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Short communication

Mutation of Cysteine-115 to Alanine in *Nicotiana glutinosa* Ornithine Decarboxylase Reduces Enzyme Activity

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Ornithine decarboxylase (ODC, EC 4.1.1.17) is the first and key enzyme in eukaryotic polyamine biosynthesis. The cDNA encoding ornithine decarboxylase from *Nicotiana glutinosa* was cloned (GenBank™ AF 323910) and expressed in *E. coli.* Site directed mutagenesis were performed on several highly conserved cysteine residues. Among the mutants, C115A showed significant changes in the kinetic properties. The K_m value of the C115A mutant was 1790 μM, which was 3-fold higher than that of the wild-type ODC. There was a dramatic decrease in the k_{cat} values of the C115A mutant, compared to that of the wild-type ODC, which had a k_{cat} value of 77.75 s^{-1}. C115A caused a shift in the optimal pH from 8.0 to 8.4. Considering these results, we suggest that cys-115 is involved in the catalytic activity of *N. glutinosa* ODC.

Keywords: Glutathione-*S*-transferase (GST), *Nicotiana glutinosa*, Ornithine decarboxylase, Site-directed mutagenesis

Introduction

Polyamines are ubiquitous and required for cell growth and differentiation in eukaryotes. In plants, polyamines are involved in the initiation and control of cell division (Serafinin-Tracassini, 1991) and affect patterns of cell differentiation and morphogenesis (Evans and Malmgerg, 1989; Galston and Flores, 1991).

Ornithine decarboxylase (ODC, EC 4.1.1.17) catalyzes the conversion of ornithine to putrescine, the first committed step in polyamine synthesis (Tabor and Tabor, 1984; Pegg, 1986; Heby and Persson, 1990). Constitutive ODC activity has been observed in cancer cells (Pegg, 1988). Its uncontrolled expression confers cancer phenotype on some cells (Auvinen *et al.*, 1992). ODC has generated interest as a potential drug target for the treatment of cancer and parasitic infections (e.g., trypanosomiasis). It has been the subject of extensive investigation. A structure-based approach for designing novel inhibitors of ODC was possible by the X-ray crystal structure of truncated mouse (Kern *et al.*, 1999), *Trypanosoma brucei* (Grishin *et al.*, 1999), and human (Almrud *et al.*, 2000) ODCs. A primary sequence alignment and structural comparison revealed extensive similarities of these three species. ODC is a pyridoxal 5-phosphate (PLP) dependent homodimer. The 2 active sites in the homodimer are formed at the interface between the N-terminal domain of one monomer and the C-terminal domain of the other monomer (Osterman *et al.*, 1997). Sixteen residues, identified as the conserved architectural core of the active site of ODC, were reported (K69, D88, R154, K169, H197, G235-237, E274, R277, Y323, D332, Y389, C360', D361' and N398') The Lys-69 residue interacted with PLP (Poulin *et al.* 1992). The positive charge on the pyridine nitrogen of PLP was maintained by the positive charge of the Glu-274 (Osterman *et al.*, 1995). Arg-277 was required for high affinity PLP binding through interaction with the 5'-phosphate of the cofactor (Osterman *et al.*, 1997). Cys-360 bound to the specific ODC inhibitor, α-difluoromethylornithine. Other residues, such as Lys-115, Lys-169 and His-197, were also shown to be important for ODC activity by forming a part in the active site (van Kranen *et al.*, 1987; Wen *et al.*, 1989; Bassez *et al.*, 1990).

In contrast to the extensive work on *T. brucei*, human, and mouse ODC, there are no reports on the structure of the plant ODC that was determined by protein crystallization and X-ray crystallography. In addition, little work has been done on the active site residues of plant ODCs. By comparing the amino acid sequence of *N. glutinosa* with other eukaryotic ODCs, we chose several highly conserved cysteine residues and changed them into alanine, a smaller amino acid without a sulfur atom. Among the site-directed mutants, cysteine-115 (cys-89 of human ODC) showed significant changes in the kinetic properties. Even though this cysteine residue was conserved among other species, there are no reports on the functional properties of this residue. In this work, the wild type and

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C115A mutant of *N. glutinosa* ODC were expressed in *E. coli* and the kinetic properties of these proteins were determined. Although cys-115 is not one of the 16 core active site residues of ODC, it illuminates several important features on the catalytic mechanism of ODC. In addition, this is the first report on site directed mutagenesis of active site residues in plant ODC.

