Chemical modification of biocatalyst for function in supercritical CO₂: *in silico* redesign of stable lipase

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Abstract:

In spite of excellent properties of supercritical CO₂ in enzyme catalyzed reactions, destabilizing effects of CO₂ molecules on the enzyme structure limits the industrial applications of this green solvent in the field of biocatalysis. Here, based on the substantial role of charged surface residues such as lysines in enzyme inactivation, we introduced for the first time, Post Translational Modifications (PTMs), a famous concept in molecular biology, as a protein stabilization strategy in supercritical condition. Lysine groups were modified using PTM templates to find out more about the exact mechanism of enzyme inactivation in supercritical CO₂ and to explore a new way for protein stabilization in this solvent. We used MD simulation as common tool for *in situ* examining enzyme structure in supercritical fluids, for the investigation of structural consequence of modifications. Different modifications including acetylation, methylation, phosphorylation, and carboxylation have been applied on the model enzyme. For comparison to CALB structure in supercritical CO₂, the acetylated enzyme was also simulated in aqueous solvent at 300 and 353 K. Interestingly, acetylation of lysine residues prevents enzyme from denaturation at high temperatures in water, which is in agreement with

experimental observation in aqueous solution. However, acetylation is not so useful for stabilization of enzyme in supercritical CO₂. In contrast, methylation and carboxylation efficiently stabilize enzyme in supercritical CO₂. Phosphate groups in phosphorylated lysines destabilize enzyme by formation of excess hydrogen bonds by inappropriate groups and perturb enzyme conformation. Moreover, it was found that modification of surface arginine residues was not so satisfactory in stabilization of the enzyme. This finding supports the mechanism of lipase inactivation through direct interaction of CO₂ molecules on lysine residues and formation of carbamates. We think this new exploration not only can open new window for developing enzyme catalyzed reaction in supercritical fluids, but it can shed some light on the mechanism of enzyme inactivation in scCO₂.

Keywords: supercritical, enzyme, modification, stabilization

Introduction

Post-translational modifications (PTMs) are covalent processing events which modulate the activity of most eukaryote proteins.[1] These PTMs change the properties of a protein by proteolytic cleavage or by addition of a modifying group to one or more amino acids. Protein modification is of major interest for both biologists and chemists. These modifications are specific chemical changes that drives the change in both protein structure and function [2]. Nowadays, many biologists and chemists use chemical modifications based on PTMs to change the structure, function and stability of proteins for different applications.

Coupling chemical modification and other techniques such as immobilization improves the catalytic performance and stability of the enzymes [3, 4]. Chemical modifications with different modifying reagents improved significantly the stability and activity of *Candida antarctica*

Lipase B (CALB) [5-8] and other enzymes [9-11]. Moreover, covalent modification of enzyme using ionic liquids has been used recently to improve enzyme stability and activity [12-16].

Enzyme catalyzed reactions in Supercritical CO₂ (scCO₂) is a promising technique which can overcome to industrial problems of non-aqueous biocatalysis [17]. The ability to control the physical properties of the solvent by simply changing the pressure or temperature, the easy and complete removal of the solvent, high reaction rates, and good control of selectivity are the advantages of using scCO₂ in enzymatic reactions[18-24]. Moreover, scCO₂ is a green, cheap, and readily available solvent. However, inactivation and short half-life of the enzyme in scCO₂ is a major problem that limits the biotechnological applications of scCO₂. [25][26-31]

