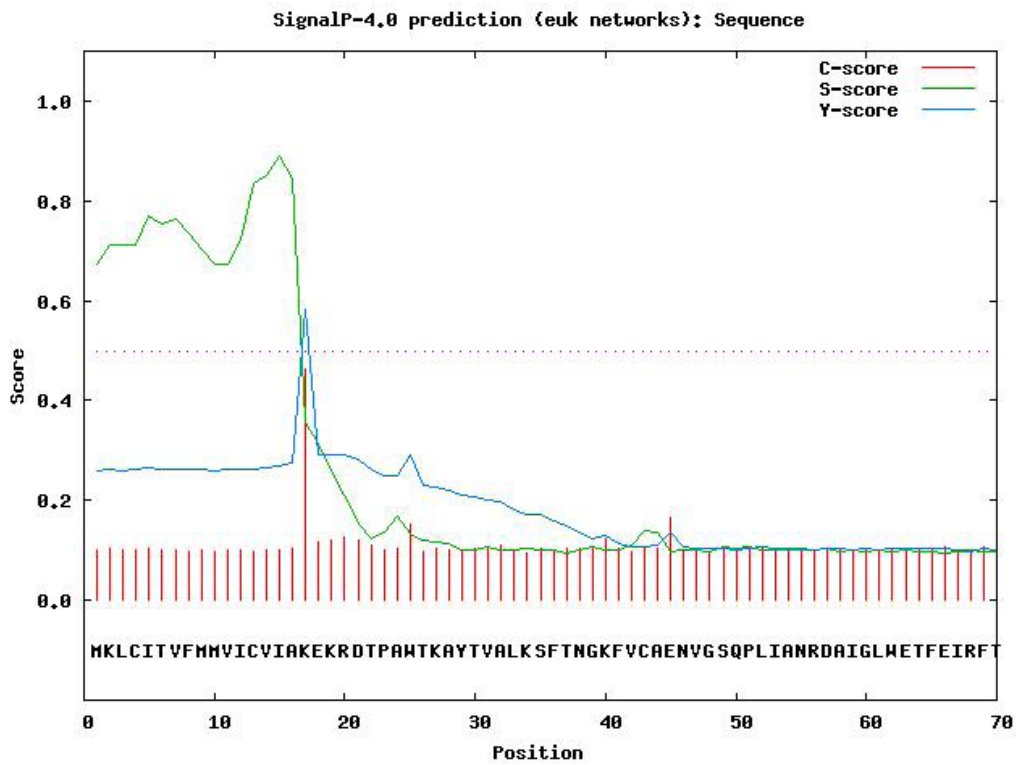


Figure 5. Signal peptide prediction result of PPOD1 from SignalP 4.0 Server analysis.

Analysis of putative conserved domains of PPOD1

The putative conserved domains in the PPOD1 of *H. magnipapillata* from the GenBank Protein Blast server showed that PPOD1 contains 2 fascin-like domains: domain 1 (amino acids 38-154) and domain 2 (amino acids 117-244) (Figure 6). Sequence analysis indicated that the 2 fascin-like domains of *H. magnipapillata* showed 41.86% amino acid identity. Further, fascin-like domains 1 and 2 of *H. magnipapillata* shared 17.69 and 22.30% amino acid identities, respectively, with the domain 1 of human fascin-1 (Figure 7).

A BLAST search in GenBank revealed that, in addition to the fascin-like domain being present in the fascin proteins of Chordata and Hemichordata, it is also present in other proteins (such as dehydrogenase, glycoside hydrolase, peroxidase, and carbohydrate binding protein) of Bacteria and Insecta (Figure 8). The resulting phylogeny based on these amino acid sequences of the fascin-like domains in *H. magnipapillata* and other species showed that the fascin-like domains of *Hydra* are grouped with the fascin-like domains of Bacteria and Insecta, while the Chordata and Hemichordata fascin-like domains form a separate monophyletic clade (Figure 8).