Materials and Methods

Materials Restriction enzymes and T4 DNA ligase were purchased from Promega. Isopropyl-1-thio-β-D-galactopyranoside (IPTG) and ampicillin were purchased from Sigma. The *N. glutinosa* cDNA library was constructed by using a Stratagene uniZAP XR vector. Pfu DNA polymerase was also purchased from Stratagene. DNA sequencing was performed, both with an automatic sequencer (ABI PRISIM 377 DNA Sequencer) and with the Sequenase 2.0 system from United States Biochemical. The pGEX-2T vector, glutathione, glutathione Sepharose 4B, thrombin, and L-[carboxy^{14}C]ornithine were obtained from Amersham Pharmacia Biotech. All of the oligonucleotides were acquired from TaKaRa Biomedicals. All of the other reagents were obtained from commercial sources.

Cloning of ODC cDNA and site-directed mutagenesis The ODC cDNA was amplified by PCR using the *N. glutinosa* cDNA library. The oligonucleotides contained *BamHI* and *SmaI* sites, respectively, in order to facilitate cloning (Sambrook *et al.*, 1989). The PCR reaction volume (50 μl) contained 500 nM primer each, 200 μM dNTPs, and 5 units of pfu DNA polymerase. The PCR reaction included an initial denaturation for 2 min at 94°C, then forty cycles of denaturation for 30 s at 94°C, annealing for 45 s at 53°C, and extension for 2 min at 72°C. The PCR product was digested with *BamHI* and *SmaI* and then ligated to the *BamHI-SmaI* backbone fragment of pGEX-2T, which contained a T7 lac promoter and Glutathione-*S*-transferase (GST) that preceded the N-terminus of the recombinant protein. An *E. coli* strain BL 21 (DE3) was transformed with the ligation product. Ampicillin-resistant transformants were selected, and plasmid DNA was purified from individual candidates. The identity of the cloned cDNA, and the fidelity of the PCR, were confirmed by DNA sequencing of the plasmid inserts by the dideoxy termination method (Sanger *et al.*, 1977) and an automatic sequencer (ABI PRISIM 377 DNA Sequencer). Two oligonucleotides were designed to replace cysteine-115 to alanine (Table 1), which were chosen by examination of the conserved sequences of other eukaryotic ornithine decarboxylases (Fig. 1). Mutagenesis was performed by sequential PCR (Dieffenbch and Dveksler, 1995). In the first reaction, either the terminal sense and internal antisense primers, or the internal sense and terminal antisense primers were used as a primer set. A recombinant pGEX-2T plasmid, containing a *N. glutinosa* ODC cDNA sequence, was used as a template. PCR was performed for 30 cycles with initial denaturation for 10 min at 94°C, then 45 s at 94°C, 45 s at 53°C and 2 min at 72°C. In the second round, a terminal sense primer and terminal antisense primer were used as a primer set, and two PCR products from the first round reactions were used as templates to produce a single annealed PCR product. The condition for PCR was as follows: initial denaturation for 2 min at 94°C and then thirty cycles of denaturation for 1 min at 94°C, annealing for 45 s at 53°C, and extension reaction for 2 min at 72°C. The final PCR product was digested with *BamHI* and *SmaI*, and ligated to the pGEX-2T vector that was linearized with *BamHI* and *SmaI* and dephosphorylated by calf intestinal alkaline phosphatase. This ligation mixture was used to transform *E. coli* BL 21 (DE3). Verification of all mutants was analyzed by performing DNA sequencing of plasmid inserts by the dideoxy termination method (Sanger *et al.*, 1977).

