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Molecular investigation on the interaction of spermine with proteinase K by multispectroscopic techniques and molecular simulation studies

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ABSTRACT

The alteration in structure, function and stability of proteinase K in the presence of spermine was investigated using spectroscopic methods and simulation techniques. The stability and enzyme activity of proteinase K-spermine complex were significantly enhanced as compared to that of the pure enzyme. The increase in the value of V_{max} and the catalytic efficiency of Proteinase K in presence of spermine confirmed that the polyamine could bring the enzyme hyperactivation. UV–vis spectroscopy, intrinsic fluorescence and circular dichroism methods demonstrated that the binding of spermine changed the microenvironment and structure of proteinase K. The fluorescence studies, showing that spermine quenched the intensity of proteinase K with static mechanism. Thermodynamic parameters analysis suggested that hydrogen bond and van der Waals forces play a key role in complex stability which is in agreement with modeling studies. The CD spectra represented the secondary structure alteration of proteinase K with an increase in α-helicity and a decrease in β-sheet of proteinase K upon spermine conjugation. The molecular simulation results proposed that spermine could interact with proteinase K spontaneously at single binding site, which is in agreement with spectroscopic results. This agreement between experimental and theoretical results may be a worth method for protein-ligand complex studies.

1. Introduction

Enzymes are biomacromolecules which attract a lot of attention because of their catalytic activity in biochemical and chemical reactions [1]. Therefore, increase in the stability and activity of enzymes has extended their practical applications [2]. Various cosolvents are widely utilized in the studies on activity properties and stability of the enzymes. Recently enzymes are greatly used in different solvent media because of a number of advantages [3,4].

Polyamine compounds (such as spermine) found in prokaryotes and eukaryotes are biogenic amines with low molecular weight nitrogenous base [5–7]. The polycationic and polymethylene structure of polyamines make them appropriate to interact with different biomacromolecules such as proteins and nucleic acids [8,9]. Literature reviews show that the in vitro use of polyamines as a solution additive can be involved in stabilization and enzyme hyperactivation [9–12]. As cosolvents, polyamines cause water molecules to favorably interact, thereby stabilizing intramolecular interactions in proteins and other macromolecules [13].

Spermine or 1, 12 diamino- 4, 9- diazadodecane is a naturel polyamine with low molecular weight. Similar to other polyamines, spermine can interact with macromolecules such as proteins. In addition to multiple cellular functions, spermine has many industrial applications. The spermine content which exists in food, lead to small intestine mucosa maturation [14–16].

Serine proteases have important biomedical and industrial functions, especially in digestion [17]. Proteinase K (E.C. 3.4.21.64) is a well-characterized serine protease of subtilisin-like family from the fungus *Tritirachium album* Limber. Proteinase K is a stable endopeptidase in the presence of dodecyl sulfate and urea, and can easily hydrolyze native proteins without significant specific sequence for their cleavage [18]. High stability and no specify of proteinase K have led to attracting considerable research attention in biotech-

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nology, industry and agriculture. Because of such strict attentions, proteinase K became a model enzyme to study protein properties studies such as structure, stability and catalytic activity in different media [19–22].

X-ray crystal structure studies on proteinase K have shown that the catalytic triad and oxyanion hole of proteinase K are formed by Asp 39-His 69-Ser 224 and Asn 161, respectively. Furthermore, two segments Asn 99-Tyr 104 and Ser 132-Gly 136 formed substrate recognition site of proteinase K [21,23].

Proteinase K is a monomeric and globular protein with 279 amino acid residues [19,24]. This enzyme belongs to α/β proteins without any distinguished domains. Proteinase K has two tryptophans make it appropriate for spectroscopic assays [24,25]. Despite the intensive use of this enzyme in biology, industry and agriculture, to this date, there are no reports showing the effect of polyamines on the structure, catalytic activity and stability of proteinsae K. The aim of this study was to investigate the binding affinity of spermine as a natural polyamine to proteinase K. Therefore, the present research reports the result of kinetic, structural and theoretical studies of proteinase K in the presence of spermine by employing UV–visible, fluorescence, circular dichroism and molecular dynamic techniques.

2. Materials and methods

2.1. Materials

Proteinase K from *Tritirachium album* (catalogue no. P8044, MW 28900 Da) was purchased freeze dried from Sigma Company and was used without further purification. ρ-nitrophenyl acetate (a synthetic substrate of proteinase K), Tris–HCl buffer and spermine tetra hydrochloride (catalogue No. S2876) were also obtained from Sigma. All enzyme solutions were made in the 50 mM Tris–HCl buffer. Spermine was dissolved in deionized water. ρ-nitrophenyl acetate was dissolved in methanol and was diluted using deionized water. All solutions were provided on the same day and used uninterruptedly.