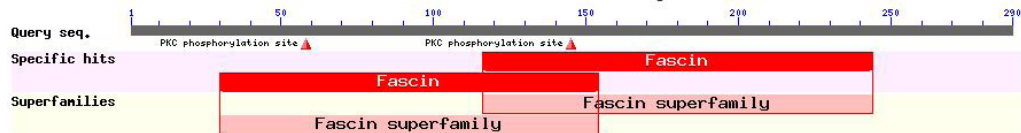


Figure 6. Putative conserved domains of PPOD1 in *Hydra magnipapillata* detected by GenBank Protein Blast server.

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A
hydra_domain_1 -TVALKSFTNGKFKVCAENVGSQLIANRDAICLWETFEIRFTN--AQTFALKSHANGKFF 60
hydra_domain_2 GTFGFKSNGNAKFVAEEAGSKSLAANRNALD VWEMFSLVYVWFSVHKVAIKALVNGLYY 60

hydra_domain_1 SASNGGNLLIANKDQAAVWETFIL-VPNYGTFGFKSNGNAKFVAEEAGSKSLAANRNA 120
hydra_domain_2 CAENAGKQSLIANRQQIGPWETFETIRFDHQTFITLKSLANGKLVCAENNGQSPLIANRDQ 120

hydra_domain_1 LDVWEMFSL 129
hydra_domain_2 IGPWETFITL 129

B
homo_fascin-1 QFGLINCGN-KYLTAFAFG-FKVNASASSLKKKIWILEQPPDEAGSAAVCLRSHL-GRY 60
hydra_domain_1 TVALKSFTNGKFKVCAENVGSQLIANRDAICL--WETFEIRFTNAQ-TFALKSHANGKF 60

homo_fascin-1 LAADKDG--VTCEREVPGDCRFLIVAHDDGRWSLQSEAHRRYFGGTEDRLSCFAQTVS 120
hydra_domain_1 VSAENAGKQSLIANRQQIGPWETFETLVPN-YGTFGFKSNGNAKFVAEEAGSKSLAANRN 120

homo_fascin-1 PAEKW---SV 130
hydra_domain_1 ALD VWEMFSL 130

C
homo_fascin-1 -QFGLINCGN-KYLTAFAFG-FKVNASASSLKKKIWILEQPPDEAGSAAVCLRSHLGRY 60
hydra_domain_2 GTFGFKSNGNAKFVAEEAGSKSLAANRNALD VWEMFSLVYVWFSVHKVAIKALVN-GLY 60

homo_fascin-1 LAADKDG--NVTCEEREVPGDCRFLIVAHDDGRWSLQSEAHRRYFGGTEDR---LSCFAQ 120
hydra_domain_2 VCAENAGKQSLIANRQQIGPWETFETIRFDHQTFITLKSLANGKLVCAENNGQSPLIANRD 120

homo_fascin-1 TVSPAEEKWSV 130
hydra_domain_2 QIGPWETFITL 130

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Figure 7. Alignment of amino acid sequences of fascin-like domains of PPOD1 from *Hydra magnipapillata* and *Homo sapiens*. Identical amino acids are shown on a gray background. *Hydra_domain_1* = fascin-like domain 1 of *Hydra magnipapillata*; *hydra_domain_2* = fascin-like domain 2 of *Hydra magnipapillata*; *homo_fascin-1* = domain 1 of human fascin-1.

