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ORIGINAL ARTICLE

NovQ is a prenyltransferase capable of catalyzing the addition of a dimethylallyl group to both phenylpropanoids and flavonoids

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NovQ is a member of a recently identified CloQ/NphB class of prenyltransferases. Although NphB has been well characterized as a prenyltransferase with flexibility against aromatic substrates, few studies have been carried out on characterization of NovQ. Hence, in this study, we investigate the kinetics, substrate specificity and regiospecificity of NovQ. The corresponding *novQ* gene was cloned from *Streptomyces niveus,* which produces an aminocoumarin antibiotic, novobiocin. Recombinant NovQ was overexpressed in *Escherichia coli* and purified to homogeneity. The purified enzyme was a soluble monomeric 40-kDa protein that catalyzed the transfer of a dimethylallyl group to 4-hydroxyphenylpyruvate (4-HPP) independently of divalent cations to yield 3-dimethylallyl-4-HPP, an intermediate of novobiocin. Steady-state kinetic constants for NovQ with the two substrates, 4-HPP and dimethylallyl diphosphate, were also calculated. In addition to the prenylation of 4-HPP, NovQ catalyzed carbon–carbon-based and carbon–oxygen-based prenylations of a diverse collection of phenylpropanoids, flavonoids and dihydroxynaphthalenes. Despite its catalytic promiscuity, the NovQ-catalyzed prenylation occurred in a regiospecific manner. NovQ is the first reported prenyltransferase capable of catalyzing the transfer of a dimethylallyl group to both phenylpropanoids, such as *p*-coumaric acid and caffeic acid, and the B-ring of flavonoids. This study shows that NovQ can serve as a useful biocatalyst for the synthesis of prenylated phenylpropanoids and prenylated flavonoids.
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Keywords: chemoenzymatic synthesis; prenylated phenylpropanoids; prenylated flavonoids; prenylation; prenyltransferase; *Streptomyces*; substrate specificity

INTRODUCTION

Natural products with one or more prenyl groups have been isolated predominantly from higher plants. These compounds, originating from multiple natural product classes, such as flavonoids, phenylpropanoids and polyketides, often possess anti-microbial, anti-oxidant, anti-inflammatory, anti-viral or anti-cancer activities.[1–4] For example, prenylated phenylpropanoids, drupanin, artepillin C and baccharin, which are *p*-coumaric acid derivatives with one or two prenyl groups, have been shown to induce an apoptotic event in human leukemia cell line HL60 and colon cancer cell line SW480.[5] Moreover, it has been reported that the oral administration of these prenylated phenylpropanoids to mice allografted with sarcoma S-180 causes a significant reduction in tumor growth.[6] In contrast, *p*-coumaric acid, which is non-prenylated, does not show these anti-cancer activities. Thus, given their enhanced and distinct activities, prenylated flavonoids and phenylpropanoids show promise as lead compounds for the development of nutraceuticals in plants and as new pharmacological agents for the treatment of human diseases.[1–4] However, as prenylated compounds often exist at trace levels in natural sources, they are seldom amenable to cost-effective synthesis.

Biocatalysts with relaxed substrate specificity displaying regiospecificity in prenyl group transfer can serve as an alternative production platform for prenylated compounds. Recently, we identified a newly described functional class of prenyltransferases, NphB and SCO7190 in *Streptomyces* strains.[7] These prenyltransferases accept a diverse collection of hydroxyl-containing aromatic substrates, such as flavonoids, to yield the corresponding prenylated products.[8]

Two prenyltransferases belonging to the same class as NphB, CloQ and NovQ have been identified. Both CloQ, isolated from *Streptomyces roseochromogenes*,[9] and NovQ, from *S. spheroides*,[10] are involved in the biosynthesis of the aminocoumarin antibiotics clorobiocin and novobiocin, respectively. They share 84% amino acid sequence identity. Purified recombinant CloQ catalyzes the formation of 3-dimethylallyl-4-HPP, a common intermediate of the aminocoumarin antibiotics, from 4-hydroxyphenylpyruvate (4-HPP) and dimethylallyl diphosphate (DMAPP) independently of divalent cations (Figure 1).[9] However, the enzymatic function of NovQ has not been fully elucidated and few studies have been carried out on substrate specificities of the CloQ/NovQ class of enzymes.

