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Short Communication

Experimental evidence on interaction between xenon and bovine serum albumin

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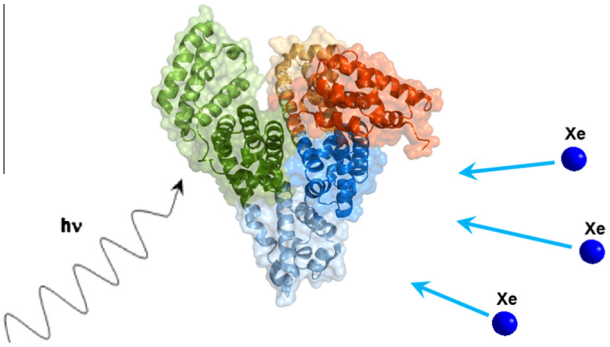
HIGHLIGHTS

- Xe gas interacts reversibly with BSA and acts as the fluorescence quencher.
- Xe guest is probably docked near the Trp212 residue and at additional places.
- Xe gas probably induces structural variations of BSA.
- BSA–Xe interactions are more complex than predicted by docking simulations.

GRAPHICAL ABSTRACT



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ABSTRACT

Xenon gas interacts with bovine serum albumin (BSA) dissolved in a physiological buffer solution. The fluorescence quenching related to the Trp emission is reversible and depends linearly on the time of saturation by Xe. The most probable site of this interaction is Trp212. The common emission of all BSA fluorophores is also influenced by Xe but this quenching is more complex and suggests: (i) at least two sites occupied by Xe and related to the Tyr and Trp residues; (ii) structural variations of BSA induced by the Xe guest atoms.

Introduction

Methods of investigation of very weak interactions between xenon and proteins are especially important due to the general anesthetic properties of the Xe gas [1]. It should be mentioned that xenon is compared to 'the ideal anesthetic agent', e.g. it has the lowest blood/gas partition coefficient (equal to 0.115) of currently available inhalational agents, so the processes of the anesthesia initiation and ending are very short in its case [2]. Although amazing features of the Xe gas in this field are well known, investigators are still trying to study and describe the mechanisms, which are responsible for its anesthetic action [3–6]. The key problem is to identify sites of the Xe docking [7]. In this report we focus our attention on the Xe interactions to albumin. This protein is of special importance in biology. It acts: (1) as a transport molecule which binds reversibly to an ions as well as a wide range of metabolites and drugs; (2) as an oncotic agent which in normal health contributes a large component to the total intravascular colloid oncotic pressure [8]; (3) as a free radical scavenger which is a major source of reduced sulfhydryl groups, the thiols (these are

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themselves involved in nitric oxide metabolism) [9,10]; etc. Bovine serum albumin (BSA), for its sequence is treated like a model for human serum albumin (HSA) [11,12]. The BSA–Xe and HSA–Xe interactions have been discovered by analysis of the NMR relaxation of hyperpolarized ^{129}Xe [13]. Yet this NMR experiment has not directly revealed places of the Xe – albumin contacts. For this reason we examine the Xe–BSA solutions using the fluorescence spectroscopy based on the light emission from strictly localized sites – from the BSA fluorophores. The aim of our work is to demonstrate the fluorescence quenching due to the BSA–Xe interactions and to indicate the regions of the Xe binding. Our conclusions are based on two different experiments on the BSA solutions mixed with xenon.

Materials and methods

Materials

All samples were prepared by dissolving bovine serum albumin (Albumin from bovine serum ⩾98%; Sigma–Aldrich) in physiological buffer (pH = 7.4) in room temperature. The concentration was 0.66 mg/mL (10^{-5} M). The solutions were also saturated with gaseous Xe (xenon 4,0; Messer Austria GmbH), at a rate of two bubbles per second. All samples were kept at 37 °C in a Ultrathermostat MLW U2c during 24 h before the experiments.

Apparatus

Fluorescence spectroscopy experiments were carried out on an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer. The fluorescence spectra were recorded in a range from 310 to 500 nm with excitation and emission slits equal to 5 and 2.5 nm, respectively. Two wavelengths of excitation were used: (1) λ_{exc} = 280 nm for the excitation of all fluorophores existing in BSA and (2) λ_{exc} = 298 nm for selective excitation of tryptophan. The maximum of both emissions was detected at 348 nm. The integral intensity of the emission was taken into account. The measurements were performed in a quartz cuvette (l = 10 mm).

