Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Biochemistry

ISSN 1815-9923
DOI: 10.3923/ajb.2017.9.15

Research Article

Chemical Modification of Oxalate Oxidase Produced from *Ochrobactrum intermedium* CL6 Gave New Insight on its Catalytic Prowess

Kunal Kumar and Prasanna Devarabhat Belur

Department of Chemical Engineering, National Institute of Technology Karnataka (NITK), Surathkal, Srinivasanagar, 575 025 Mangalore, India

Abstract

Background: Use of chemical modifications to identify important amino acid residues, which are crucial for the catalytic activity of oxalate oxidase produced from novel endophytic bacterium *Ochrobactrum intermedium* CL6, isolated from *Colocasia esculenta* tubers. **Methodology:** Oxalate oxidase produced from a newly isolated endophytic bacterium, *Ochrobactrum intermedium* CL6 was purified and subjected to chemical modifications using amino-acid specific reagents. The modification reactions were optimized to find the specific reagent concentration and reaction time. **Results:** The results revealed cysteine, carboxylates, histidine and tryptophan residues are part of the active site. Moreover, modification of cysteine and histidine residues had resulted in the enhancement of enzyme activity. A two-fold Increase in oxalate oxidase activity was observed when histidine residues were modified with 15 mM diethylpyrocarbonate for 60 min. Histidine-modified enzyme exhibited K_{cat} value of 140 S^{-1} against 91 S^{-1} of native enzyme. However, catalytic efficiency did not increase substantially (1.82%) due to the counteracting increased K_m value. **Conclusion:** Kinetic studies revealed that chemical modification of histidine residues resulted in the enhancement of turnover number (K_{cat}) by 1.53 times compared to native enzyme. The catalytic efficiency was 2.5 times higher than the oxalate oxidase from Barley roots showing the potential of this enzyme for clinical and industrial applications. Hence, it is worthwhile improving catalytic efficiency of OxO produced from *Ochrobactrum intermedium* CL6 by site directed mutagenesis.

Key words: Catalytic efficiency, chemical modification, *Ochrobactrum intermedium*, oxalate oxidase

Received: October 20, 2016 Accepted: November 18, 2016 Published: December 15, 2016

Citation: Kunal Kumar and Prasanna Devarabhat Belur, 2017. Chemical modification of oxalate oxidase produced from *Ochrobactrum intermedium* CL6 gave new insight on its catalytic prowess. Asian J. Biochem., 12: 9-15.

Corresponding Author: Prasanna Devarabhat Belur, Department of Chemical Engineering, National Institute of Technology Karnataka (NITK), Surathkal, Srinivasanagar, 575 025 Mangalore, India Tel: +919483035265 Fax: +91824-2474033

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oxalate oxidase (OxO, EC 1.2.3.4) is one of the enzymes that can generate H_2O_2 and CO_2 on the catalytic breakdown of oxalic acid in the presence of molecular oxygen[1]. This enzyme was first isolated and characterized from barley (Hordeum vulgare) and wheat (Triticum aestivum)[2,3] and functionally belongs to diverse protein superfamily known as Cupins and is concerned with cell wall, fungal defence and salt tolerance[4].

Oxalate oxidase activity has been reported in white rot basidiomycete, *Ceriporiopsis subvermispora*[5] and a bacterium, *Pseudomonas* sp. OX-53[6] as well. Among all, OxO found in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) seedlings are extensively studied and most of the commercially available OxO is purified from barley and wheat plants. Most recently, a new source of the enzyme having diverse characteristics was produced from novel endophytic *Ochrobactrum intermedium* CL6, isolated from *Colocasia esculenta* tubers[7].

Oxalate is a major component of kidney stones in blood and urine and its clinical assays are accomplished enzymatically by oxalate oxidase[8]. Regular assessment of oxalate levels in urine helps to monitor and control hyper oxaluria and Urolithiasis[1]. The undesirable scaling caused by bleaching filtrate of pulp and paper industry due to the presence of oxalic acid can be prevented by the enzyme oxalate oxidase[9].