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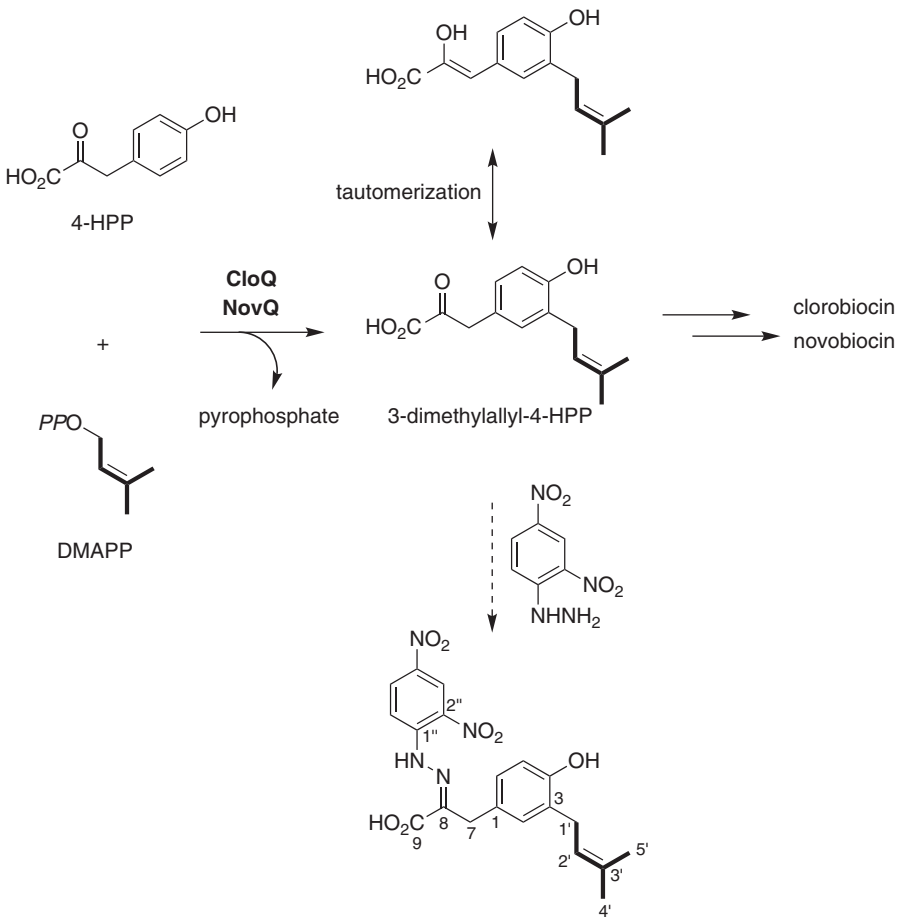


Figure 1 CloQ- and NovQ-catalyzed reaction in clorobiocin and novobiocin biosyntheses. The dashed line represents a derivatization reaction with 2,4-dinitrophenylhydrazine.

Here, we report the cloning of the *novQ* gene from another novobiocin-producing *Streptomyces* strain, *S. niveus* 16259,[11] and the kinetics and relaxed substrate specificity of the purified recombinant NovQ enzyme. NovQ catalyzes carbon–carbon-based and carbon–oxygen-based prenylations of a diverse collection of phenylpropanoids, flavonoids and dihydroxynaphthalenes (DHNs). Despite its catalytic promiscuity, NovQ-catalyzed prenylation occurs in a regiospecific manner. This is the first report showing that the bacterial enzyme NovQ can be used as a biocatalyst for the synthesis of both prenylated phenylpropanoids and prenylated flavonoids.

RESULTS

Expression and purification of recombinant NovQ protein

The *novQ* gene from *S. niveus* 16259 was overexpressed in *E. coli* as an N-terminal His_8-tagged protein, and the recombinant protein was purified to apparent homogeneity (Figure 2). The molecular mass of NovQ was estimated to be 40 kDa by SDS-polyacrylamide gel electrophoresis (Figure 2) and by gel filtration chromatography, suggesting that NovQ is likely a monomer.

