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Pesticide Biochemistry and Physiology 87 (2007) 23–29
PESTICIDE
Biochemistry & Physiology
www.elsevier.com/locate/ypest

Human serum albumin interaction with paraquat studied using spectroscopic methods

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Received 18 February 2006; accepted 11 May 2006
Available online 19 May 2006

Abstract

The interaction between herbicide paraquat and human serum albumin (HSA) was investigated by fluorescence and UV/Vis absorption spectroscopy. Paraquat can strongly quench the intrinsic fluorescence of HSA by static quenching and nonradiative energy transferring; The hydrophobic and electrostatic interactions play a major role in stabilizing the complex. The binding site number n and apparent binding constant K_A, corresponding thermodynamic parameters ΔG, ΔH, ΔS at different temperatures, were calculated. The distance r between donor (HSA) and acceptor (paraquat) was obtained according to fluorescence resonance energy transfer. The effect of paraquat on the conformation of HSA was analyzed using synchronous fluorescence spectroscopy.

Keywords: Paraquat; Human serum albumin–paraquat interaction; Intrinsic fluorescence; Binding thermodynamics; Fluorescence resonance transfer

1. Introduction

The herbicide paraquat (PQ) (1,1′-dimethyl-4,4′-bipyridinium dichloride) is a quaternary nitrogen compound, active as a redox drug inhibiting reduction of NADP to NADPH during photosynthesis [1]. This broad-spectrum herbicide has been widely used in agriculture for over 40 years. It is approved for use in more than 130 countries [2]. It is known to generate superoxide anions in mitochondria and cytosol of yeast and mammalian cells leading to the formation of several reactive oxygen species [3,4]. It is used as a nonselective contact herbicide, in presowing and interrow weed control of ploughed land, for stubble cleaning, and as a spray for weed control in maize, vineyards, and orchards. Because of its unavailability and its resistance to microbial degradation and breakdown by sunlight, PQ is long-lived. The bound residues transported in runoff with the sediment persist indefinitely [1]. Because of this widespread use, it often translates from foliage to foodstuffs and to the environment. It is known to exert toxic effects both in humans and animals and has been regarded as a genotoxic agent, inducing oxidative damage and strand breaks in DNA [5,6]. Immuno-histochemical studies have demonstrated the distribution and localization of paraquat in the lungs and brain, skin, stomach and esophagus, and in the immune and hematopoietic system [7].

Albumin represents 52–60% of the total plasmatic protein content and plays an important role in transport endogenous ligands and xenobiotics mostly through the formation of noncovalent complexes at specific binding sites, actuating in the regulation of their plasmatic concentrations [8,9]. Investigating the interaction of drugs to human serum albumin (HSA) can elucidate the properties of drug–protein complex, as it may provide useful information of the structural features that determine the therapeutic effectiveness of drugs [10]. The molecular interactions between HSA and many drugs have been investigated successfully [10–13]. However, the binding of the pesticides to proteins has seldom been investigated [14,15]. Furthermore, the interaction of paraquat with human serum albumin has not been thoroughly investigated.

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doi:10.1016/j.pestbp.2006.05.003

Fluorescence quenching is an important method to study the interaction of substances with protein because it is sensitive and relatively easy to use. Fluorescence spectroscopy is essentially a probe technique sensing changes in the local environment of the fluorophore, which distinguishes it from generalized techniques, such as calorimetry, far-ultraviolet circular dichroism (CD), and infrared (IR) spectroscopy. Also, various possibilities of structural rearrangements in the environment of the fluorophore may lead to a similar fluorescence signal; they can complicate interpretation of the experimental result and be exploited to obtain unique structural and dynamic information [16–18]. In the present work, we demonstrated the affinity of paraquat to HSA and the thermodynamics of their interaction. In order to attain these objectives, we planned to carry out detailed investigation of paraquat–HSA association using fluorescence spectroscopy and UV/Vis absorption spectroscopy. Through fluorescence resonance energy transfer and synchronous fluorescence spectroscopy, we planned to further investigate the effect of the energy transfer and the effect of paraquat on the conformation of HSA.