Enzymes are able to perform many chemical and biochemical transformations with efficiencies that are typically unparalleled by chemical catalytic agent. However, these evolved systems may lack breadth or utility in other non-natural application. Altering enzyme and protein scaffolds through covalent chemical modification can expand the usefulness of native biocatalysts in a wide arena, as well as open new horizon of applications[10], as diverse range of functionality can be introduced in natural enzymes by means of chemical modification methods[11]. Chemical modification of biocatalysts can alter affinity, specificity or stability, which in turn makes it superior to its native form. The most common use of covalent modification is to identify amino acid residues associated with catalytic site of enzymes. Covalent modification often leads to the inactivation of enzymes and thus identifies functionally important aminoacids[12]. This new insight has stimulated the several study groups to employ chemical techniques to modify enzymes, an altogether different approach compared to site-directed mutagenesis[13]. By using specific chemical modifiers, amino acids, disulphide bonds and bound metal ions essential for enzyme activity was identified in number of enzyme like xylanase, L-asparaginase, lactase, proteases and oxalate oxidase from barley and wheat[14-19].

Current study describes the use of chemical modifications to identify important amino acid residues, which are crucial for its catalytic activity of OxO produced from novel endophytic bacterium *Ochrobactrum intermedium* CL6, isolated from *Colocasia esculenta* tubers. Further, an effort was made to enhance its kinetic properties by modifying a key amino acid residue by employing chemical modification.

MATERIALS AND METHODS

Enzyme production, quantification and purification: The enzyme oxalate oxidase (OxO) was produced by the *Ochrobactrum intermedium* CL6, an endophytic bacterium isolated from *Colocasia esculenta* as previously described[7]. The organism was cultured in a medium containing the following components (gL^{-1}): sucrose-20, NH_4Cl-2, sodium oxalate-5, KH_2PO_4-3, Na_2HPO_4-6, NaCl-5, NH_4Cl-2, $MgSO_4.7H_2O$-0.1, $MnSO_4$-0.05, biotin-0.0015, with an initial pH of 6.5 and 2% (v/v) inoculum volume. Sterilized production medium (25 mL) in 250 mL Erlenmeyer flasks was incubated at 30°C, 150 rpm in an incubator shaker for 65 h. Cell-free supernatant was collected after the centrifugation of fermentation broth at 2500×g for 10 min. The oxalate oxidase (OxO) enzyme present in cell free supernatant was purified by ethanol precipitation followed by gel filtration chromatography using Sephadex G-100 column[7].

Oxalate oxidase activity (OxO) was determined spectrophotometrically (UV 5200, Labomed India Pvt. Ltd.) as described by Kumar and Belur[7] with some modifications. The total enzyme substrate reaction mixture of 2.74 mL, consisting of 1.7 mL of sodium succinate buffer (50 mM, pH 3.8), 0.04 mL of 200 mM oxalic acid solution and 1 mL of enzyme solution was incubated for 30 min at 80°C. After incubation, the reaction was stopped by adding 0.1 mL of 100 mM EDTA solution, followed by the addition of 1 mL of colour development reagent comprising 0.1 mM MBTH (3-Methyl-2-Benzothiazolinone hydrazone) and 0.72 mM DMA (N,N-Dimethylaniline) along with 3 U of 20 µL of peroxidase (Sigma-Aldrich) solution. The hydrogen peroxide produced due to the OxO catalysed hydrolysis of oxalic acid, reacts with MBTH and DMA in the presence of peroxidase enzyme to form purple colour indamine dye. The amount of indamine dye produced from the sample due to oxalate oxidase activity on oxalic acid was determined by extrapolating the standard curve prepared using commercially available hydrogen peroxide. One unit of OxO is the amount of enzyme, which

liberates 1 μmol of H_2O_2 in 1 min at 80°C. Total protein content was estimated by Lowry's method with Bovine Serum Albumin (BSA) as standard[20].

Chemical modification of oxalate oxidase: Purified OxO was taken for chemical modifications. Cysteine, carboxylates, histidine, serine and tryptophan residues were subjected to modifications in five different sets of experiment and their effect on enzyme activity was studied. Modification reactions were carried out in a reaction volume of 1 mL and incubated at 37°C. Strength of important reagents used in each reaction and reaction time were varied to find the optimum reaction conditions. The modified enzymes were then assayed for activity as per the method described in this study and compared with the activity of the native enzyme. Data shown in the results are mean ± standard error of three replicates.