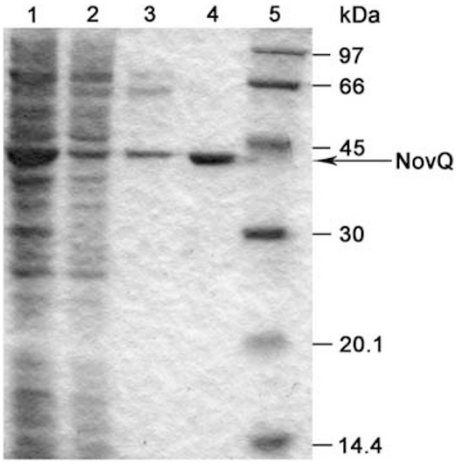


Figure 2 Expression and purification of the recombinant NovQ enzyme. A total of 12% SDS-polyacrylamide gel electrophoresis of the enzyme. Lanes: 1, total protein after the induction; 2, soluble protein after the induction; 3, fraction eluted with 250 mM imidazole from a Ni-NTA Superflow column; 4, purified enzyme after gel filtration chromatography and 5, low molecular mass markers.

Identification of the reaction product of NovQ

We next attempted to detect the formation of 3-dimethylallyl-4-HPP by the action of NovQ on 4-HPP and DMAPP. Initial efforts to directly detect the formation of 3-dimethylallyl-4-HPP were carried out using HPLC, but the results were inconclusive. This was thought to be because of an equilibrium between the keto and enol forms of 3-dimethylallyl-4-HPP synthesized by NovQ. Therefore, we decided to derivatize the NovQ reaction product with 2,4-dinitrophenylhydrazine to yield a dinitrophenylhydrazone, because 2,4-dinitrophenylhydrazine

is often used to detect compounds, such as 4-HPP with a keto group. After derivatization, a major dinitrophenylhydrazone derivative was purified by HPLC and identified as 3-dimethylallyl-4-HPP-dinitrophenylhydrazone from the HR-MS, ^{1}H NMR and ^{13}C NMR spectral data, unequivocally showing that the NovQ cloned in this study catalyzes the addition of a dimethylallyl group to 4-HPP to yield 3-dimethylallyl-4-HPP (Figure 1).

Steady-state kinetic studies of NovQ

For steady-state kinetic studies of NovQ, we used a spectrophotometric NovQ prenyltransferase assay using a coupled system with a pyrophosphate reagent, because the NovQ prenyltransferase forms a pyrophosphate anion co-product during catalysis (Figure 1). This assay quantifies product formation by the concomitant oxidation of NADH. From initial velocity measurements, we determined that the NovQ reaction followed Michaelis–Menten kinetics. Apparent K_m values were determined to be $33.3 \pm 4.7\,\mu M$ for 4-HPP and $15.1 \pm 1.9\,\mu M$ for DMAPP at fixed saturating concentrations of DMAPP (1 mM) and 4-HPP (1 mM), respectively (Supplementary Table S1). The turnover number of the reaction was $5.0 \pm 0.1\,min^{-1}$.

Substrate specificity of NovQ

Next, we examined the substrate specificity of NovQ. We focused on the following prenyl acceptors: phenylpropanoids, including *p*-coumaric acid and caffeic acid; the plant polyketides, including resveratrol and olivetol; the (iso)flavonoid sub-class, including naringenin, apigenin, daidzein and genistein; and DHNs, including 1,3-DHN, 1,6-DHN and 2,7-DHN. We first incubated NovQ with naringenin in the presence of DMAPP (C_5) or geranyl diphosphate (C_{10}). The resultant prenyltransferase activity of NovQ was detected only in the presence of DMAPP. Therefore, in the further experiments, each substrate was incubated with NovQ in the presence of DMAPP. All substrates, except for olivetol and 1,3-DHN, underwent facile prenylation, yielding between one and three products. The prenylation activity was independent of divalent cations, such as Mg^{2+}. Even in the presence of 5 mM EDTA, NovQ possessed full enzymatic activity (Figure 3). Thus, we concluded that NovQ, similar to CloQ,[9] is a magnesium-independent prenyltransferase (Supplementary Table S1).

NovQ-catalyzed reaction products were purified by preparative HPLC, and their structures were elucidated primarily by comparison of the ^{1}H NMR spectra of the isolated products with their corresponding substrates. Most reaction products contained a dimethylallyl group in the *ortho* position with respect to a neighboring hydroxy moiety. In addition to this carbon–carbon-based prenylation, NovQ catalyzed carbon–oxygen-based prenylation in some cases.