But, there is not a clear reason at the molecular levels for these observations.[32] One possible hypothesis for inactivation of enzyme in scCO₂, is the formation of carbamates between CO₂ and lysine residues on the protein surface.[25] Although, some reports have suggested that carbamate formation is useful in enzyme catalyzed reactions[33, 34], others concluded that it leads to the enzyme inactivation.[27, 35] Altogether, there is a lack of consistency in the literature about enzyme inactivation in scCO₂ which can be attributed to the weakness of the available experimental techniques to describe the atomic level phenomena in supercritical/macromolecule system at high pressures.[25] Inability of spectroscopic measurements of macromolecule microstructure with the enough resolution at high pressures of supercritical fluids leads to develop Molecular Dynamics (MD) simulation to study protein structure in scCO₂. CALB is the most studied enzyme in non-aqueous media such as organic solvents and supercritical fluids. For the first time, we reported the detailed structural properties of *Candida antarctica* Lipase B (CALB) and its conformational reliability in scCO₂ using MD simulations.[36] Afterward, similar results were obtained by other investigators.[37] We also showed that how adding ionic

liquids (ILs) can stabilize protein conformation in scCO_2 . [38] It was found that CO_2 molecules interact directly with the surface residues which leads to protein instability. These residues are the initial points of enzyme denaturation. ILs hamper the interaction between surface residues such as lysine with CO_2 molecules which can limit the number of formed carbamates on enzyme surface. Now, we can say that each factor which can limit the CO_2 interactions with charged residues i.e. carbamate formation, can potentially stabilize enzyme. This fact is shown systematically in our recent paper. [39] In this case, PTMs may also be useful candidates. Can modifying lysine residues reduce instability of the enzyme? If so, which modifications can potentially stabilize enzyme? What are the structure consequence on enzyme conformation by these modifications? What is the coordinates of active site residues after enzyme modifications? This questions limit the applications and research about PTMs. There are many PTMs which can modify any residue on the enzyme. Despite the great importance of PTMs for biological function, their PTMs study has been limited by a lack of suitable methods, and many key modifications have only been discovered late in the elucidation of various biological processes. [1] If we use experiments before analyzing the structural consequences of each modification, the procedure become very time-consuming and expensive. Moreover, structural data which can be obtained for modified enzymes at high pressure conditions of supercritical fluids is limited. MD simulation can be a useful tool for studying PTMs. A large library of PTMs can be evaluated by MD simulation at the first step. MD simulation can give structural consequence of PTMs on the enzymes and proteins and compare the stability of native and modified forms. Afterwards, few selected candidates which are active and stable in MD analysis can be produced and evaluated by experiments. To the best of our knowledge, enzyme modification has not used in supercritical condition to this time. This despite the fact that enzyme

suffer from harsh condition of (Super Critical Fluids) SCFs and modification of lysine residues on the enzyme surface may be a useful solution for limiting the formation of carbamates. So, in this work, we have evaluated the applications of PTMs on enzyme residues in supercritical condition.

Theoretical Methods

The crystal structure of CALB (PDB: 1TCA)[40] was taken from the Protein Data Bank as an initial structure of protein in all simulations. Modifications on enzyme residues were performed by Vienna PTM 2.0[41]. Chemical structure of different modifications of lysine group which were used in this work are shown in Fig. 1. MD simulations were performed in scCO₂ at 305 K and 200 bar. These values of pressure and temperature are based on experimental values of scCO₂ in which protein denaturation was observed.[42] CALB was also simulated in water at 1 bar and two temperature of 300 and 353 K. The total charges of each simulation box were neutralized with sodium and chloride ions. The equilibration time for each simulation was 20 ns with a time step of 0.2 fs. Simulation results for CALB in scCO₂ were satisfactory in this simulation time. In simulation studies in such media, CALB reach to equilibrium at few nanoseconds. Therefore, applying more simulation lead to produce waste computational costs. Thus, no longer simulation time is required. To avoid any dependencies on the initial conditions and to increase the accuracy of the simulations, each simulation was repeated four times with the different initial conditions and the mean results were reported. The well-tested SPC/E[43] and EPM2[44] models were used for water and CO₂, respectively. All MD calculations were carried out using GROMACS 5.0.1[45] and GROMOS 54a7[46] force field. GROMOS 54a7 was tested as an accurate force field for simulation of PTMs.[47] A steepest-descent algorithm was

performed to minimize the energy of each system and to relax the solvent molecules. LINCS[48] algorithm was employed to fix the chemical bonds between the atoms of the protein and SETTLE[49] algorithm in the case of solvent molecules. To maintain a constant temperature and pressure for various components during simulations, the Berendsen[50] coupling algorithm was used. Particle Mesh Ewald (PME)[51] algorithm was applied for each component of the systems to estimate the electrostatic interactions. Molecular graphics were constructed by PyMOL[52] and all graphical representation structures were derived from final 2ns of simulation