Modification of cysteine residues: Cysteine residues of OxO were modified using DTNB (5,5-dithio-bis-(2-nitrobenzoic) acid) as described by Hu and Guo[19]. A stock of 10 mM DTNB solution was prepared fresh in 100 mM phosphate buffer (pH 8.0) for use. The modification reaction mixture had comprised of 20 mM phosphate buffer of pH 8, with 0.017 U OxO and 0.2-0.8 mM DTNB. Modified OxO activity was then estimated at two different temperatures (55 and 80°C).

Modification of carboxylates: The importance of carboxylate groups in the activity of OXO was investigated by modifying them using EDAC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)[18]. Freshly prepared stock of 500 mM EDAC in ethanol was used for the reaction. The modification reaction was carried out in 10 mM potassium acid phthalate/NaOH buffer of pH 4.1 containing 0.017 U OxO and 10-100 mM EDAC in 1 mL reaction volume. Modified OxO activity was then estimated at 80°C.

Modification of histidine residues: Histidine residues were modified using 150 mM DEPC (diethylpyrocarbonate) stock solution prepared in ethanol. The reaction was carried out by incubating 0.017 U OxO with 1-15 mM DEPC in 20 mM phosphate buffer of pH 8 in a reaction volume of 1 mL[19]. Modified OxO activity was then estimated at two different temperatures (55 and 80°C).

Modification of serine residues: For modification of serine residues, a stock of 150 mM PMSF (phenylmethane sulfonyl fluoride) prepared in ethanol was used. The reaction was conducted at 37°C by incubation of 0.017 U OxO with 1-30 mM PMSF in 20 mM phosphate buffer of pH 8 in a reaction volume of 1 mL[19]. Modified OxO activity was then estimated at 80°C.

Modification of tryptophan residues: For modification of tryptophan residues, a stock of 1 mM NBS (N-bromosuccinimide) was prepared in 100 mM phosphate buffer (pH 8.0). The reaction was carried out at 37°C by incubation of 0.017 U OxO with 0.01-0.05 mM NBS in 20 mM phosphate buffer of pH 8 in a reaction volume of 1 mL[14,19]. OxO activity was then estimated at 80°C.

Estimation of kinetic parameters: The kinetic parameters K_m and V_{max} were determined by Lineweaver-Burk double reciprocal plot and the turnover number (K_{cat}) were calculated for native OxO and histidine-modified OxO. Oxalic acid in the range of 0.09-10.0 mM was used as the substrate for native enzyme whereas 0.025-10.0 mM oxalic acid was used for histidine-modified enzyme under optimal assay conditions (pH 3.8 and 80°C). Turnover number (K_{cat}) was calculated by the equation:

$$K_{cat} = \frac{V_{max}}{[E_0]}$$

where, $[E_0]$ is the molar concentration of enzyme in the reaction mixture. The concentration of enzyme was maintained at 0.073 μM for the entire assay. The catalytic efficiency of native and histidine modified enzyme was calculated by computing the ratio of K_{cat} to K_m.

RESULTS AND DISCUSSION

Effect of chemical modifications of amino acid residues of enzyme

Modification of cysteine residues: Modification of cysteine residues of *Ochrobactrum intermedium* CL6 OxO resulted in the slight increase in activity. Modification carried out by using different concentrations of DTNB (0.2-0.8 mM) for different time intervals (0-40 min) showed that 0.4 mM of DTNB with incubation for 20 min was optimum, which resulted in an increase in activity by 10% when assayed at 55°C (Fig. 1a). However, modified enzyme did not show any enhancement of activity when assayed at 80°C (as compared to native enzyme), indicating modified enzyme is not thermostable unlike its native form[7]. The increase in activity and loss of thermostable property could be attributed to considerable changes in conformation of the enzyme. This could be possible due to the reaction between thiol groups of proteins and DTNB leads to the formation of mixed disulphide[21]. The mixed disulphide formed are themselves highly reactive