Phenylpropanoids. With two phenylpropanoids, *p*-coumaric acid and caffeic acid, NovQ catalyzed the prenylation of a hydroxy group, leading to the formation of an *O*-prenyl linkage (Table 1). Interestingly, NovQ appended two dimethylallyl groups to caffeic acid to yield 3-*O*,4-*O*-di-(dimethylallyl) caffeic acid. In addition to this *O*-prenylation, NovQ prenylated the C-3 of *p*-coumaric acid to yield drupanin (3-dimethylallyl coumaric acid).

Plant polyketides. Of the two plant polyketides tested, resveratrol and olivetol, NovQ used only resveratrol as a substrate, prenylating it at C-3′ (Table 1).

Flavonoids. NovQ prenylated all flavonoids tested as substrates (Table 2). Incubation with naringenin and genistein yielded two products with a dimethylallyl group at C-3′ or *O*-4′ in the B-ring. In contrast, C-3′ specific prenylation in the B-ring occurred with apigenin and daidzein.

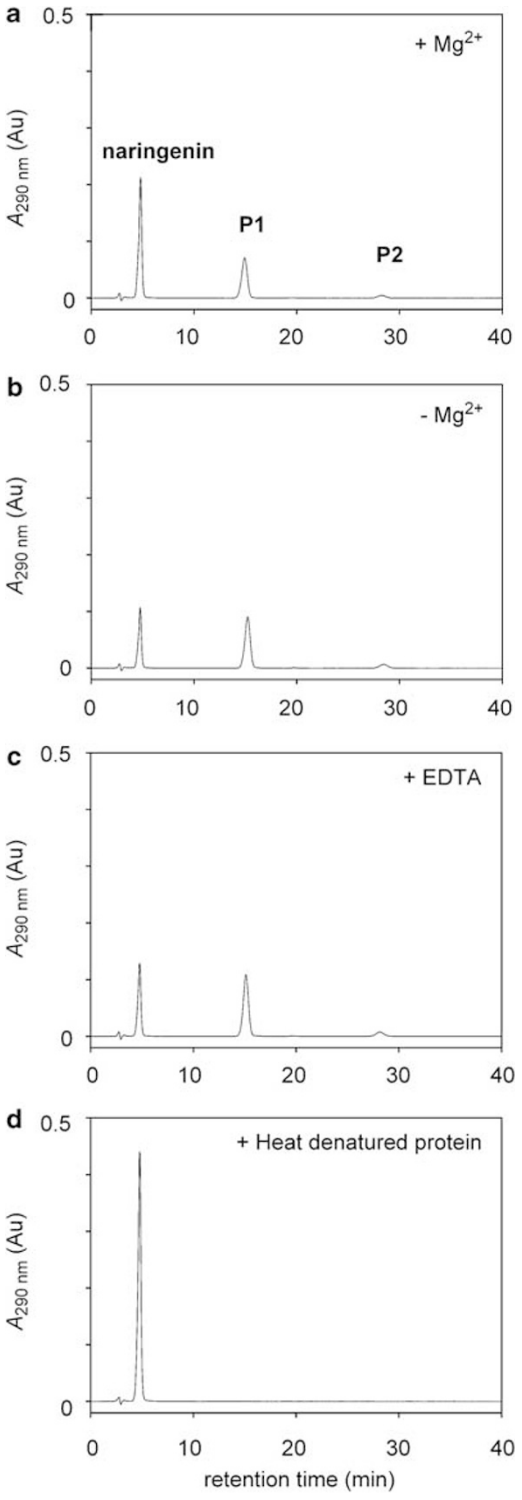
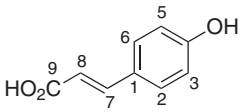
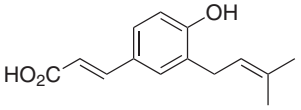
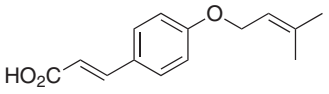
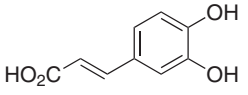
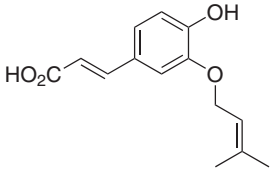
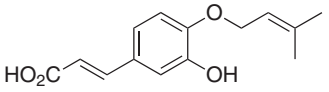
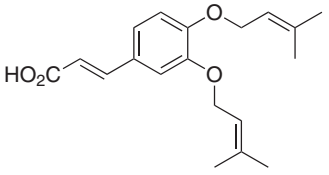
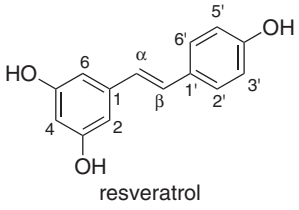
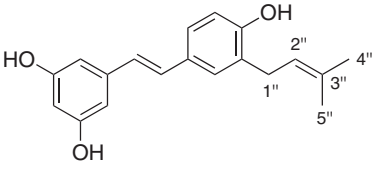
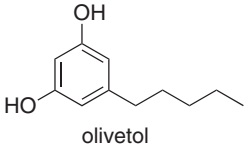


