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Spectrophotometric studies on the interaction between nevadensin and lysozyme

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Abstract

Interaction between nevadensin and lysozyme (Lys) was studied using spectrophotometric techniques such as steady fluorescence, synchronous fluorescence, circular dichroism (CD) and UV–vis absorption. The fluorescence emission intensity of Lys was strongly quenched by the addition of nevadensin. Spectrophotometric observations are rationalized in terms of a static quenching process at lower concentration of nevadensin (less than 8 μM) and a combined quenching process at higher concentration of nevadensin (8–20 μM). Binding constants and binding sites for the nevadensin–Lys system were evaluated. The distance of 2.28 nm and the energy transfer efficiency of 0.586 between nevadensin and Lys, evaluated from the Förster non-radioactive resonance energy transfer theory, indicated that the energy transfer from Lys to nevadensin occurred with higher possibility. Thermodynamic data showed that nevadensin was included in the hydrophobic cavity of Lys via hydrophobic interactions. UV/vis measurements on the enzymatic activity of Lys in the absence and presence of nevadensin indicated that the interaction between nevadensin and Lys led to a reduction in the activity of Lys. Furthermore, nevadensin binding to Lys had no influence on the molecular conformation of Lys.

Keywords: Nevadensin; Lysozyme; Fluorescence quenching; Combined quenching process

1. Introduction

Nevadensin (Scheme 1), belonging to the flavonoid family, has been widely used in traditional Chinese medicine for the treatment of lymph node tuberculosis, cough with tachypnoea and rheumatic pains [1]. It broadly distributes in plants of *lysiontous pauciflorus* Maxim, which are growing in a vast area of southern China. Otherwise, the therapeutical action of drugs is exerted by the storage in proteins and transportation by proteins in blood plasma. Therefore, studies on it will provide an insight into the chemical nature of the interaction of biomacromolecule with small drug molecules.

Lysozyme (Lys), a small monomeric globular protein, consists of 129 amino acid residues and contains six tryptophan (Trp) and three tyrosine (Tyr) residues [2]. With a molecular weight of about 14,306, its tertiary structure is compact with several helices surrounding a small beta sheet region. Since Lys was recognized by Fleming in 1922 as a bacteriolytic agent having an ability to hydrolyze bacterial cell walls, it has become one of the most intensively studied proteins. The action of Lys on bacteria works cooperatively and synergistically with antibiotics, which has a very important practical value in medicine area. Moreover, Trp or Tyr residues can cure abscess, stomatitis, rheum, etc. *via* binding to antibiotics. Therefore, studies on the interaction between drug and Lys are of importance in view of realizing disposition, transportation and metabolism of drug as well as efficacy process involving drug and Lys. A large amount of results have been published for the interaction of drug with human serum albumin. Yet, few results have been involved in drug binding to Lys.

In this work, we will report our studies on the interaction of nevadensin with Lys using spectrophotometeric spectra. Efforts were made to investigate the quenching mechanism, binding constants, binding sites, binding mode, binding location and the effect of nevadensin on the conformation of Lys.

2. Materials and methods

2.1. Materials and preparation of solutions

Lys was purchased from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Its

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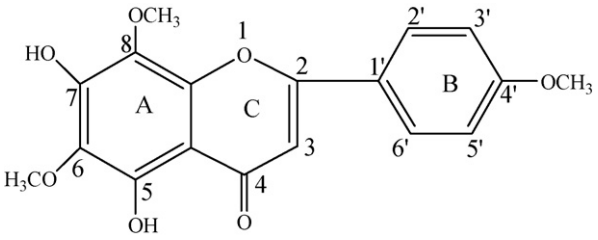

Scheme 1. Chemical structure of nevadensin.

molecular weight was assumed to be 14,306. An assay kit including *Micrococcus lysodeikticus* powder and standard Lys (20000 *U*/mg) with a special solvent for *Micrococcus lysodeikticus* was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Nevadensin was of analytical grade and purchased from the National Institute for Control of Pharmaceutical and Bioproducts, China.

A solution of 1 mol L^{-1} NaCl (analytical grade) was used to maintain the ion strength of samples at 0.1. A buffer solution of 0.05 mol L^{-1} Tris–HCl containing 0.1 mol L^{-1} of NaCl was used to keep the pH of solution at 7.40. A stock solution of Lys was prepared with the Tris–HCl buffer solution and stored in the dark at 4 °C for use. The stock solution of 1×10^{-3} mol L^{-1} nevadensin was prepared by dissolving nevadensin in 50 mL of anhydrous methanol. The suspension of 0.25 mg mL^{-1} *Micrococcus lysodeikticus* and the solution of 0.05 mg mL^{-1} standard Lys were prepared with a special solvent for *Micrococcus lysodeikticus* and doubly distilled water, respectively. All other reagents were of analytical grade. Doubly distilled water was used throughout all experiments.