compound[22] and might have been used to link to other thiols to form a new disulphide. Some reversible thiol-disulphide interchange reactions among proteins precede via mixed disulphide intermediates and can lead to migration of disulphide bonds to other locations in the same or separate protein, which further forms disulphide linkages with other free thiols in a protein[23]. Formation of new disulphide bonds might bring functional change through a conformational change or steric blockage[24] and might have enhanced the activity of the enzyme oxalate oxidase towards the substrate oxalate and this change in conformation might have contributed to its loss of thermostability. A similar phenomenon was reported in the case of Fructose 1,6-Diphosphatase, where modification of the enzyme by DTNB led to the increase in enzyme activity [25].

Modification of carboxylate residues: The modification of carboxylate residues of OxO using 10-100 mM EDAC resulted in complete loss of activity (Fig. 1b). The result indicated that with an increase in the concentration of EDAC, there is a progressive decrease in activity of oxalate oxidase. The enzyme lost its complete activity at 100 mM of EDAC with 30 min of reaction time. Complete loss of activity was also observed with 30 mM EDAC with 90 min reaction time. The result points to the fact that carboxylate residues are essential for OxO activity, which is in agreement with wheat and barley, root OxO[18,19]. In the initial reaction, carbodiimide reacts with the carboxyl group of a protein to form an intermediate compound, O-acylisourea complex, subsequent reaction of the intermediate with amine forms an amide linkage[26]. This reaction causes inactivation of the modified proteins and decreases the catalytic efficiency.

Modification of histidine residues: There was an enhancement of catalytic activity of OxO when DEPC at 1-15 mM concentration was used to modify the enzyme's histidine residues. A two-fold increase in OxO activity was observed when the enzyme was reacted with 15 mM concentration of DEPC for 60 min (Fig. 1c). Interestingly the modified enzyme showed same activity at 55 and 80°C, the trend seen in its native form. Oxalate oxidase purified from wheat seedlings and barley root, on chemical modification of their histidine residues were also found uninhibited[18,19] but unlike *Ochrobactrum intermedium*, such modification did not lead to any improvement of enzyme's catalytic prowess. Modification of histidine by DEPC results in carbethoxylated derivatives[27], which may lead to loss of acid-base behaviour of histidine and the ability of imidazole ring to coordinate with metal ions[28-30]. The OxO from *Ochrobactrum intermedium* CL6 might be having Mn^{2+} incorporated in the active site of the enzyme like most other oxalate oxidases[31]. Modification of histidine prevents the coordination of Mn^{2+} with its imidazole ring and thus Mn^{2+} becomes partially free to interact more effectively with the substrate oxalic acid. It has been reported that Mn^{2+} takes part in catalytic interaction with the substrate during the catalysis process by oxalate oxidase[31]. Modification of histidine resulted in the two-fold increase in activity in glutamate dehydrogenase[32] and 2.6-fold increase in pancreatic α-amylase[33] to that of native form.

Modification of serine residues: Modification of serine residues by reacting OxO with PMSF did not result in any loss of activity. At the same time, no enhancement and reduction in enzyme activity were noticed even after changing PMSF concentration and reaction time (Fig. 1d). This shows that serine residue is not a part of the active site of OxO.

Modification of tryptophan residues: Tryptophan residues of *Ochrobactrum intermedium* CL6 OxO was modified by reacting with NBS of varying strength (0.01-0.05 mM). About 65% of native activity was inhibited within 15 min of reaction time with 0.05 mM NBS. The enzyme lost its activity completely when it was reacted with 0.025 mM NBS for 45 min (Fig. 1e). The result shows that tryptophan residues are essential as they could be present at the active site of the enzyme. There are no reports on inhibition of OxO by modification of tryptophan residues of OxO.