Figure 3 HPLC analysis of prenylated products formed from naringenin by the action of NovQ. After incubation for 1 h with NovQ (**a**) in the presence of 2.5 mM Mg^{2+}, (**b**) in the absence of Mg^{2+} or (**c**) in the presence of 5 mM EDTA. (**d**) After incubation for 1 h with heat-denatured NovQ. **P1**, 3′-dimethylallyl naringenin; **P2**, 4′-*O*-dimethylallyl naringenin.

Table 1 Prenylated phenylpropanoids and plant polyketides synthesized by NovQ

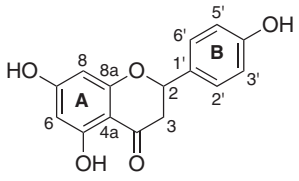
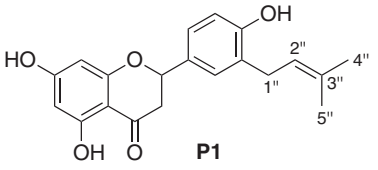
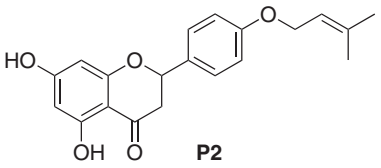
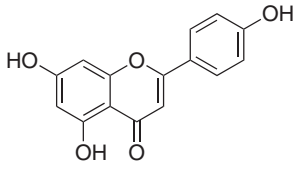
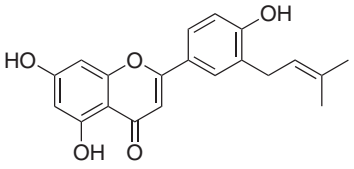
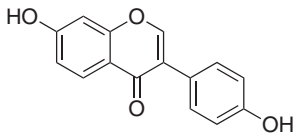
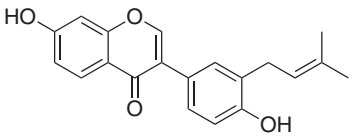
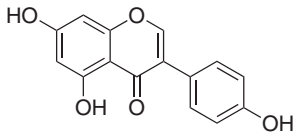
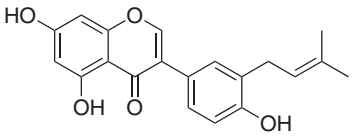
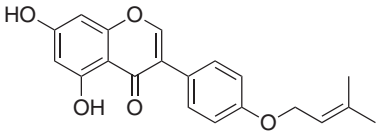
Substrate	*Product*	*Yield (%)*
 *p*-coumaric acid		16.8
		34.4
 caffeic acid		17.3
		26.1
		20.0
 resveratrol		12.3
 olivetol	N.P.	-

Abbreviation: N.P., no products.

Dihydroxynaphthalenes. NovQ accepted 1,6-DHN and 2,7-DHN as substrates to yield three and one products, respectively, but it did not accept 1,3-DHN (Table 3). The main product of 1,6-DHN was 1-*O*-dimethylallyl-1,6-DHN. Other products were prenylated at C-4 or C-2 of 1,6-DHN. Interestingly, NovQ catalyzed attachment of the C-3′ carbon of the dimethylallyl group to 1,6-DHN. Reaction with 2,7-DHN yielded only 1-dimethylallyl-2,7-DHN.