Expression and purification of wild type and mutant ODC *E. coli* BL 21 (DE3) was transformed with the pGEX2T-ODC plasmid. Transformants were tested for the ODC expression upon induction with IPTG. *E. coli* BL 21 (DE3) cells, carrying the expression plasmid that contained the wild type or mutant ODC, were grown overnight at 37°C in a LB medium (containing 50 μg/ml ampicillin) (Sambrook *et al.*, 1989). The cells were diluted 100-fold into the same medium and allowed to grow until A_{600} reached 0.5. To induce expression, 1 mM IPTG was added to the culture. The cells were harvested 4 h after induction by centrifugation (5,000 × *g*; 10 min), sonicated in phosphate-buffered saline (PBS), and the cell lysate was centrifuged at 13,000 × *g* for 20 min. The resulting supernatant was used for purification on GST Sepharose 4B resin in a batch procedure, according to the manufacturer's recommendations. Recombinant mutated or wild type ODC was recovered from fusion protein by thrombin cleavage and then purified. The purity of the recombinant protein was assessed by a sodium dodecyl sulfate-polyacrylamide gel electrophoresis and polyacrylamide gradient gel electrophoresis (Laemmli *et al.*, 1970).

ODC activity assay ODC activity was assayed at 37°C for 60 min by the liberation of $^{14}CO_2$ from L-[carboxy^{14}C]ornithine as a substrate (Kim and Cho, 1993). A Tris-HCl buffer (pH 8.0) was used at a concentration of 20 mM that contained 1 mM PLP, 1 mM DTT, and 50 μM EDTA. One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 nmole $^{14}CO_2$ per h.

Kinetic characterization of wild type and mutant ODC Substrate saturation studies of the wild type and mutant ODC activity were performed to determine K_m and V_{max} values for ornithine. The initial rates were determined by plotting the amount of product *versus* time. The K_m, V_{max} and k_{cat} values were calculated from $1/V_i$ *versus* 1/[S] plot (where V_i represents initial velocity and [S] indicates substrate concentration) (Segel, 1975). Optimum pH of the ODC was determined, described previously (Kim and Cho, 1993; Park and Cho, 1999).

Protein analysis SDS-polyacrylamide gel electrophoresis was performed by the Laemmli method (Laemmli, 1970) using a Tris-Glycine buffer [25 mM Tris, 200 mM, Glycine, 0.1% SDS (w/v), pH 8.3] and a 12.5% separating gel. The protein concentration was determined by the method of Bradford (Bradford, 1976) with bovine serum albumin as a standard.

Results and Discussion

Expression and purification of wild type and C115A mutant ODC A lambda Uni-ZAP XR cDNA library of *N. glutinosa* was screened with oligonucleotide pairs that represent the conserved amino acid motifs of *Nicotiana tabacum* and other ODCs. The PCR product yielded a single band of 1299 bp. The open reading frame consisted of 432 amino acids (GenBank™ AF 323910). The eukaryotic ODC sequences show very little similarity to those of *E. coli*s biosynthetic and biodegardative ODCs (Applbaum *et al.*, 1975), or the *Lactobacillus* sp. 30a ODC (Guirard and Snell, 1980). However, *Nicotiana glutinosa* ODC appeared to have similarities with other eukaryotic ODCs. The deduced amino acid of *N. glutinosa* ODC (GenBank™ AF323910) had a 90% identity with another plants ODC, *Datura stramonium* (GenBank™ X87847) (Fig. 1). But, the homology decreased between plant and mammalian ODCs; 44% identity with human (Moshier *et al.*, 1990) and 43% identity with rat ODC (van Kranen *et al.*, 1987; Wen *et al.*, 1989).

Mutation of cys-115 to alanine was performed by sequential PCR. A pair of sense and antisense oligonucleotides was designed to replace the cysteine residue to alanine (Table 1). Induction of pGEX-2T, GST fusion vector, with 1 mM IPTG lead to overexpression of 72.5 kDa GST-fused wild type and C115A ODCs (Fig. 2A). Thrombin treated wild type and C115A GST-ODC fusion proteins showed 46,000 dalton bands on 12.5% SDS-polyacrylamide (Fig. 2B). The native molecular weights of the two proteins were 92,000 daltons estimated by polyacrylamide-gradient gel electrophoresis (data not shown). This indicates that the mutation at cys-115 had no effect on forming the homodimeric structure.