Results and Discussion:

Acetylation of lysines

Lysine acetylation was discovered on histones about half a century ago[53] and is a key PTMs in different kind of cells, which naturalize the positive charge of this amino acid, changing protein structure and function in different manner.[54] There are many reports of protein acetylation which involved in diverse biological processes, suggesting that lysine acetylation has broad regulatory functions. It has a key role in the regulation of gene expression by modification of core histone tails.^[55] Some modified lysines have some roles in modulation of chromatin architecture by specifically binding to bromodomain containing proteins.[56] This PTM is also important for p53 functions and interactions and for microtubule stabilization.[57] Structural feature of unacetylated/ acetylated CALB is shown in Fig. 2(a). There are 9 lysine residues in CALB which are modified with different PTMs (residue number: 13, 32, 98, 124, 136, 208, 271, 290, and 308).

These structures are energetically minimized and there are no distinct difference between secondary and tertiary structure of CALB upon modification. The local conformation of one of modified lysine residues is shown in Fig. 2 (b).

Effects of acetylation on conformational stability of CALB in water

To evaluate the effects of acetylation on conformational stability in aqueous solution, the structure of modified and unmodified CALB are simulated at the elevated temperature. The root mean square deviation (rmsd) values which computed on C-alpha atoms for acetylated and un-acetylated CALB at the 353K and 1 bar were shown in Fig. 3 (a). As shown in this figure, rmsd values increase dramatically for unmodified CALB in 353 K, in comparison with those which obtained for enzyme at 300 K. Interestingly, for acetylated CALB at 353 K, rmsd values remain very close to those obtained for native CALB at 300 K. This shows that acetylation prevent thermal denaturation of the enzyme at elevated temperatures. Root mean square fluctuation (rmsf) values for each residue in acetylated and native form of CALB have been calculated and are shown in Fig. 3 (b). This figure is also imply that although the fluctuations are comparable in acetylated CALB at 353 K and un-acetylated CALB at 300 K, these values approach to very high amounts in un-acetylated CALB at 353 K. Based on rmsd and rmsf values we can conclude that acetylation of CALB can lead to produce very stable structure and can help to conformational integrity even at elevated temperatures. These results are in complete agreement with experimental observations of Shaw et al.[58] in which acetylation of lysine in α -amylase- one of the most important hydrolytic enzymes used in industry-produces highly negatively charged variants that are enzymatically active, thermostable, and more resistant than the wild-type enzyme to irreversible inactivation on exposure to denaturing conditions (e.g., 1 h at 90°C in solutions containing 100-mM sodium dodecyl sulfate). Although this is the first study which

reports that “CALB acetylation can produce stable biocatalyst”, it was found by experimentalist that other chemical modifications by ethylene glycol bis(succinimidyl succinate) (EGNHS) and glutaraldehyde can enhance CALB stability[6] . Moreover, CALB modification can alter enzyme enantioselectivity.[6, 7]

Catalytic activity of acetylated CALB

It is possible that a modification can stabilize 3D conformation of an enzyme but in the same time has unfavorable effects on catalytic activity. Modifications on the enzyme surface can alter the structure of active center, which can lead to enzyme deactivation. Catalytic properties of CALB are related to the situation of a catalytic triad, Ser105, Asp187, and His224. In the study of the organic solvent effects on the catalytic activity and stability of CALB, the structure of this catalytic triad was considered as major factor affecting the activity of CALB.[59] For function of the enzyme, the formation of a transition state is essential. This transition state is formed by attacking Ser105 as nucleophile to the carbonyl carbon of substrate which promoted by His224 as proton acceptor. The hydrogen bonding and the arrangement of catalytic triad has the most important factor in the formation of this transition state. The arrangements of the catalytic triad of CALB are compared in Fig. 4, between native forms of CALB at low and high temperatures and acetylated form at high temperature. It is clear that at the elevated temperature, the arrangement of the catalytic triad was destroyed and the distances between active site residues are far from each other to form appropriate hydrogen bonds. In contrast, even at the elevated temperature, the places of each residues in acetylated enzyme are very similar to the native enzyme at room temperature. This shows that acetylation not only stabilize tertiary structure of