In addition, we determined the yields of prenylated products formed after a 24-h incubation with NovQ. Naringenin was most highly converted to its prenylated derivatives, with a yield of 98.3%. On the other hand, the yield of the prenylated products of the other substrates ranged from 2.5 to 63.4%. The ratio of *C*-prenylated and *O*-prenylated products was dependent on substrates. For example, the major product in the presence of the naringenin substrate was

Table 2 Prenylated flavonoids synthesized by NovQ

Substrate	*Product*	*Yield (%)*
 naringenin	 **P1**	87.2
	 **P2**	11.1
 apigenin		7.6
 daidzein		2.5
 genistein		2.3
		5.3

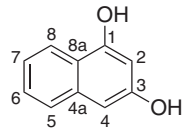
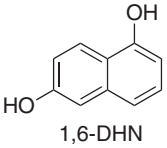
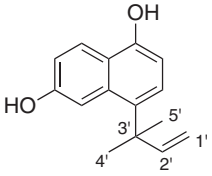
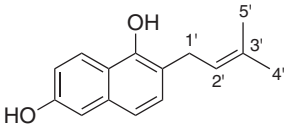
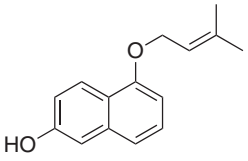
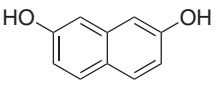
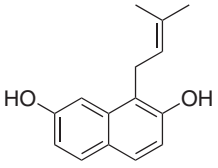
C-prenylated, whereas *O*-prenylated forms were the major products of genistein, *p*-coumaric acid and 1,6-DHN. With caffeic acid, only *O*-prenylated products were synthesized at a high yield (63.4%).

DISCUSSION

In this study, we examined the kinetics and substrate specificity of the NovQ gene, which was cloned from a novobiocin-producing *S. niveus* 16259. The recombinant NovQ enzyme showed enzymatic properties similar to those of the previously characterized CloQ,[9] as summarized in Supplementary Table S1, probably reflecting the high sequence identity (84%) between these enzymes.

To date, CloQ/NovQ enzymes have been considered prenyltransferases that are highly specific to 4-HPP.[9,10] The recombinant CloQ enzyme did not accept related aromatic compounds, such as 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, and L-tyrosine, as substrates,[9] and nor did a partially purified NovQ enzyme from *S. spheroides* NCIMB 11891 accept *p*-coumaric acid or 4-hydroxybenzoic acid as substrates.[10] These results had prevented us from thoroughly elucidating the substrate specificity of the CloQ/NovQ enzymes, although these enzymes are potential biocatalysts for the production of diverse prenylated compounds. However, in this study, we clearly showed for the first time that NovQ also accepts a diverse

Table 3 Prenylated DHNs synthesized by NovQ

Substrate	*Product*	*Yield (%)*
 1,3-DHN	N.P.	-
 1,6-DHN		1.5
		6.4
		49.8
 2,7-DHN		31.0

Abbreviations: DHNs, dihydroxynaphthalenes; N.P., no products.

collection of hydroxy-containing aromatic substrates, such as phenylpropanoids, flavonoids and DHNs, to yield the corresponding prenylated products. It is noted that NovQ showed substrate specificities and regiospecificities that were substantially different from those of NphB.[8] For example, NovQ catalyzed the prenylation of phenylpropanoids, such as *p*-coumaric acid and caffeic acid (Table 1), neither of which is prenylated by NphB.[8] In addition, NovQ appended a dimethylallyl group to the B-ring of flavonoids (Table 2), whereas NphB appends a geranyl group to the A-ring of flavonoids.[8] These differences may be ascribed to the low sequence identity (19%) between NovQ and NphB. On the other hand, the substrate specificity of CloQ is probably similar to that of NovQ, because of the high sequence identity of these enzymes.

We recently solved an architecturally new crystal structure of NphB.[7] Keller *et al.*[12] reported the crystallization and preliminary X-ray analysis of CloQ. Thus, the potential of employing these biocatalysts using structure-based enzyme engineering provides a convenient starting point for exploring novel prenylation chemistry and the bioactivity of aromatic compounds.[13–15] In addition, such rationally based engineering of NphB and CloQ/NovQ will provide another powerful synthetic tool with which to expand the diversity and bioactivities of many synthetic and natural compounds through enzyme-directed regiospecific prenylation.