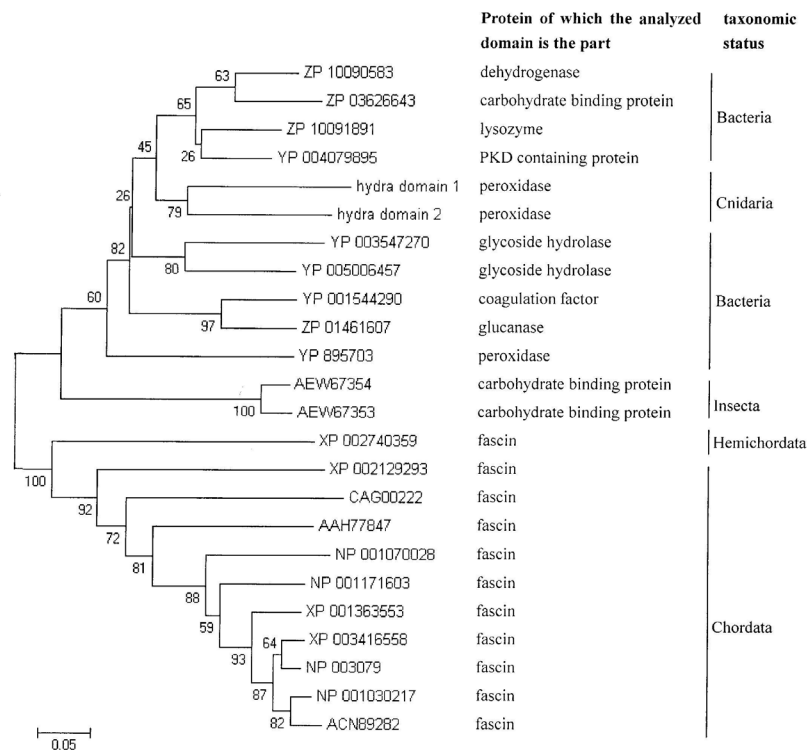


Figure 8. Neighbor-joining (NJ) tree based on amino acid sequences of fascin-like domains in *Hydra magnipapillata* and other species. Bar equals 0.05 units of Kimura's two-parameter distance. Numbers above branches in these trees are Bootstrap values at least 50% of the 1000 bootstrap replications.

Tertiary structure prediction of PPOD1

The 3-D protein model constructed in this study was predicted by SWISS-MODEL automated protein modeling, CPHmodels 3.2, and Geno3D, based on the 3p53A template (human fascin-1). Although the major model structures of these 3 methods are obviously different, they all shared a common characteristic, in which the N-terminal subdomains stretch out from the main domains (Figure 9).

Prokaryotic expression of recombinant PPOD1

The recombinant PPOD1 was expressed in *E. coli* using pET-GST plasmids carrying strong promoter and terminator sequences derived from phage T7. Expression was tested by SDS-PAGE analysis of the protein extracts from the recombinants in *E. coli* BL21 strains (Figure 10). The results indicated that the molecular mass of the fused PPOD1 protein incorporating an N-terminal GST tag and C-terminal His tag was about 40 kDa, as expected, and that the fusion protein formed inclusion bodies. The obtained expression product could be used to purify the protein, prepare PPOD1 antibody, and further study its function.

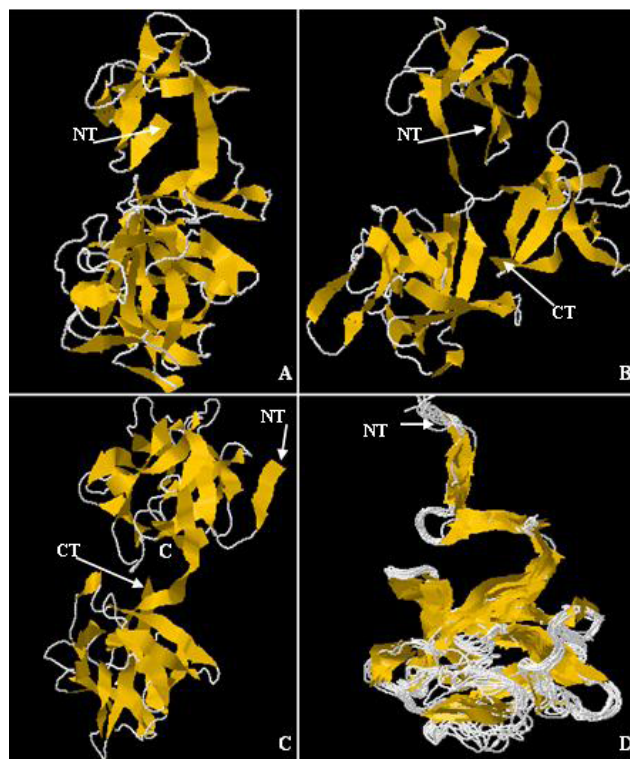


Figure 9. Predicted 3-D structure of PPOD1 from *Hydra magnipapillata*. **A.** 3-D structure prepared with SWISS-MODEL Server; **B.** 3-D structure prepared with SWISS-MODEL Server, and Figure A was turned ninety degrees to become figure B; **C.** 3-D structure prepared with CPHmodels 3.2 Server; **D.** 3-D structure prepared with Geno3D Server; NT = N-terminal subdomain; CT = C-terminal subdomain.