2.2. Apparatus and methods

Fluorescence measurements were performed on an LS-55 spectrofluorophotometer (Perkin Elmer, America) following an excitation at 282 nm. Fluorescence spectra were recorded over a wavelength range of 290–500 nm. Both excitation and emission bandwidths were adjusted at 10 nm. Synchronous fluorescence spectra were obtained by scanning simultaneously the excitation and emission monochromator. The wavelength interval ($\Delta\lambda$) is fixed individually at 15 and 60 nm, at which the spectrum only shows the spectroscopic behavior of Tyr and Trp residues of Lys, respectively. Circular dichroism (CD) spectra were recorded on an Olis DSM-1000 automatic recording spectropolarimeter (USA) over the range of 200–250 nm at an interval of 0.5 nm in a 1-mm cell equipped with a temperature controlling unit. Each CD spectrum given was an average of three scans at 298 K. CD determinations of pure Lys and nevadensin-Lys mixtures were carried out using the buffer solutions of nevadensin at a corresponding concentration as the reference. Results are expressed as ellipticity (mdeg), which was obtained in mdeg directly from the instrument.

The activity of Lys in the absence and presence of nevadensin was measured on a UV-260 UV–vis spectrophotometer (Japan) by the absorption change at 450 nm of *Micrococcus lysodeikticus*, which was decomposed by Lys along time. The absorptions were recorded at an interval of 15 s within a time span of 5 min at 298 K. The specific activity (*U*/mg) of Lys was calculated to evaluate the effect of drug on the activity of Lys upon addition of nevadensin.

2.3. Fluorometric titration experiments

Into a 3.0 mL of Tris buffer solution (pH 7.40) containing 8×10^{-6} mol L^{-1} Lys was successively titrated using trace syringe the nevadensin stock solution, reaching a final nevadensin concentration of 2×10^{-5} mol L^{-1}. The fluorescence intensities were recorded at 347 nm following an excitation at 282 nm. The measurements were performed at 298, 308, and 318 K.

3. Results and discussion

3.1. Fluorescence quenching studies of Lys

Fig. 1 shows the fluorescence emission spectra of Lys with various amounts of nevadensin following an excitation at 282 nm. Lys exhibits a strong fluorescence emission band at 347 nm. Its intensity decreased gradually with the addition of nevadensin, i.e., the excited Lys was quenched by nevadensin. It was also observed that an increase in the fluorescence intensity at 426 nm assigned to nevadensin, which was well observed at a nevadensin concentration of 20 μM in the absence of Lys. These observations may be referred to a strong binding of nevadensin to Lys and a radiationless energy transfer between nevadensin and Lys [3]. Furthermore, an isoactinic point at 405 nm at less than 8 μM of nevadensin was observed in Fig. 1. It indicated the existence of both bound and free nevadensin at equilibrium [4]. When the concentration of nevadensin rose above 8 μM, no identical isoactinic points were present. This observation implies that the fluorescence quenching mechanism involved may be rationalized in terms of a combined quenching (both static

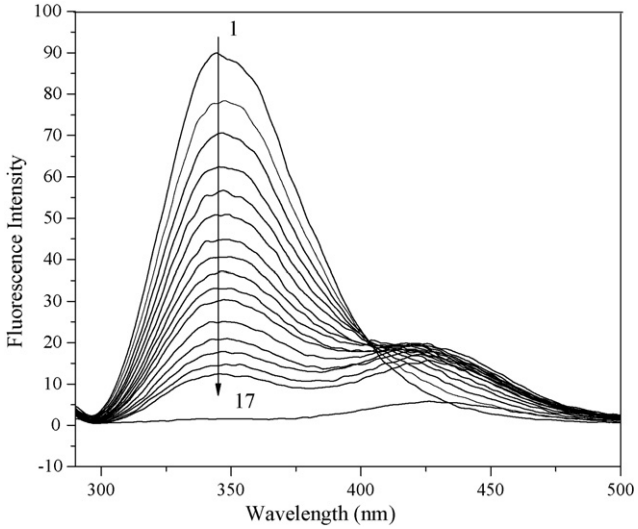

Fig. 1. Fluorescence spectra of Lys (8 μM) following the excitation at 282 nm, at pH 7.40 and 298 K with various amounts of nevadensin: [nevadensin] = 0 (1), 1 (2), 2 (3), 3 (4), 4 (5), 5 (6), 6 (7), 7 (8), 8 (9), 9 (10), 10 (11), 12 (12), 14 (13), 16 (14), 18 (15) and 20 μM (16), respectively. (17) shows the emission spectrum of pure nevadensin at 20 μM.