pH dependent activity of wild-type and mutant ODC The ODC activity of the wild type and C115A mutant was assayed by using two buffer systems, potassium phosphate (pH 6.4-7.4) and Tris-HCl buffers (pH 7.4-8.6). The pH rate profiles for the purified wild type ODC showed its highest catalytic activity toward L-ornithine at pH 8.0 (Fig. 3).

```
                 B1-bb          B2-bbbb         H1-hhhhhhhhhhhhhhh  B3-b H2-hhhhhhh
N.glutinosa     MAGQTIIVSGLNPAAILQSTIGGGASPTAAAAENGTRKVIPLSRDALQDFMLSIITQKLQDEKQPFYVLDLGEVVSLMDQ   80
D.stramonium    MAGQTVIVSGLNPAAILQSTIGG-ATPAPAAENDHTRKVVPLSRDALQDFMVSIITQKLQDEKQPFYVLDLGEVVSLMDQ   79
T.brucei        ----------------------------------------------RDALCKKISMNTCDEGDPFFVADLGDIVRKHET   74
human           ------------------------------------------------------------DDKDAFYVADLGDILKKHLR   54
mouse           ------------------------------------------------------------DDKDAFYVADLGDILKKHLR   54
                                                     ↓
                hhhh    B4-bbbb  H3-hhhhhhh  B5-  H4-hhhhhh     B6-bb    H5-hhhhhhh  B7-b
N.glutinosa     WKSSLPNIRPFYAVKCNPEPSFLSILSAMGSNFDCASRAEIEYVLALGISPDRIVFANPCKPESDIIFAAKVGVNLTTYD  160
D.stramonium    WNAGLPNIRPFYAVKCNPEPSFLSMLSAMGSNFDCASRAEIEYVLSLGISPDRIVFANPCKPESDIIFAAKVGVNLTTFD  159
T.brucei        WKKCLPRVTPFYAVKCNDDWRVLGTLAALGTGFDCASNTEIQRVRGIGVPPEKIIYANPCKQISHIRYARDSGVDVMTFD  154
human           WLKALPRVTPFYAVKCNDSKAIVKTLAATGTGFDCASKTEIQLVQSLGVPPERIIYANPCKQVSQIKYAANNGVQMMTFD  134
mouse           WLKALPRVTPFYAVKCNDSRAIVSTLAAIGTGFDCASKTEIQLVQGLGVPAERVIYANPCKQVSQIKYAASNGVQMMTFD  134

                  H6-hhhhhhh    B8-bbbb                   H7-hhhhhhhhhh   B9-bbbbb     H8-hhhhhh
N.glutinosa     SEDEVYKIRKHHPKSELLPRIKPMFDGNARCPMGPKYGALPEEVEPLLRAAQAARLTVSGVSFHIGSGDADSNAYLGAIA  240
D.stramonium    SEDEVYKIRKHHPKCELLLRIKPMDDGNARCPMGPKYGALPEEVEPLLRTAQAARLTVSGVSFHIGSGDADSKAYLGAIA  239
T.brucei        CVDELEKVAKTHPKAKMVLRISTD-DSLARCRLSVKFGAKVEDCRFILEQAKKLNIDVTGVSFHVGSGSTDASTFAQAIS  233
human           SEVELMKVARAHPKAKLVLRIATD-DSKAVCRLSVKFGATLRTSRLLLERAKELNIDVVGVSFHVGSGCTDPETFVQAIS  213
mouse           SEIELMKVARAHPKAKLVLRIATD-DSKAVCRLSVKFGATLKTSRLLLERAKELNIDVIGVSFHVGSGCTDPDTFVQAVS  213

                hhhhhhhhhhh      B10-               H9-hhhhhhhhh      B11-b  H10-  B12-bbbbbbbb
N.glutinosa     AAKEVFETAAKLGMSKMTVLDVGGGFTSGHQFTTAAVAVKSALKQHFDDEPELTIIAEPGRFFAETAFTLATTVIGKRVR  320
D.stramonium    AAKGVFETAARFGMSKMTVLDIGGGFTSGHQFTTASAAVRSALEQHFHDEQELTIIAEPGRFFAETAFTLATTIIGKRVR  319
T.brucei        DSRFVFDMGTELGFN-MHILDIGGGFPGTRKFEEIAGVINNALEKHFPPDLKLTIVAEPGRYYVASAFTLAVNVIAKKVT  316
human           DARCVFDMGAEVGFS-MYLLDIGGGFPGSEKFEEITGVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKIE  299
mouse           DARCVFDMATEVGFS-MHLLDIGGGFPGSEKFEEITSVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKTE  299

                  B13-bbb                                        B14-bbbb      B15-b          B16-
N.glutinosa     GELREYWINDGLYGSMNCVLYDHATVNATPLAVLSNRTNVTCGGSKTFPTTVFGPTCDALDTVLRDYQLPELQVNDWLVF  400
D.stramonium    GELREYWINDGLYGSMNCVLYDHATVNATPLACMSNRSNLNCGGSKTFPSTVFGPTCDALDTVLRDYQLPELQVNDWLIF  399
T.brucei        GAHAEYYVNDGVYGSFNCILYDHAVVRPLP-----QREPI--PNEKLYPSSVWGPTCDGLDQIVERYYLPEMQVGEWLLF  403
human           DESSEYYVNDGVYGSFNCILYDHAHVK--PL-LQKRPKPD----EKYYSSSIWGPTCDGLDRIVERCDLPEMHVGDWMLF  383
mouse           DESNEYYVNDGVYGSFNCILYDHAHVKA---LLQKRPKPD----EKYYSSSIWGPTCDGLDRIVERCNLPEMHVGDWMLF  383

                b    B-17         B18-bbb
N.glutinosa     PNMGAYTKAAGSNFNGFNTSTIVTHLAYTYPS                                                  432
D.stramonium    PNMGAYTKAAGSNFNGFNTSAIVTHLAYAYPS                                                  431
T.brucei        EDMGAYTVVGTSSFNGFQSPTI----------                                                  425
human           ENMGAYTVAAASTFNGFQRPTI----------                                                  405
mouse           ENMGAYTVAAASTFNGFQRPNI----------                                                  405
```