the enzyme, but it also help to enzyme activation. Moreover, these results confirm that acetylation do not change the native arrangement of the active center.

Effects of acetylation on the stability of CALB in scCO₂

Rmsd values for acetylated and un-acetylated CALB in scCO₂ were compared in Fig. 5 (a). As shown in the figure, rmsd values are comparable and very high in both cases in scCO₂. Rmsd reach to about 0.45 nm in scCO₂ which is very close to those values obtained in hot water in the foregoing section. Therefore, CALB has an instable structure in scCO₂ which is in agreement with our previous studies. Although acetylation of enzyme in scCO₂ reduces the rmsd values, this reduction is negligible. This shows that acetylation, unlike to what that seen in hot water, cannot be a distinct useful modification for stabilization of enzyme in scCO₂. Rmsf values of acetylated and native CALB residues are in similar ranges (Fig. 5 (b)) which also implicated that structural fluctuations of both forms are similar in scCO₂.

Carboxylation of lysines

Here, carboxylation of lysine is especial case in PTMs. In actual, lysine carboxylation in nature is the formation of carbamate from reactions between lysine and CO₂, which is the strongest hypothesis in enzyme deactivation in scCO₂. Thus, converting all of lysine to carboxylated form can be very important both in case of the mechanistic insights and also producing a method for enzyme stabilization in scCO₂. Rmsd values for carboxylated and native form of CALB are compared in Fig. 6(a). Interestingly, after lysine carboxylation rmsd values strongly reduced in comparison with native enzyme in scCO₂. This shows that lysine carboxylation leads to producing very stable enzymes in scCO₂. Rmsf values are also show different behavior in carboxylated and native forms of the enzyme (Fig. 6(b)). Structural fluctuation of CALB

residues is reduced by carboxylation especially at high fluctuating residues, which can be seen in Fig. 6(b). These regions include $\alpha 5$ helix (residues 139-150), which play a critical role in CALB activity. As shown in Fig. 7 the secondary structure of $\alpha 5$ (highlighted as red) is destroyed in native form of CALB, while helicity is remained in carboxylated form. These results show that carboxylation can be useful modification for CALB in scCO_2 . By carboxylation of lysine residues at the protein surface, the net charge of enzyme surface is converted from positive to negative. It has been shown that proteins with unusually high negative charge have more resistance toward negatively charged molecules and surfactants.[58, 60]

By increasing negative charges on the enzyme surface CO_2 molecules cannot form appropriate interaction with positive lysines at the enzyme surface. So, CO_2 molecules cannot perturb enzyme structure. Now, this fundamental question may be asked that why carbamate formation in scCO_2 is considered as destabilizing effects for enzyme in scCO_2 ? Firstly, it should be noted that there is a dichotomy between the experimental data about the effects of carbamate formation on the activity of the enzyme. [27, 33, 34, 61] Besides, an important issue about carbamate formation in scCO_2 is that the formed carbamate is very unstable. At one moment carbamate is formed and then is dissociated, which this process repeated until termination of the enzyme treatment in scCO_2 . Therefore, those sections of enzyme which have the potential of carbamate formation become labile structures. This phenomenon can be occur especially in decompression step in scCO_2 solvent in which the concentration of CO_2 molecules are suddenly reduced and the decarboxylation of lysine can be occurred. The results which obtained here showed that carbamate formation in scCO_2 is not a destroying by its own. The instability of formed carbamate may be the major reason for destabilization of enzyme in scCO_2 .