Kinetic studies of native OxO and histidine modified enzyme: The K_m value obtained from the Lineweaver-burk plot for native and histidine modified enzyme was found to be 0.45 and 0.68 mM respectively and V_{max} was 413 and 625 μM min^{-1}, respectively (Fig. 2). There is a slight decrease in the affinity of the histidine-modified enzyme towards its substrate oxalic acid as evident from the increase in K_m value. The turnover numbers (K_{cat}) of native and histidine-modified enzyme were 91 and 140 S^{-1}, respectively. The catalytic efficiency of histidine-modified enzyme was found to be 20.59×10^4 against 20.22×10^4 $M^{-1}S^{-1}$ of native enzyme. Thus, the chemical modifications of histidine residues lead to the increase in turnover number (K_{cat}) which was however counteracted by increase in K_m value. As a result, a marginal increase (1.82%) in catalytic efficiency was found due to chemical modification. Nevertheless, the catalytic efficiency recorded here is 2.5 times that of OxO from Barley roots[34] indicating its better catalytic prowess. Moreover, the *Ochrobactrum intermedium* CL6 OxO, which is studied

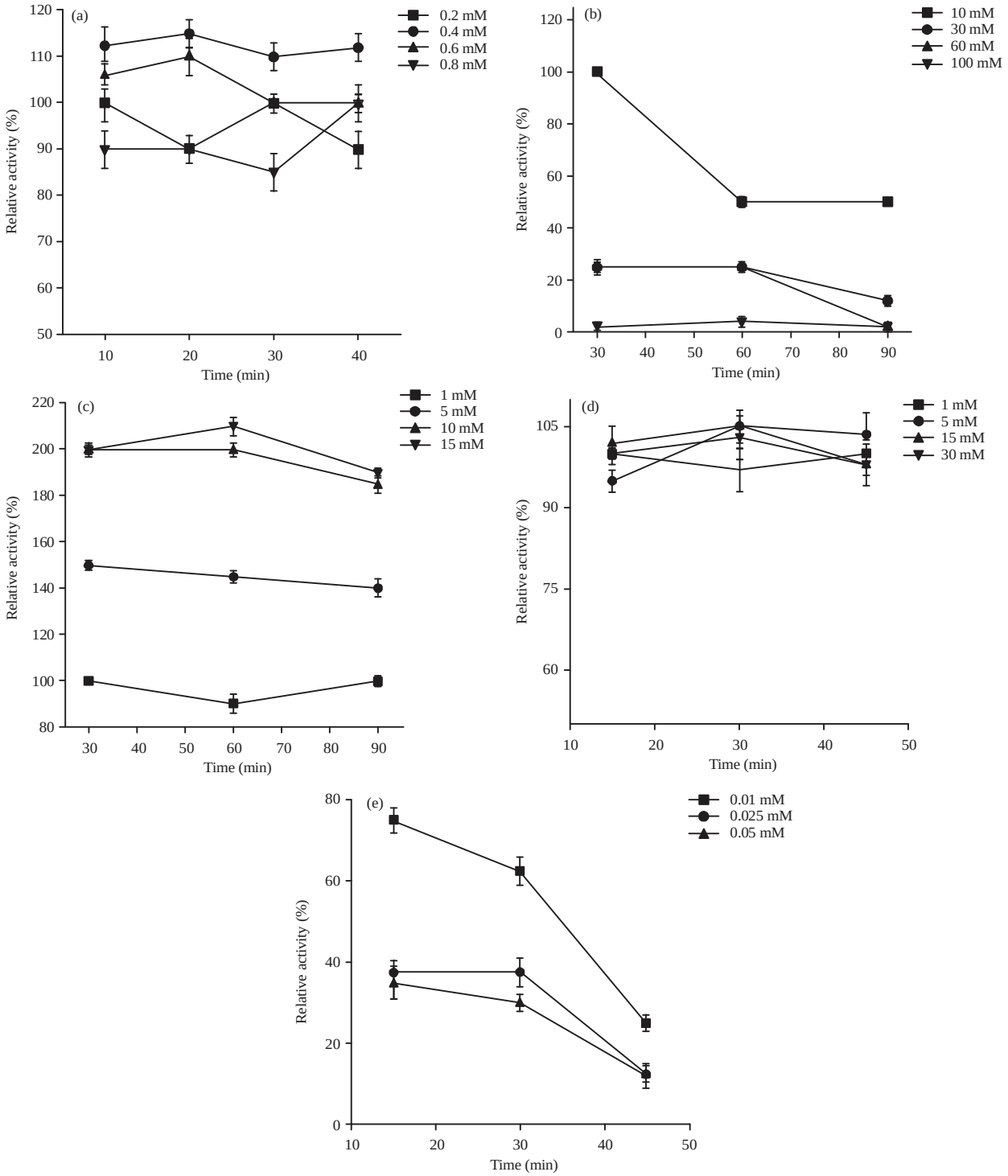

Fig. 1(a-e): Effect of reagent concentration and reaction time on the activities of Oxalate oxidase (OxO) of *Ochrobactrum intermedium* CL6. (a) Cysteine modification: Effect of 5,5-dithio-bis-(2-nitrobenzoic) acid (DTNB) concentration and reaction time on the activity of enzyme, (b) Carboxylate modification: Effect of 1- ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) concentration and reaction time on the activity of enzyme, (c) Histidine modification: Effect of diethylpyrocarbonate (DEPC) concentration and reaction time on the activity of enzyme, (d) Serine modification: Effect of sulfonyl fluoride (PMSF) concentration and reaction time on the activity of enzyme and (e) Tryptophan modification: Effect of N-bromosuccinimide (NBS) concentration and reaction time on the activity of enzyme