Fig. 1. Comparison of the primary amino acid sequence of *N. glutinosa*, *Datura stramonium*, *Trypanosoma brucei,* human and mouse ODC. Sequences were deduced from papers describing the *N. glutinosa* (GenBank™ AF323910), *D. stramonium* (GenBank™ X87847), *T. brucei* (GenBank™ J02771), and human (GenBank™ J02771) and mouse (Kahana and Nathans, 1985) ODC. Regions of primary sequence similarity were shown in the shaded area. The arrow indicates the Cys-115 residues in the *N. glutinosa* sequence, which were mutated as described under "Materials and Methods". The secondary structural elements of *N. glutinosa* ODC are given above its sequences, H, α-helix; B, β-sheets.

Table 1. Mutagenic oligonucleotides for site-directed mutagenesis of ODC. Nucleotides that were exchanged in order to obtain the desired mutation are underlined and in bold letters. Numbers in superscript indicate the position of nucleotides in the coding region of the gene. Restriction sites are underlined (sense; *Bam HI*, antisense; *Sma I*). Mutation at Cysteine-115 is indicated by a one-letter amino acid abbreviation. The first letter is the wild type residue. The last letter is the amino acid to which it is changed by the mutated codon. WT, wild type; S, sense; AS, antisense.

5 GGATCCATGGCCGGCCAAACAATAATCGTTTCCG	Sense
5 CCCGGGTCAGCTTGGATAAGTATAAGCGAGGTGAG	Antisense
5[331]CTCAAATTTTGATTGTGCTAGCCGAGCTG	C115WT
5 CTCAAATTTTGAT**GC**TGCTAGCCGAGCTG	C115A (S)
5 GCTCGGCTAGCA**GC**ATCAAAATTTGAGCC	C115A (AS)

