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Conformational study of papain in the presence of sodium dodecyl sulfate in aqueous medium

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Abstract

The interactions between a globular protein, papain and the anionic surfactant, sodium dodecyl sulfate (SDS) have been investigated in aqueous medium using fluorimetric, circular dichroism, Fourier transform infra-red, UV–vis spectrophotometric, dynamic light scattering, and nuclear magnetic resonance techniques. The conformational change of papain in aqueous solution has been studied in the presence of SDS. The results show the high α-helical content and unfolded structure of papain in the presence of SDS due to strong electrostatic repulsion leading to a "necklace and bead model" in protein–surfactant complexes.

Keywords: Conformational study; Protein–surfactant interaction; Papain; SDS

1. Introduction

The study of surfactant–protein interaction creates much interest for many physicochemical as well as conformational phenomena. Such interaction has been widely studied for many years because of its applications in industry, chemical, biological, pharmaceutical and cosmetic laboratories [1–15]. Now-a-days, proteolytic enzymes (peptide-bond cleaving enzymes) are extensively used in soap and detergent industries for their very effective role to remove any stain, particularly stains of blood, egg-yolk, meat-soup, etc. In most of the interactions between proteins and surfactants, the "surfactant binding" to a single protein is considered, which can unfold and sometimes denature the globular protein [3,5]. Two general cases may appear for denatured proteins [16]: mixtures of anionic surfactants with proteins below and above the isoelectric point (IEP). Below the IEP, the protein is considered as a "cationic biopolymer" where the interactions with anionic surfactants are dominated by precipitation phenomena. Above the IEP, the interactions can form stable, fully solubilized complexes, which can change the topology and conformation of the protein molecule in solution. The most widely used anionic surfactant, SDS unfolds and denatures proteins more than that of cationic surfactants [5,7–8,17].

The water-soluble globular protein, papain (EC 3.4.22.2) is a thiol enzyme obtained from the latex and unripe fruit of *Carica papaya* (tropical melon or papaw). Papain is a carbohydrate free, basic, single chain protein. Papain has molecular weight of 23,350 Da and consists of 212 amino acid residues (methionine absent; IP 8.75) with four disulfide bridges and catalytically important cysteine (position 25) and histidine residues (position 158) [18]. Papain is used medically for fetal as well as postnatal brain regions to provide maximal dissociation and viability of the neurons and for the treatment of necrotic tissue and eczema.

Usually, various modes of association are observed for surfactant–protein interactions due to dipole–dipole, ion–dipole or ion–ion forces. Six different types of associations are discussed by Nagarajan et al. [19] on polymer–surfactant interactions involving either individual surfactant molecules or surfactant clusters along with the chains of polymers. The protein–SDS complex has been proposed by different models, such as rod shaped model

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[2], necklace model [14,15], deformable prolate ellipsoid model [4], etc. Among these, the 'necklace model' seems to have the strongest support. Originally, results from free boundary electrophoresis proposed that an unfolded protein binds SDS in the form of micelle-like clusters [12]. The proposal of necklace model is based on the results from free boundary electrophoresis [20], SANS [21], quasi-elastic light scattering [22], NMR [23], ESR [24], viscometry [14,25], circular dichroism [14,15] and fluorescence technique [24,26], where the partially or fully unfolded protein wraps around the micelle-like aggregates. This type of model is also observed between surfactants with polymers [20,27] and polyelectrolytes [28].

The physicochemical properties have already been studied [15] by tensiometry, conductometry, calorimetry, etc. for the interaction between SDS and papain. In this paper, the work has been done to study the conformation of papain in presence of SDS using a number of techniques like fluorimetry, circular dichroism, Fourier transform infra-red, UV–vis absorption and dynamic light scattering spectrophotometry, and nuclear magnetic resonance spectrometry. This investigation helps to determine the shape of the protein–surfactant aggregates.