Although the inappropriate interaction of CO₂ with carboxylic group may be a reason for stabilization of the structure, the conformation consequence of the intra-chain interactions as a result of carboxylation may be another important reason. It is clear that by carboxylation, the appropriate interaction of CO₂ with the positive amine side chain of lysine groups is disappeared and in contrast, CO₂ molecules cannot favorably interact with added carboxyl groups. However, to obtain the role of carboxylation on the structural properties of the enzyme and the possible mechanism of stabilization, the mean structure of some carboxylated lysine residues were analyzed. After structural analysis, it was found that carboxylated lysine on the enzyme surface make good interactions, which cannot be seen in unmodified lysine residues.

For example, the mean structural situations of native and carboxylated Lys 32 were compared in Fig.8. Carboxylated Lys 32 interacts strongly with Tyr 61 and Arg 242 through hydrogen bonding (Fig. 8 (a)). These hydrogen bonds immobilize surface structures and stabilize them. However, there are not such stabilizing interactions for unmodified Lys 32 (Fig. 8 (b)) and this residue can move freely. It was also found that carboxylated Lys 98 has an important role in stabilization of the loop structure between two important structural helices in CALB structure. As can be seen in Fig. 9 carboxylated lysine form different kinds of hydrogen bond and a rigid structure is constructed between two helices. Such loop structures are commonly fluctuating regions in protein structures. Moreover, carboxylation may help enzyme to be more active in destabilizing condition of scCO₂. It was known that some helical structures such as helix5 (residues 139-150) and helix10 (residues 266-289) have major roles in activation of CALB.[62] We found, in our earlier simulation works that one of the initial regions, which decomposed by exposing to scCO₂ and urea 8M is helix5.[63, 64] Other researcher have obtained similar result about that and found that modifying helix5 leads to obtaining more active and stable

enzymes[62]. Here, we found that carboxylation of Lys 136 can stabilize helix5 from denaturation in scCO₂. As shown in Fig. 7(a), when an unmodified CALB is simulated in scCO₂ the helical structure of helix5 is decomposed. However, this helix remains native after in carboxylated form of CALB in scCO₂ (Fig. 10). We attributed this stabilization on the role of Lys136. Carboxylation of Lys 136 leads to its strong interaction by Arg 238. As can be seen in the Fig. 6(b), this interaction reduces the rmsf values of helix5 (residues 139-150) to some extent.

Phosphorylation of lysines

Reversible phosphorylation is the most common PTM which plays fundamental regulatory roles in almost every aspect of cell life.[65] The widespread protein phosphorylation has been studied on serine, threonine and tyrosine that form acid-stable phosphomonoesters.[66] However, lysine residues also may undergo phosphorylation.[67] The acid liability of the P-N bond has prevented this modification from being studied extensively.[68] The rmsd values for phosphorylated and native CALB which are shown in Fig. 11 (a) are in similar ranges. This imply that no stability gain can be achieved by phosphorylation of lysine residues. This may be due to inappropriate interactions of phosphate groups at the enzyme surface. Although phosphorylation, same as carboxylation, leads to change the enzyme net charges from positive to negative, the amounts of negative charge in first case is very high for three negative oxygen atoms of phosphate group. This high negative charges on the enzyme surface may lead to intrinsic instability in 3D conformation of the enzyme. Fluctuations of enzyme residues in phosphorylated and native CALB in scCO₂ also confirm this fact because they are in a similar range (Fig. 11 (b)) The negative oxygen atoms in phosphate groups have strong ability for accepting hydrogen bonds. This high value of tendency can perturb protein structure and form

unexpected complex structures at modifying regions. When a group with very high ability of hydrogen bond accepting attached to the protein surface, some residues near this group may release their initial situations in tertiary structure and compete with other residues for donating of hydrogen bonds to this attached group. If any of these residues be one of important component of a helix, then this helix may be deconstructed as a results of attenuation of intra-residue interactions. One example of such phenomena can be seen in Fig. 12. As shown in Fig. 12 (a), Lys 136 interacts with Asp 17 through hydrogen bonding of its side chain. After modifying the side chain of lysine by phosphate group, phosphate group not only cannot form appropriate interaction with Asp 17, but it also inclined to form a strong hydrogen bond by Thr 159 (Fig. 12 (b)). This tendency can denature one helical structure in CALB conformation (Fig. 12(b)). Altogether, these data show that phosphorylation cannot be an appropriate modification for stabilization of enzyme in scCO_2 .