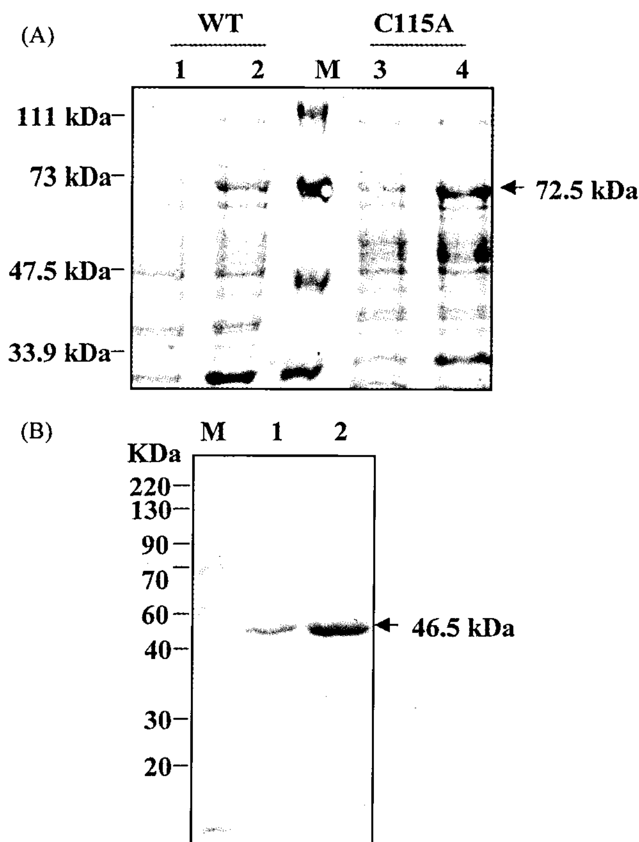

Fig. 2. SDS-PAGE analysis of *N. glutinosa* ODC (A) IPTG-induction of recombinant wild type and C115A ODC protein. The bacterial cell lysate, induced by 1 mM IPTG, were analyzed on 12% SDS-PAGE, described in "Materials and Methods". Lane 1, control wild-type lysate; lane 2, IPTG-induced overexpression of wild-type ODC; lane 3, control C115A lysate, lane 4, IPTG-induced overexpression of C115A ODC. (B) SDS-PAGE analysis of thrombin cleaved wild type and C115A ODC. Wild type and mutant ODC purification and thrombin proteolysis were performed as described in "Materials and Methods". The purified enzyme (5 μg) was analyzed by 12.5% SDS-PAGE. Lane 1, thrombin-cleaved wild type ODC; lane 2, thrombin-cleaved C115A ODC.

However, C115A ODC showed an altered pH optimum from 8.0 to 8.4. In addition, C115A showed a slower decrease in the ODC activity, while there was a 50% decrease in the wild type ODC at pH 8.6.

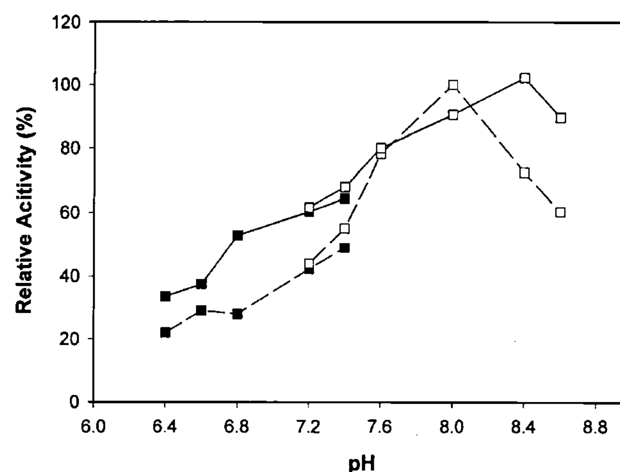

Fig. 3. The pH profiles for the ornithine decarboxylase activity of wild type and C115A ODC protein. The ODC activity of purified wild type (-----) and C115A (——) ODC were assayed in various ranges of pH buffers, described under "Materials and Methods". A 40 mM potassium phosphate buffer; 6.4-7.4 (■) and 40 mM Tris buffer; 7.4-8.6 (□) were used for its effective buffer range.

