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Candida albicans NADPH-P450 reductase: Expression, purification, and characterization of recombinant protein

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ABSTRACT

Candida albicans is responsible for serious fungal infections in humans. Analysis of its genome identified *NCP1* gene coding for a putative NADPH-P450 reductase (NPR) enzyme. This enzyme appears to supply reducing equivalents to cytochrome P450 or heme oxygenase enzymes for fungal survival and virulence. In this study, we report the characterization of the functional features of NADPH-P450 reductase from *C. albicans*. The recombinant *C. albicans* NPR protein harboring a 6×(His)-tag was expressed heterologously in *Escherichia coli*, and was purified. Purified *C. albicans* NPR has an absorption maximum at 453 nm, indicating the feature of an oxidized flavin cofactor, which was decreased by the addition of NADPH. It also evidenced NADPH-dependent cytochrome *c* or nitroblue tetrazolium reducing activity. This purified reductase protein was successfully able to substitute for purified mammalian NPR in the reconstitution of the human P450 1A2-catalyzed O-deethylation of 7-ethoxyresorufin. These results indicate that purified *C. albicans* NPR is an orthologous reductase protein that supports cytochrome P450 or heme oxygenase enzymes in *C. albicans*.

1. Introduction

Candida albicans is a well-known pathogenic yeast that causes the opportunistic oral and vaginal infections in humans [1–3]. Systemic fungal infections have emerged as important causes of morbidity and mortality in immunocompromised patients, including those suffering with HIV, cancer chemotherapy, or organ/bone marrow transplantation [4,5]. We recently purified and characterized the first cytochrome P450 (P450, CYP), CYP52A21, and heme oxygenase (CaHmx1) from *C. albicans* and initially regarded these enzymes as targets for the treatment of *Candida* infections [6,7]. An analysis of the genome of *C. albicans* was recently completed [8]. It was found to contain approximately 10 putative cytochrome P450 enzymes (including CYP51, CYP52, and CYP61), and NADPH-P450 reductase (NPR) allowing for the transfer of electrons to cytochrome P450 enzymes (http://www.candidagenome.org/). Several NPR genes have been previously discovered and characterized in soil yeasts, including *C. bombicola* and *C. maltosas* [9,10]. These NPRs provide *Candida* P450 enzyme-reducing equivalents to assimilate a variety of hydrocarbon compounds. Similarly, NPR from *C. albicans* appears to be involved in metabolic processes that use the host lipid in animals necessary for its pathogenesis or survival.

Yeast P450 enzymes are generally class II family; they are associated with the endoplasmic reticulum and a single NPR drives all P450 enzymes, as is also the case in humans [11]. These enzymes are flavoproteins that contain FAD and FMN as cofactors [12]. The crystal structure of NPR from *Saccharomyces cerevisiae* was previously determined by Lamb et al. and the roles of two different FMN-binding sites were proposed as a mechanism underlying electron transfer [13]. Homologous NPRs from *C. bombicola* and *C. maltosas* were expressed successfully and the reduction activities of cytochrome *c* were noted, although their characterizations were analyzed only at the cell lysate levels [9,10].

In this study, we have successfully cloned, overexpressed, and purified for the first time an NADPH-P450 reductase from *C. albicans*, and characterized this protein in terms of its structural, spectroscopic, and catalytic properties.

2. Materials and methods

2.1. Materials

Nitroblue tetrazolium, 7-ethoxyresorufin, sodium dithionite, cytochrome *c* (from horse heart), and NADPH were purchased from Sigma (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI). Ni^{2+}–nitrilotriacetate agarose was purchased from Qiagen, (Valen-

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cia, CA). Other chemicals were of the highest commercially available grade. *Escherichia coli* DH5α cells were purchased from Invitrogen (Carlsbad, CA).

2.2. Construction of expression plasmids

The general approach has been previously described [7,14]. The genomic DNAs from *C. albicans* were generously provided by the laboratory of Professor Won-Ki Huh of Seoul National University. The open reading frame for NADPH-P450 reductase, and an added 6×His-C-terminal tag, were amplified using PCR with forward and reverse primers (5′-cgaatCATATGgcattagacaaatta-3′, 5′-caagaagatgtttggcatcaccatcaccatcactaaaagcttccggTCTAGAaataat-3′) and the amplified PCR fragment was cloned into the pCW(Ori$^+$) vector using the NdeI and XbaI restriction sites. The cloned vectors were verified by nucleotide sequencing analysis and restriction digestion.

2.3. Enzymes expression and purification

The expression and purification of the *C. albicans* NPR enzyme were conducted as described previously, with some modifications [15,16]. In brief, the *E. coli* DH5α strains transformed with pCW(Ori$^+$) vectors were inoculated into TB medium containing 100 μg/ml ampicillin and 1.0 mM IPTG. The expression cultures were grown for 4 h at 37 °C and then for 16 h at 28 °C with shaking at 200 rpm in 1 l Fernbach flasks. Bacterial inner membrane fractions containing NPR from *C. albicans* were isolated and prepared from 1 l of TB (with ampicillin, 100 μg/ml) expression cultures. The NPR enzyme was purified using a Ni^{2+}–nitrilotriacetate column as described previously [16,17]. Briefly, the membranes were solubilized overnight at 4 °C in 100 mM Tris–HCl buffer (pH 7.4) containing 20% glycerol, 0.3 M NaCl, 1 mM EDTA, 1 mM DTT, riboflavin (0.1 μg/ml), 1.0% CHAPS (w/v), and 0.5% Tergitol NP-10 (v/v). The solubilized fraction was then loaded onto a Ni^{2+}–nitrilotriacetate column and the purified protein was acquired with an elution buffer containing 300 mM imidazole. The NPR-containing eluted fraction was subsequently dialyzed at 4 °C against 50 mM potassium phosphate buffer (pH 7.7) containing 20% glycerol, and 0.1 mM EDTA.