Methylation of lysine and arginine

Methylated lysine and arginine residues in histones are crucial parts of the histone code, and recognition of these methylated residues by protein interaction domains modulates transcription.[69] Moreover, methylation of lysine and arginine residues on non-histone proteins has considered as an important regulator of cellular signal transduction in cells.[70] Since both lysine[71, 72] and arginine[73, 74] methylation are very important and predominant PTMs in cell, here, we constructed both all-arginine and all-lysine-methylated CALB and simulated them. This can help to have a comparison between the effects of lysine and arginine amino group blocking on the enzyme stability in scCO_2 and obtaining more about the mechanism of enzyme instability in scCO_2 . Rmsd values for all-arginine-methylated and all-lysine-methylated forms of CALB and also native CALB in scCO_2 were compared in Fig. 13 (a). As shown in the figure,

although blocking lysine with methyl group leads to significant reduction in rmsd values of CALB, methylation of arginine cannot stabilize enzyme structure in scCO₂. Rmsf values are also confirmed that methylation of lysine is more potent in reduction of residues dynamics in comparison with methylation of arginine (Fig. 13 (b)). Blocking lysines leads to stabilization but blocking arginines do not. These results amplify the hypothesis which conclude the attacks of CO₂ molecules on lysines and not arginines.

After structural analysis of the average conformation of all-Lys-methylated CALB after MD simulation, it was found that the local structures of modified lysine residues are very similar to those of in native form. Fig. 14 shows some of these local structures for some modified lysine groups. As shown in this figure, methylation of lysine residues do not change the local structure about these groups and therefore, do not form further interactions similar to those seen in carboxylation or phosphorylation. These data imply that methylation only prevents CO₂-lysine interaction and the possible formation of carbamylated lysine residues. This insight again confirm that the main probable mechanism of enzyme/protein inactivation in scCO₂ is carbamate formation on surface lysine residues. Moreover, these results show that methylation of lysine residues is the best modification, which can prevent enzyme denaturation in scCO₂. Because methylation of lysine does not alter the 3D conformation by multiple interaction similar to what that has been seen in phosphorylation. Many soluble proteins resist crystallization because of the intrinsic flexible nature of the surface residues such as lysine. Lysine methylation has considered as routine rescue strategy for protein crystallization.[75, 76] Addition of methyl groups to the side chain amine of lysines has been shown to promote crystallization presumably by immobilizing these side chains.[76] It was shown that by this routine protocol, complete modification of all available lysine residues can be achievable.[77] Therefore, based on this

experimental data and also our theoretical results, it can be concluded that lysine methylation by blocking the available amine side chain of lysine can prevent CO₂ interaction and formation of carbamate. The actual methylation of all lysines is feasible. Accordingly, it is possible to produce large scale biocatalysts by this method and solve the problems which there are in the way of industrialize the biocatalytic reactions in scCO₂. The proposed stabilization method discussed here, not only show that enzyme remains robust and stable in supercritical fluids by using PTMs, but it can be completely practical in large scale enzyme production. PTMs are natural modification in nature. So, microorganisms such as fungus cells can produce modified enzymes in large industrial scales. CALB is also produced from yeast cells and can modified economically. It seems that cooperation of supercritical biocatalysis in one side and using PTMs for producing stable enzyme in other side, can establish a promising new way in biocatalyst industry.