EXPERIMENTAL SECTION

Cloning of the *novQ* gene from *S. niveus* 16259

On the basis of the nucleotide sequences 80 bp upstream and 58 bp downstream of the *novQ* gene previously cloned from *S. spheroides* NCIMB 11891 (accession no. AF170880),[9] oligonucleotide primers pNOVQN1 (5′-GAACT GATCACGATCGACCG-3′) and pNOVQC1 (5′-TCGAACACCGGCGGCT GACG-3′) were synthesized (Operon Biotechnologies, Tokyo, Japan). These primers were used to amplify the DNA fragment containing the *novQ* gene from the *S. niveus* 16259[11] genome by PCR. The PCR-amplified 1.1-kb DNA fragment was cloned into a pT7Blue vector (Takara Bio, Tokyo, Japan) to give pT7BnovQ. Sequence analysis of the 1.1-kb DNA fragment revealed one complete open reading frame that is completely identical to that of the *novQ* gene from *S. spheroides* NCIMB 11891.[9]

Construction of an expression plasmid for the *novQ* gene

PCR amplification using pT7BnovQ and oligonucleotides for ligation into the *E. coli* expression vector pHIS8[16] was carried out with the forward primer 5′-GGGGGGCCATGGACCCGCACTCCCGATGAATC-3′ (*Nco*I site underlined) and the reverse primer 5′-GGGGGGGGATCCTCATCGGGCACCTC

CGGTG-3′ (*Bam*HI site underlined) (Operon Biotechnologies) to generate pHis8novQ. The pHis8novQ construct was transformed into *E. coli* BL21(DE3), and the *novQ* gene was overexpressed as previously described.[16]

Recombinant NovQ purification

For protein extraction, cells were suspended in lysis buffer (50 mM Tris-HCl (pH8.0), 500 mM NaCl, 20 mM imidazole, 20% (w/v) glycerol and 1% Tween 20). The cell suspensions were sonicated with a Branson Sonifier 250 (Emerson Japan, Tokyo, Japan). To separate the cellular debris from the soluble protein, the lysate was centrifuged at 17 000 rpm at 4 °C for 20 min. NovQ was purified from the resulting supernatant with a Ni-NTA Superflow resin (Qiagen, Tokyo, Japan). After washing with wash buffer containing 50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 20% (w/v) glycerol and 20 mM imidazole, NovQ was eluted using the same buffer containing 250 mM imidazole. After elution, NovQ was dialyzed against 75 mM Tris-HCl (pH 7.5) buffer containing 100 mM NaCl to remove glycerol and imidazole.

Molecular weight analysis

The molecular weight of His_8-NovQ was determined by gel filtration chromatography on a HiLoad 26/60 Superdex 75pg column (GE Healthcare Bio-Sciences, Tokyo, Japan) that had been equilibrated with 75 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl. The column was calibrated with a Gel Filtration Calibration Kit LMW (GE Healthcare Bio-Sciences) that included conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa). The proteins were eluted with 75 mM Tris-HCl (pH7.5) containing 100 mM NaCl.

Conditions for enzymatic reactions

All NovQ assays were carried out in 75 mM Tris-HCl (pH 7.5) containing 2.5 mM $MgCl_2$, 0.25 mM aromatic substrate, 0.5 mM DMAPP and 1 mg ml^{-1} NovQ, in a total volume of 300 µl. The reaction mixtures were incubated at 30 °C for 24 h. After incubation, the reaction mixtures were freeze-dried, and the residues were dissolved in 100 µl of methanol. The reaction products were analyzed by HPLC equipped with an MD-2010 Plus photodiode array (JASCO, Tokyo, Japan) with PEGASIL ODS (4.6×250 mm, Senshu Scientific, Tokyo, Japan) using an isocratic elution of 70% methanol with 0.1% acetate or a linear gradient (30 min) of 50–100% methanol with 0.1% acetate. The yield of each product was defined as the ratio of the peak area of the prenylated product to the sum of those of the remaining substrate and all prenylated products.