Kinetic parameters of wild type and mutant ODC Mutations of several important cysteine residues were studied from mouse and *T. brucei* ODC. The C70S mutant of mouse ODC still had full activity when expressed in *E. coli* (Coleman *et al.*, 1993). However, the C360A mutant of *T. brucei* had a significant effect on the k_{cat} values, a 55-fold decrease compared to the wild-type enzyme. It showed important roles in Shiff's base formation, decarboxylation, and protonation of ODC (Jackson *et al.*, 2000). Despite C360A and several other cysteine residues that were studied, there is no study on the functional roles of amino acid, Cys-115 (human Cys-89). Table 2 shows the kinetic analysis of the wild type and C115A mutant ODC of *N. glutinosa*. The K_m values for the wild-type ODC was 562 μM, which was higher than that for barley seeds (360 μM) and *Glycine max* (135 μM), but similar to *Physarum polycepalum* (600 μM) ODC. Changing Cys-115 to alanine resulted in a 3-fold increase in the K_m values for the substrate, L-ornithine. In contrast to the 3-fold increase in the K_m values, the specific constant (k_{cat}/K_m) was dramatically decreased. The decarboxylation of L-ornithine to putrescine (k_{cat}) by C115A ODC was 1200 times

Table 2. Kinetic analysis of wild type and C115A ODC by using L-ornithine as the substrate. Wild type and mutant ODC were purified as described under "Materials and Methods". K_m, V_{max} and k_{cat} values were determined from double-reciprocal plots for L-ornithine concentration and initial velocities. The assays were carried out at 37°C for 1 h with varying concentrations of L-ornithine from 100 μM to 10 mM.

Mutants	K_m	V_{max}	k_{cat}	k_{cat}/K_m
	μM	nmole/m/mg	s^{-1}	$M^{-1}s^{-1}$
WT	562±61	12.54±1.0	77.78±14.3	1.5×10^5
C115A	1790±367	6.34±0.6	0.064±0.006	8.09

slower than the wild type enzyme. It could be speculated that the exchange of cysteine-115 to a smaller alanine could provide additional space for the water molecule to enter the active site. This would probably influence a number of ordered hydrogen bonds. Changing the charged sulfur ion to a hydrophobic side chain would also impair the correct binding of L-ornithine. Based on the X-ray structure of the mouse ODC, cys-115 (human Cys-89) is located next to a cluster of acidic residues (D88, E274, D233), which bind with three water molecules and form a network of hydrogen bonds that influence the electron withdrawing properties of the cofactor (Kern *et al.*, 1999). Therefore, all of these changes on the C115A mutant would probably break the network of hydrogen bonds, decreasing dramatically the k_{cat} values and increasing the K_m values.

Recently, a three-dimensional structure of *N. glutinosa* ODC was predicted by the homology modeling server at Swiss-Model (unpublished observation). The ODC structures were quite similar, not only in the packing of the molecules in the unit cell, but also in the details of the structure with other eukaryotic ODCs. The symmetrical homodimer of *N. glutinosa* ODC was formed by a head-to-tail interaction between the barrel of one domain and the sheet domain of the other. The barrel domain was formed by the α/β barrel from residues 66-288. The rest of the residues formed the β-structure of the sheet domain (Fig. 1). The barrel domain began and ended with a helix (H2-H10), like the mouse ODC barrels (Kern *et al.*, 1999), but different from triose phosphate isomerase (TIM) barrels, which usually started with a β structure (Banner *et al.*, 1974). Cys-115 was located in the β-sheet between helix-3 and helix-4, which forms a part in the cavity of the active site that is presented by the first subunit. Thus, the highly conserved region 113-117 (-FDCAS-), containing Cys-115, would also be involved in the catalytic activity of the ODC protein.

This is the first report on the identification of functionally important regions and residue of *N. glutinosa* ODC by sitedirected mutagenesis. Since there is no report on the crystal structure of plant ODC, a X-ray crystallographic analysis of *N. glutinosa* ODC will provide further insight into the structure-functional relations of this enzyme. This will provide a basis for the design of specific regulators of polyamine biosynthesis in plants.

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