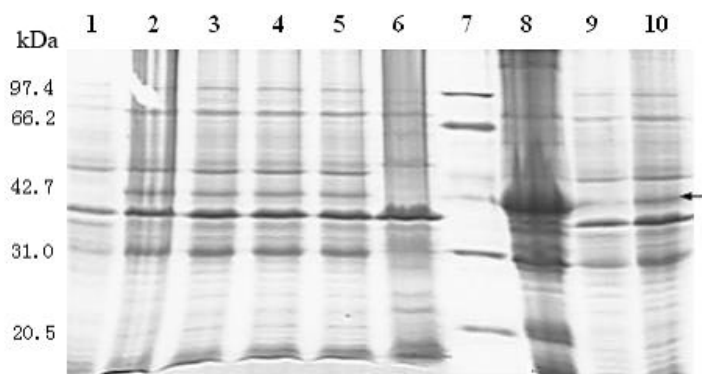


Figure 10. Expression and solubility of recombinant PPOD1 fusion protein analyzed by SDS-PAGE gel stained with Coomassie blue R250. Arrow, pointing to PPOD1 fusion protein; lanes 1-5 = the whole cell protein of BL21(DE3)/pET-GST-PPOD1 induced by IPTG for 0, 1, 2, 3, and 4 h; lane 6 = the whole cell protein of BL21(DE3) induced by IPTG for 4 h; lane 7 = molecule weight marker of protein; lane 8 = the insoluble fraction of BL21(DE3)/pET-GST-PPOD1 induced by IPTG for 4 h after sonic treatment; lane 9 = the soluble fraction of BL21(DE3)/pET-GST-PPOD1 induced by IPTG for 4 h after sonic treatment; lane 10 = the whole cell protein of BL21(DE3)/pET-GST-PPOD1 induced by IPTG for 4 h.

Assay of the region-specific expression of PPOD1

This study shows the PPOD1 expression patterns at the whole-mount level (see Figure 11A). However, the precise expression pattern of PPOD1 on the adhesive face of basal disc, in which the animals use to directly attach to a substrate, is difficult to determine. To investigate the PPOD1 expression pattern on the adhesive face of basal disc, we first observed the attachment process of the basal disc on a glass slide (Figure 11B and C). It was found that ectodermal epithelial cells located on the edge of the adhesive face of the basal disc extended lamellipodia and filopodia when the basal disc was attached tightly to glass slide (Figure 11D). Interestingly, the result showing the PPOD1 expression pattern on the adhesive face of basal disc indicated the presence of high-level PPOD1 activity in the ectodermal epithelial cells located on the edge of the adhesive face, but not in the ectodermal epithelial cells located in the central region of the adhesive face (Figure 11E).