Steady-state kinetic parameters

A spectrophotometric NovQ prenyltransferase assay using a coupled system with a pyrophosphate reagent (Sigma-Aldrich Japan, Tokyo, Japan) was used for steady-state kinetic studies of NovQ, because the NovQ prenyltransferase forms a pyrophosphate anion co-product during catalysis (Figure 1). Prenyltransferase activity was assayed in 75 mM Tris-HCl (pH 7.5) containing 2.5 mM $MgCl_2$, 4-HPP, DMAPP and 267 µl of the pyrophosphate reagent in a total volume of 800 µl. When the concentration of DMAPP was fixed at 1 mM, the concentrations of 4-HPP were varied at 25, 50, 100 and 500 µM. With a fixed concentration of 1 mM 4-HPP, the concentrations of DMAPP were varied: 12.5, 25, 50 and 100 µM. After the reaction mixture containing no enzyme was incubated at 30 °C for 5 min, the reaction was started by adding 117 µg of NovQ. NovQ-dependent oxidation of NADH was monitored in a UV-1600PC spectrophotometer (Shimadzu, Kyoto, Japan) equipped with a cell holder CPS-240A (Shimadzu) adjusted at 30 °C. Initial velocities were determined from the slope of a plot of NADH consumption versus incubation time. The molar extinction coefficient (ε) of NADH at 340 nm was 6220. Steady-state kinetic parameters were calculated using the SigmaPlot 10.0 software and Enzyme Kinetics Module 1.3 (Systat Software, Point Richmond, CA, USA).

Product analysis

Large-scale production of prenyl products was carried out in 75 mM Tris-HCl (pH 7.5) containing 2.5 mM $MgCl_2$, 2.5 mM aromatic substrate, 5 mM DMAPP and 1 mg ml^{-1} NovQ, in a total volume of 10 ml. The reaction mixtures were incubated overnight at 25 °C, and then extracted thrice with 10 ml ethyl acetate. When 4-HPP was used as a substrate, the reaction mixture was incubated at 30 °C for 1 h and then mixed with an equal volume of a 2,4-dinitrophenylhydorazine solution (0.25 mM in 2 M HCl) for derivatization. After 1 h of incubation with continuous stirring, the reaction mixture was extracted thrice with 10 ml of ethyl acetate. After drying over Na_2SO_4, the ethyl acetate extract was evaporated *in vacuo* and the residue was dissolved in 1 ml of methanol. Prenylated products were further purified by preparative HPLC with PEGASIL ODS (20 x 250 mm, Senshu Scientific) using an isocratic elution of 70% methanol containing 0.1% acetate. For the dinitrophenylhydrazone derivative, 80% methanol containing 0.1% trifluoroacetic acid was used. The structures of the reaction products were analyzed by their ^{1}H NMR, ^{13}C NMR and HMBC spectroscopic data (600 MHz, JEOL ECA-600, JEOL, Tokyo, Japan) and HR-MS data (JEOL JMS-T100LC, JEOL, Tokyo, Japan). MS analysis was performed using electrospray ionization (ESI) in negative ion mode.

3-dimethylallyl-4-HPP-dinitrophenylhydrazone

The above product was synthesized from 4-HPP and DMAPP by the recombinant NovQ and derivatized with 2,4-dinitrophenylhydrazine. HR-MS (ESI^-) *m/z* 427.12249 (calcd for $C_{20}H_{19}N_4O_7^-$ 427.12537). ^{1}H NMR (600 MHz, MeOH-*d*4) δ 1.63 (s, 3H, H-5′), 1.66 (s, 3H, H-4′), 3.20 (d, J = 7.5 Hz, 2H, H-2′), 3.98 (s, 2H, H-7), 5.24 (t, J = 7.6 Hz, 1H, H-2′), 6.66 (d, J = 8.2 Hz, 1H, H-5), 6.94 (d, J = 8.3 Hz, 1H, H-6), 7.01 (s, 1H, H-2), 8.20 (d, J = 9.6 Hz, 1H, H-6″), 8.40 (dd, J = 2.0, 9.6 Hz, 1H, H-5″), 8.98 (d, J = 2.0 Hz, 1H, H-2″). ^{13}C NMR (MeOH-*d*4) δ 14.9 (C-5′), 23.1 (C-4′), 26.2 (C-1′), 29.2 (C-7), 113.4 (C-5), 115.8 (C-6″), 120.7 (C-2′), 120.7 (C-3″), 122.9 (C-1), 124.9 (C-6), 127.1 (C-3), 127.9 (C-2), 128.1 (C-5″), 129.7 (C-2″), 130.4 (C-3′), 138.1 (C-4″), 142.4 (C-1″), 145.6 (C-8), 152.4 (C-4), 165.4 (C-9).

Accession number

The nucleotide sequence of the 1.1-kb DNA fragment including the *novQ* gene from *S. niveus* 16259 has been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under accession number AB496950.

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