Conclusions

In this work, we evaluate the hypothesis of “can blocking lysine side chains with post-translation modifications prevent enzyme denaturation in scCO₂?” We obtained interesting results about this hypothesis. It was found that how acetylation of lysine side chain can improve thermal stability of the enzyme in aqueous solution, which the result are in good accordance with experimental observations. However, acetylation of lysine side chain was not so useful in scCO₂. In contrast, carboxylation of lysine side chains significantly improve the enzyme stability in scCO₂. By carboxylation, the net charge on the enzyme surface convert from positive to negative. In the presence of these negative charges on the enzyme surface CO₂ molecules cannot form appropriate interaction with the enzyme surface. So, CO₂ molecules cannot perturb enzyme

structure. Although carboxylation leads to stability gain in scCO₂, the phosphorylation of lysine residues do not improve the enzyme stability. This is due to the very high negative charges on the enzyme surface as a result of phosphorylation which can lead to unfavorable intermolecular interactions in between enzyme side chains. Although most of hypotheses about enzyme deactivation in scCO₂ have concluded that lysine residues are the most probable interaction sites of CO₂ molecules, arginine residues can also interact with CO₂ through their amine side chains. For comparison between lysine and arginine residues, both side chains of these amino acids were blocked by standard methylation PTM. Interestingly, methylation can stabilize enzyme in case of lysine but not for arginine. This shows that CO₂ molecules destabilize enzyme predominately through its lysine residues. We found that the most appropriate and also experimentally feasible modification for enzyme working in scCO₂ is methylation of all lysine residues. To the best of knowledge, this is the first time which applied modified enzyme in scCO₂ for stabilization of 3D conformation. It is also the first study which investigated deeply the effects of PTMs on the protein structure and resulting conformations in scCO₂. Although this work is a molecular modelling simulation and is a prediction, the results is very interesting and applicable. Moreover, studying modified structures at high pressures is impossible with the current experimental techniques. We think that using modified enzymes in scCO₂ can be a key tool for overcoming to problem of enzyme instability in scCO₂ and for converting the research about scCO₂ biocatalysis from academic to industrial scales.

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Figures

Native lysine

Acetylated lysine

Carboxylated lysine

Phosphorylated lysine

Methylated lysine

Figure 1: Different modification of lysine group which were used in this work (hydrogen atoms attached to carbons were removed for clarity).

a)

b)

Figure 2. a) Graphical representation of native and acetylated CALB structure and b) structure of native and acetylated Lys1

a)

b)

Figure 3: (a) rmsd values of 3D structure of CALB and (b) rmsf values for each residues of CALB in aqueous solvent

a)

b)

c)

Figure 4. Conformation of the active site residues of CALB a) in native form at 300 K b) in native form at 353 K and c) in acetylated form at 353 K

a)

b)

Figure 5: (a) rmsd values of 3D structure of CALB and (b) rmsf values for each residues of CALB in supercritical phase

a)

b)

Figure 6: (a) rmsd values of 3D structure of CALB and (b) rmsf values for each residues of CALB in supercritical phase

a)

b)

Figure 7: Graphical representation of helix5 a) in native form scCO_2 and b) in carboxylated form in scCO_2

a)

b)

Figure 8: Structures of a) carboxylated Lys 32 and b) native Lys 32

Figure 9: hydrogen bonds of carboxylated lysine leads to stabilizing a loop between two helix structures

Figure 10: The role of Lys 136 in stabilizing of helix5.

a)

b)

Figure 11: (a) rmsd values of 3D structure of CALB and (b) rmsf values for each residues of CALB in supercritical phase

a)

b)

Figure 12: a) interaction of Lys 136 and Asp 17 for stabilizing a helical structure of CALB and b) formation of a strong hydrogen bond between phosphorylated Lys 136

a)

b)

Figure 13: (a) rmsd values of 3D structure of CALB and (b) rmsf values for each residues of CALB

Figure 14: Structural alignment of lysines in crystal (green) and methylated (blue) forms. Methylated structures were driven after simulation in scCO_2

a)

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b)

Figure 15. Conformation of the active site residues of CALB a) in native form at 300 K and b) in methylated form at 353 K