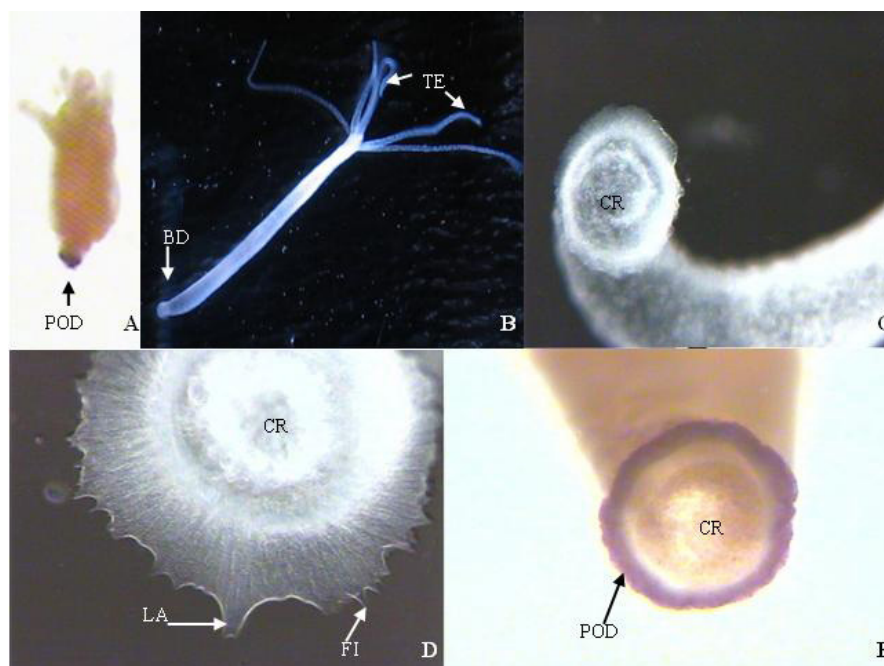


Figure 11. Attachment process of basal disc to glass slide and the assay of region-specific expression of PPOD1. **A.** PPOD1 expression model in a *Hydra* individual; **B.** the *Hydra* body of which basal disc did not attach on glass slide; **C.** the adhesive face of basal disc when basal disc attached just on glass slide; **D.** the adhesive face of basal disc when basal disc was attached tightly to glass slide; **E.** PPOD1 expression model on the adhesive face of basal disc; BD = basal disc; CR = central region; FI = filopodia; LA = lamellipodia; POD = the PPOD1 expression region.

DISCUSSION

Peroxidase activity usually involves donating electrons to other substrates (such as ferrocyanide and ascorbate), which facilitates binding by breaking them into harmless components. Of note, peroxidase enzymes degrade hydrogen peroxide, which is a naturally occurring

byproduct of oxygen metabolism in the body (Fontaine et al., 1990; Rabe and Hillier, 2003). As a result, this substance is converted into water and oxygen. Peroxidases are widely distributed in both plants and animals and contribute to various metabolic tasks. The most important function of peroxidase is probably the protection of cells from oxidative stress; however, peroxidases also contribute to a range of cell processes, such as growth and differentiation, inflammation, phagocytosis, and apoptosis (Behl et al., 1994; Nelson et al., 1994). The basal disc of *Hydra* is the most proximal region of the polyp, and it is the area used by the animal to attach to various substrates. The basal disc is also one of the extremities at which cells die and are sloughed off. Hence, foot-specific peroxidase might be involved in the defense mechanisms of this exposed body region and/or might be involved in the differentiation or aging processes of these cells (Hoffmeister et al., 2002).

However, our observations indicated that high concentrations of foot-specific peroxidase PPOD1 are only present in ectodermal foot mucous cells located on the edge of the adhesive face of the basal disc (Figure 11E). This observation raises the question of what is the biological significance of the region-specific expression pattern of PPOD1. This question requires answering to explore the physiological functions of foot-specific peroxidase. It is likely that studies combining PPOD1 bioinformatic analyses with morphological characteristic assessments of the ectodermal epithelial cells on the edge of the adhesive face of the basal disc might provide novel insights about the physiological functions of foot-specific peroxidase.

The results of our study indicated that PPOD1 contains 2 fascin-like domains (Figure 6). Fascin is a small, globular actin-bundling protein that contributes to the formation of a variety of cell protrusions and cytoplasmic actin bundles (Adams, 2004). For instance, a moving cell attached to a substrate extends lamellipodia and filopodia. Basically, at the tip of a lamellipodium, certain protein factors, such as small G-protein Rac, initiate the dendritic network of actin filaments that push the cell membrane in an outward direction. Subsequently, the clustering of the barbed ends of actin filaments at the lamellipodial tip represents initial step in the formation of filopodia. Then the bundling of these actin filaments supports the protrusion of filopodia from the lamellipodia (Schafer et al., 2009). Existing evidence indicates that fascin is the main actin filament bundling protein in filopodia (Svitkina et al., 2003), and that it tends to localize on the filopodia of various types of cells from invertebrates to mammals (Khurana and George, 2011).

In the present study, we demonstrated that the ectodermal epithelial cells located on the edge of the adhesive face of the basal disc harbor peroxidase activity (Figure 11E), in addition to extending lamellipodia and filopodia when the basal disc tightly attaches to a glass slide (Figure 11D). Therefore, the fascin-like domains of *Hydra* PPOD1 probably contributes to the bundling of the actin filament of these cells, which ultimately leads to the formation of filopodia. This phenomenon might contribute toward strengthening the adsorbability of the basal disc to a substrate. In other words, the foot-specific peroxidase PPOD1 might have dual activities of peroxidase and fascin protein.

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