2.4. Reduction activity assays

The reduction activity of the purified *C. albicans* NPR protein was analyzed via cytochrome *c* assays with some modifications [18]. The increase in absorbance at 550 nm was evaluated after

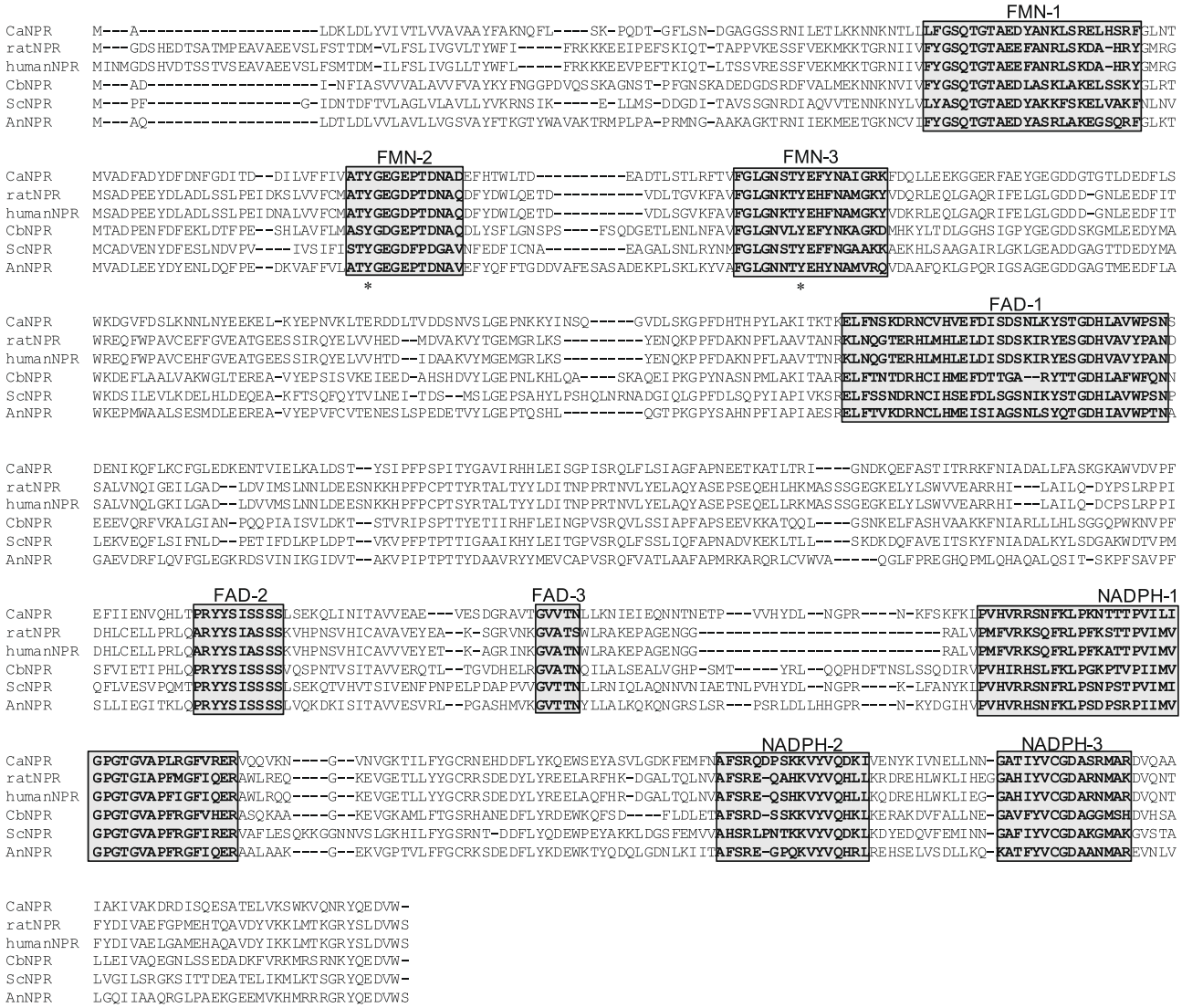

Fig. 1. Sequence alignment of *C. albicans* NPR with other NPR enzymes. The amino acid sequences were aligned using the T-Coffee software (http://www.tcoffee.org). The residues corresponding to the putative FMN-, FAD-, and NADPH-binding domains are shown in bold with boxes. The alignment scores of *C. albicans* NPR to rat, human, *C. bombicola*, *S. cerevisiae*, and *A. niger* NPRs were 85, 86, 92, 94, and 87, respectively. * indicates the conserved Tyr residues in the FMN-binding domains.