2. Materials and methods

2.1. Materials

HSA was purchased from Sigma (St. Louis, MO, USA) and Technical grade paraquat was obtained from Shandong Dacheng Pesticide Co., LTD (Zibo, China). The buffer Tris was purchased from Acros (Geel, Belgium), and NaCl, HCl, etc. were all of analytical purity. HSA solution (1.0×10^{-6} mol L^{-1}) was prepared in pH 7.40 Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.1 mol L^{-1} NaCl). The paraquat solution (5.0×10^{-4} mol L^{-1}) was prepared in pH 7.40 Tris–HCl buffer.

2.2. Equipments and spectral measurements

The UV/Vis spectrum was recorded at room temperature on a GBC UV/Vis916 spectrophotometer (Australia) equipped with 1.0 cm quartz cells. All fluorescence spectra were recorded on LS-50B spectrofluorimeter (Perkin-Elmer USA) equipped with 1.0 cm quartz cells and a thermostat bath. The widths of both the excitation slit and the emission slit were set to 5.0 nm.

2.3. Procedures

A 2.5 mL solution, containing appropriate concentration of HSA, was titrated by successive additions of a 5.0×10^{-4} mol L^{-1} stock solution of paraquat (to give a final concentration of 1.6×10^{-5} mol L^{-1}). Titrations were done manually by using trace syringes. The fluorescence spectra were then measured (excitation at 290 nm and emission wavelengths of 300–500 nm) at four temperatures (288, 295, 302, 309 K). In addition, in the presence of 1.0×10^{-6} mol L^{-1} metal ions, other fluorescence spectra could be obtained. The UV/Vis absorbance spectra of paraquat with concentration of 1.0×10^{-6} mol L^{-1} was recorded at room temperature.

2.4. Principles of fluorescence quenching

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with quencher molecule. Fluorescence quenching is described by the Stern–Volmer equation [19]:

$$\frac{F_0}{F} = 1 + K_q\tau_0[\mathrm{Q}] = 1 + K_{\mathrm{SV}}[\mathrm{Q}], \qquad (1)$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, respectively. K_q, K_{SV}, τ_0, and [Q] are the quenching rate constant of the biomolecule, the Stern–Volmer dynamic quenching constant, the average lifetime of the biomolecule without quencher ($\tau_0 = 10^{-8}$ s) and the concentration of the quencher, respectively. The concentration of the quencher should be the free ligand concentration, but it is not known in experiment. So, in our analysis it was approximated by the total concentration of the quencher. For higher ligand concentration, in excess of available specific protein binding sites, this approximation is valid. Obviously,

$$K_q = K_{\mathrm{SV}}/\tau_0. \qquad (2)$$

Hence, Eq. (1) was applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q].

3. Results and discussion

3.1. Fluorescence quenching

HSA consists of 585 amino acids residues forming a single polypeptide with well-known sequence, which contains three homologous α-helix domains (I–III). Each domain contains 10 helices and is divided into antiparallel six-helix and four sub-domains (A and B) [20,21]. A valuable feature of intrinsic fluorescence of proteins is the high sensitivity of tryptophan to its local environment [19]. Changes in emission spectra of tryptophan are common in response to protein conformational transitions, subunit association, substrate binding, or denaturation [17]. So, the intrinsic fluorescence of proteins can provide considerable information about their structure and dynamics, and is often considered on the study of protein folding and association reactions. There is only one tryptophan located at position 214 along the chain, in sub-domain IIA of HSA. HSA solutions excited at 290 nm emit fluorescence attributable mainly to tryptophan residues.

The effect of paraquat on tryptophan residues fluorescence intensity is shown in Fig. 1. As the data shows, the fluorescence intensity of HSA decreased regularly with the increasing concentration of paraquat, which indicates that paraquat can bind to the HSA and the binding site