2.2. Kinetic studies of proteinase K in the presence of spermine

A Pharmacia 4000 UV–vis spectrophotometer was used for the catalytic activity assays of proteinase K. The reaction mixture contained 25 μg/ml of proteinase K in 50 mM Tris–HCl (pH 8). Substrate concentration in all assays was within the range of 0.2–1.25 mM.

Different concentrations of spermine (0–5 mM) were incubated with proteinase K at 35 °C for 15 min in 50 mM Tris–HCl buffer. Changes in the absorbance at 405 nm after addition of substrate (molar absorption coefficient of ρ-nitrophenol, $\varepsilon = 18800\ M^{-1}\ cm^{-1}$) [26] were used for the calculation of produced ρ-nitrophenol and velocity. The Lineweaver – Burk plot was obtained from the data of velocity against the substrate concentrations of ρ-nitrophenyl acetate and the kinetic parameters were calculated from Lineweaver-Burk plot [27].

2.3. Thermal stability studies of proteinase K

Thermal denaturation of proteinase K was studied by recording absorbance at a temperature range of 20–90 °C with a scan rate of 1 °C/min. A Pharmacia 4000 UV–vis spectrophotometer, equipped with an external thermostat was used for thermal stability studies at 280 nm. The T_m of denaturation process was obtained, from the temperature with $\Delta G^\circ = 0$, on the basis of two state equilibrium models.

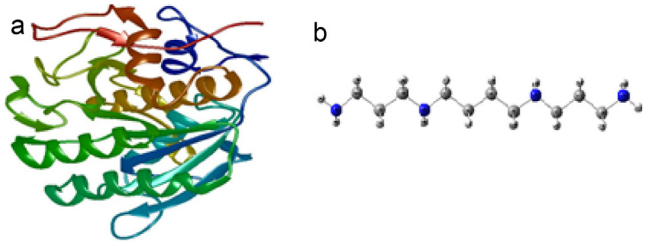

Fig. 1. (a) Structure of proteinase K (ID code 2ID8), (b) Structure of spermine.

2.4. Structural studies of proteinase K

2.4.1. UV–vis absorption studies

UV–vis spectra were obtained by a Pharmacia 4000 UV–vis spectrophotometer over the wavelength of around 245–300 nm using quartz cuvettes with 1 cm path length. Tris–HCl buffer (50 mM) was taken as the blank. Each spectrum is an average of at least 3 individual samples recorded at 35 °C. Absorption spectra were obtained at 0.1 mg/ml of proteinase K Tris–HCl buffer and different concentration of spermine (0–4 mM). All the solution systems were equilibrated at 35 °C for 15 min, and then the absorbance changes were obtained at 280 nm.

2.4.2. Fluorescence studies

The fluorescence emission of proteinase K at different concentrations of spermine was applied at 25 and 35 °C by a Shimadzu RF-5301 fluorescence spectrophotometer. The studies were performed on a quartz cell of 10 mm path length containing 0.1 mg/ml proteinase K. All measurements were recorded by exciting 278 nm in the emission range 290–450 nm. Furthermore, the excitation and emission slits were 3 and 5 nm for excitation and emission, respectively. Tris–HCl 50 mM was employed as the blank was subtracted from the obtained spectrum and was used as the correct fluorescence emission spectrum.

2.4.3. Circular dichroism studies

The secondary structural characteristics of proteinase K was measured by far-UV CD (190–260 nm) at different concentrations of spermine (0, 2, 4 mM. The Spectra of proteinase K after 15 min equilibration were run on an Aviv model 215 spectropolarimeter (USA). The CD spectra were obtained using a 1 mm cell path length. The reported CD spectra are an average of three scans, which was subtracted with spectra of buffer and of the spermine without enzyme. The results were given in ellipticity [θ] ($deg\ cm^2\ moL^{-1}$). The percentages of secondary structures were performed by means of the CDNN program, version 2.1.0.223 and secondary structure changes of enzyme at different concentrations of proteinase K were distinguished.

2.5. Theoretical studies

2.5.1. Molecular docking studies

The three dimensional structure of proteinase K with 1.27 Å resolution (as a receptor) with ID code 2ID8 was downloaded from the RCSB Protein Data Bank (http://www.rcsb.org) (Fig. 1a). All the crystal waters and hetero atoms such as Cl^- were removed; as a result, the free proteinase K was retained. The three dimensional structures of the spermine (as a ligand) were retrieved as SDF format from Pubchem compound database (http://pubchem.ncbi.nlm.nih.gov/) as a SDF format. Then opened the SDF file with Guass view software and save as PDB format (Fig. 1b). The ligand preparation was then carried out by adding hydrogen bonds and lowering energy using Hyperchem software and MM^+ force field.