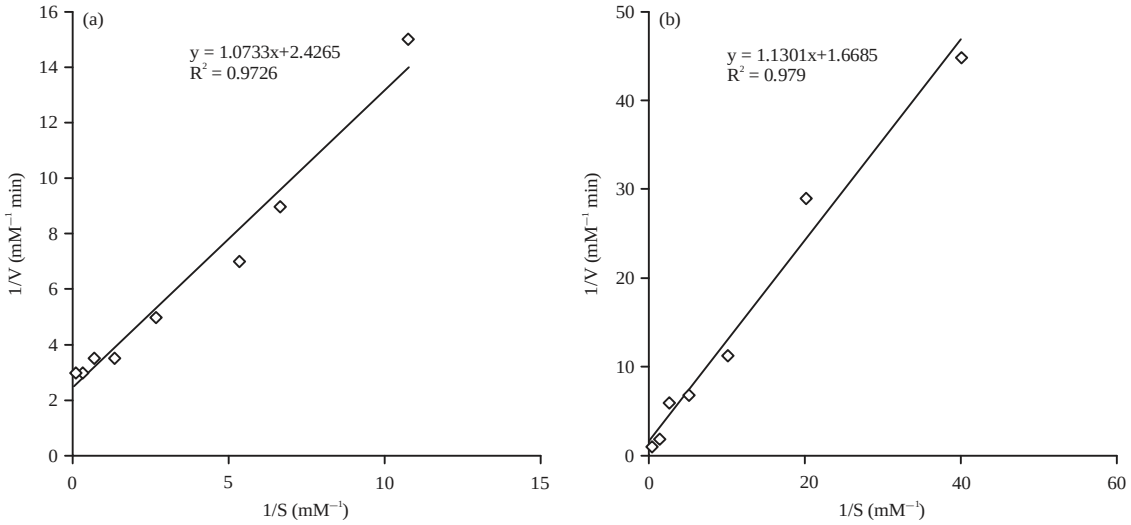

Fig. 2(a-b): Kinetic studies of native and histidine modified Oxalate oxidase (OxO) of *Ochrobactrum intermedium* CL6. Lineweaver-burk plot for (a) Native enzyme and (b) Histidine-modified enzyme

here, had exhibited no substrate inhibition up to 50 mM concentration[7] making it an interesting enzyme for commercial applications.

CONCLUSION

Chemical modification of amino acid residues of enzyme revealed that cysteine, carboxylates, histidine and tryptophan residues are part of the active site and modification of cysteine and histidine residues had resulted in the enhancement of enzyme activity. Kinetic studies revealed that chemical modification of histidine residues resulted in the enhancement of turnover number (K_{cat}) by 1.53 times compared to native enzyme. The catalytic efficiency was 2.5 times higher than the OxO from barley roots showing the potential of this enzyme for clinical and industrial applications. This study highlights the utility of chemical modification technique in identifying the critical amino acid residues present in active site. Once the critical amino acid residues are identified using these simple chemical modification technique, site directed mutagenesis targeting the desired amino acid residue present in the active site could be carried out to enhance the kinetic properties. This study also demonstrates using simple chemical modification technique in enhancing the kinetic properties of the enzymes.