2. Experimental section

2.1. Materials and methods

The globular protein, papain was crystalline powder, a product of Merck, Germany. It was dissolved in double distilled water and the pH was unadjusted. The anionic surfactant, sodium dodecyl sulfate (SDS) was a product of Sigma, USA. In all preparations, the used double distilled water has specific conductance of $3\,\mu S\,cm^{-1}$ at 303 K. All measurements were done at constant temperature in a water bath maintained at 298 ± 0.01 K.

2.1.1. Circular dichroism spectroscopy

Far- and near-UV circular dichroism (CD) measurements were performed on a Jasco, J-600 recording spectropolarimeter (Japan) attached with a chiller to control the temperature of Xe-lamp and electronic circuit. The instrument was calibrated with an aqueous solution of d-10-camphorsulphonic acid. Solutions for CD were prepared by mixing the papain solution in water with different concentrations of SDS in equal proportion. Far-UV CD spectra were measured between 200 and 250 nm wavelength with the mixture of papain and SDS in a 1 mm path length cuvette to study the conformational changes in the secondary structure of the protein. Near-UV CD measurements were done with a 10 mm path length cuvette in the region of 250–320 nm to study the changes in the tertiary structure. Five scans of each spectrum were signal averaged to increase the signal-to-noise (S/N) ratio and the scan speed was 50 nm/min.

2.1.2. Fluorimetry

The fluorescence emission spectra of native papain and of papain–SDS mixtures were measured using a F-3010 Fluorescence spectrophotometer, Hitachi (Japan) with a slit width of 1 cm. The excitation wavelength was 292 nm with the emission range of 300–400 nm. All spectra were measured thrice in a constant temperature water bath and the mean values were used for data processing.

2.1.3. Fourier transform infra-red spectroscopy

Solutions used for Fourier transform infra-red (FTIR) measurements were prepared by mixing of papain and SDS solutions in D_2O at different concentrations in equal proportion. Infra-red spectra were measured with a Nicolet, Impact 410 Fourier transform infra-red spectrophotometer (USA) at 298 K. Samples of a soluble or aggregated protein were placed between two CaF_2 discs (32 mm diameter) separated by a 25 μm thick spacer. The CaF_2 discs containing the protein sample were packed into a thermally insulated cell holder assembly placed on a mount inside the sample compartment of the FT-IR instrument, whose temperature was maintained by circulating water from a constant temperature water bath. For each spectrum, 64 interferograms were averaged and collected at absorbance mode with Happ–Genzel apodization function and Fourier transformed to give a resolution of $4\,cm^{-1}$ from 4000 to $400\,cm^{-1}$ region. The spectrum for D_2O was subtracted from the spectrum of the protein solution (or the mixture of protein–SDS) in D_2O, the resultant protein difference spectra were smoothed to remove the possible noise, and the baseline correction was made. Then Fourier self-deconvolution (FSD) was carried out using a bandwidth at half height of the peak of $20\,cm^{-1}$ and a resolution enhancement factor (K value) of 2.5, over the frequency range of $1700–1600\,cm^{-1}$. Such computational procedures were done employing the Omnic software.

2.1.4. UV–vis spectrophotometry

Spectrophotometric measurements were performed using a Shimadzu 160A UV–vis spectrophotometer (Japan) having temperature-controlling arrangement; 1 cm quartz cuvettes were used for sample holding. Initially, the absorbance reading was taken at 280 nm with a sample containing 2 ml aliquots of $0.01\,gm\,dl^{-1}$ papain solution and the reference cell containing 2 ml water; then subsequent reading was taken after each addition of SDS solution to both cells at 298 K.

2.1.5. Dynamic light scattering spectrophotometry

The particle size of microwater droplets of protein–surfactant complex was determined using a dynamic light scattering (DLS) instrument of Otsuka electronics, Japan. The used light source was 632 nm Ne laser, which acted on the solution of protein–surfactant mixture. The solution of papain or papain–SDS mixtures (1:1, v/v) in aqueous medium (millipore water) at different concentrations of SDS was taken in a cylindrical quartz cell. The DLS measurements have been taken at the angle of 90°, the scattered light from the solu-