SIGNIFICANCE STATEMENT

In the current study, an effort was made to enhance the kinetic properties of oxalate oxidase enzyme by employing chemical modifications. Oxalate oxidase produced from a newly isolated endophytic bacterium, *Ochrobactrum intermedium* CL6 was purified and subjected to chemical modifications using amino-acid specific reagents. A two-fold increase in oxalate oxidase activity was observed when histidine residues were modified with 15 mM diethylpyrocarbonate for 60 min. Kinetic studies revealed that chemical modification of histidine residues resulted in the enhancement. This study highlights the utility of chemical modification technique in identifying the critical amino acid residues present in active site and in enhancing the kinetic properties of enzymes.

ACKNOWLEDGMENT

Mr. Kunal Kumar is supported by scholarship for doctoral course by the National Institute of Technology Karnataka (NITK) Surathkal, Karnataka, India.

REFERENCES

1. Dunwell, J.M., 1998. Cupins: A new superfamily of functionally diverse proteins that include germins and plant storage proteins. Biotechnol. Genet. Eng. Rev., 15: 1-32.
2. Chiriboga, J., 1966. Purification and properties of oxalic acid oxidase. Arch. Biochem. Biophys., 116: 516-523.
3. Lane, B.G., J.M. Dunwell, J.A. Ray, M.R. Schmitt and A.C. Cuming, 1993. Germin, a protein marker of early plant development, is an oxalate oxidase. J. Biol. Chem., 268: 12239-12242.

4. Dunwell, J.M., S. Khuri and P.J. Gane, 2000. Microbial relatives of the seed storage proteins of higher plants: Conservation of structure and diversification of function during evolution of the cupin superfamily. Microbiol. Mol. Biol. Rev., 64: 153-179.
5. Aguilar, C., U. Urzua, C. Koenig and R. Vicuna, 1999. Oxalate oxidase from *Ceriporiopsis subvermispora*: Biochemical and cytochemical studies. Arch. Biochemi. Biophys., 366: 275-282.
6. Koyama, H., 1988. Purification and characterization of oxalate oxidase from *Pseudomonas* sp. OX-53. Agric. Biol. Chem., 52: 743-748.
7. Kumar, K. and P.D. Belur, 2016. New extracellular thermostable oxalate oxidase produced from endophytic *Ochrobactrum intermedium* CL6: Purification and biochemical characterization. Preparative Biochem. Biotechnol., 46: 734-739.
8. Godara, S. and C.S. Pundir, 2008. Urinary and serum oxalate determination by oxalate oxidase immobilized on to affixed arylamine glass beads. Indian J. Med. Res., 127: 370-376.
9. Sjode, A., S. Winestrand, N.O. Nilvebrant and L.J. Jonsson, 2008. Enzyme-based control of oxalic acid in the pulp and paper industry. Enzyme Microbial Technol., 43: 78-83.
10. Diaz-Rodriguez, A. and B.G. Davis, 2011. Chemical modification in the creation of novel biocatalysts. Curr. Opin. Chem. Biol., 15: 211-219.
11. Qi, D., C.M. Tann, D. Haring and M.D. Distefano, 2001. Generation of new enzymes via covalent modification of existing proteins. Chem. Rev., 101: 3081-3112.
12. Kyte, J., 2006. Structure in Protein Chemistry. Garland Science, New York, ISBN: 9781136843518, Pages: 832.
13. DeSantis, G. and J.B. Jones, 1999. Chemical modification of enzymes for enhanced functionality. Curr. Opin. Biotechnol., 10: 324-330.
14. Wen, L., Z.W. Miao and W.D. Qing, 1999. Chemical modification of xylanase from *Trichosporon cutaneum* shows the presence of carboxyl groups and cysteine residues essential for enzyme activity. J. Protein Chem., 18: 677-686.
15. Pritsa, A.A. and D.A. Kyriakidis, 2001. L-asparaginase of *Thermus thermophilus*: Purification, properties and identificaation of essential amino acids for its catalytic activity. Mol. Cell. Biochem., 216: 93-101.
16. Schroeder, M., M. Schweitzer, H.B.M. Lenting and G.M. Guebitz, 2004. Chemical modification of proteases for wool cuticle scale removal. Biocatalysis Biotransformation, 22: 299-305.
17. Talbert, J.N. and J.H. Hotchkiss, 2012. Chemical modification of lactase for immobilization on carboxylic acid-functionalized microspheres. Biocatalysis Biotransformation, 30: 446-454.
18. Kotsira, V.P. and Y.D. Clonis, 1998. Chemical modification of barley root oxalate oxidase shows the presence of a lysine, a carboxylate and disulfides, essential for enzyme activity. Arch. Biochem. Biophys., 356: 117-126.
19. Hu, Y. and Z. Guo, 2009. Purification and characterization of oxalate oxidase from wheat seedlings. Acta Physiol. Planta., 31: 229-235.
20. Nigam, A., 2007. Lab Manual in Biochemistry: Immunology and Biotechnology. Tata McGraw-Hill Education, New Delhi.
21. Riddles, P.W., R.L. Blakeley and B. Zerner, 1982. Reassessment of Ellman's reagent. Methods Enzymol., 91: 49-60.
22. Garman, A.J., 1997. Non-Radioactive Labelling: A Practical Introduction. Academic Press, London.
23. Klomsiri, C., P.A. Karplus and L.B. Poole, 2011. Cysteine-based redox switches in enzymes. Antioxidants Redox Signal., 14: 1065-1077.
24. Nagy, P., 2013. Kinetics and mechanisms of thiol-disulfide exchange covering direct substitution and thiol oxidation-mediated pathways. Antioxidants Redox Signal., 18: 1623-1641.
25. Little, C., T. Sanner and A. Pihl, 1969. Stimulation of fructose 1,6 diphosphatase by sulfhydryl reagents and interaction between the different enzymic sites in the molecule. FEBS J., 8: 229-230.
26. Wong, S.S. and D.M. Jameson, 2011. Chemistry of Protein and Nucleic Acid Cross-Linking and Conjugation. 2nd Edn., CRC Press, USA., ISBN: 9780849374913, Pages: 622.
27. Mendoza, V.L. and R.W. Vachet, 2009. Probing protein structure by amino acid specific covalent labeling and mass spectrometry. Mass Spectrometry Rev., 28: 785-815.
28. Rebek, Jr. J., 1990. On the structure of histidine and its role in enzyme active sites. Struct. Chem., 1: 129-131.
29. Zhao, X.Y. and T.W. Hutchens, 1994. Proposed mechanisms for the involvement of lactoferrin in the hydrolysis of nucleic acids. Adv. Exp. Med. Biol., 357: 271-278.
30. Eren, E., D.C. Kennedy, M.J. Maroney and J.M. Arguello, 2006. A novel regulatory metal binding domain is present in the C terminus of Arabidopsis Zn^{2+}-ATPase HMA2. J. Biol. Chem., 281: 33881-33891.
31. Opaleye, O., R.S. Rose, M.M. Whittaker, E.J. Woo, J.W. Whittaker and R.W. Pickersgill, 2006. Structural and spectroscopic studies shed light on the mechanism of oxalate oxidase. J. Biol. Chem., 281: 6428-6433.
32. Wallis, R.B. and J.J. Holbrook, 1973. The reaction of a histidine residue in glutamate dehydrogenase with diethyl pyrocarbonate. Biochem. J., 133: 183-187.
33. Yamashita, H., H. Nakatani and B.I. Tonomura, 1991. Substrate-selective activation of histidine-modified porcine pancreatic α-amylase by chloride ion. J. Biochem., 110: 605-607.
34. Kotsira, V.P. and Y.D. Clonis, 1997. Oxalate oxidase from barley roots: Purification to homogeneity and study of some molecular, catalytic and binding properties. Arch. Biochem. Biophys., 340: 239-249.