Procedure of measurements

The first experiment

The BSA solution in the physiological buffer was placed in a sealed vessel. The solution was saturated by the xenon gas by portions. Each of the portions consists of the saturation in constant rate during 20 min. After each portion of the Xe addition, the vessel was opened and the sample was taken to the measurement. After that the vessel was closed. There were possible small losses of the Xe gas caused by cyclic opening and closing the vessel.

The second experiment

The BSA solution in the physiological buffer was placed in the sealed vessel. The solution was saturated by the xenon gas only once. The saturation was ceaseless and its time equal to 40 min was assumed. After that the vessel was opened and the sample was taken to the measurement, then the vessel was closed. The next measurements were carried out after 1, 2, 3, 4, 5, 6 and 72 h after the ceaseless saturation (during 40 min). One should expect small losses of the Xe gas caused by cyclic opening and closing the vessel.

Results and discussion

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with quencher molecule [14]. We have used this method for measuring the fluorophore – Xe binding affinities. There are three kinds of fluorophores in BSA: tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe), but only Trp and Tyr have a large quantum yield and should give dominant contribution to the total BSA emission [15]. In contrast to the Tyr residues, which are distributed in whole BSA molecule, Trp is located in two positions: Trp212 in the subdomain IIA within a hydrophobic binding pocket and Trp134 in the subdomain IB on the surface of the molecule [16].

In the first experiment we were studied the fluorescence behavior of bovine serum albumin dissolved in the buffer and saturated step-by-step by the Xe gas. The spectra were recorded directly after each portion of the saturation lasting 20 min. Thus the cumulative saturation time, ST, was ST = $n \cdot 20$ min, where the experimental number n was varied from 0 to 6 (Fig. 1 and Supplementary material: Figs. S1 and S2).

Selective excitation of Trp (λ_{exc} = 298 nm) has given emission band with the maximum at 348 nm. Its integral intensity decreases with the cumulative saturation time of the solution, ST = $n \cdot 20$ min, and this quenching, Q(298), reaches about 5.24% after ST = 120 min (Fig. 1).

Similar result was recorded after the excitation of all fluorophores in BSA (λ_{exc} = 280 nm) and Q(280) = 4.40% after ST = 120 min was obtained (Fig. 1). Those both observations are experimental evidence that xenon gas acts as the fluorescence quencher and interacts with BSA. Decreasing of the emission in both cases (after the 298 and 280 nm excitations) is rather not large, but this finding should not be surprising. Gaseous xenon as the anesthetic agent should subtly and reversibly react with a living organisms [17].

Q(298) increases linearly with ST (Fig. 1; R^2 = 0.9864). This phenomenon may suggest that Xe interacts directly with one Trp residue or close to its neighborhood. The suggestion associated with the Trp134 participation might be rejected because the primary interactions of anesthetic binding in albumin are rather hydrophobic and van der Waals types [18]. Trp212 fulfills this requirement. Additionally, it is well established that different quenchers would preferentially interact with apolar regions of albumin – for example the antithyroid drug Methimazole [19]. We also know that the π-electrons system of Trp could be involved in the interaction with halothane [18]. Furthermore, hydrophobic and van der Waals forces are the major expected contributors to the binding

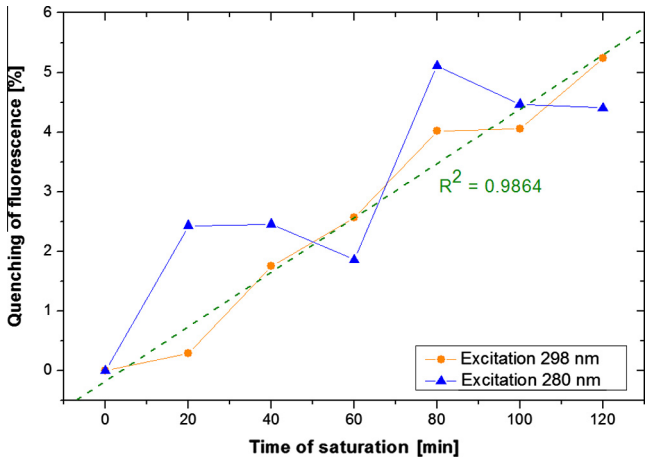

Fig. 1. The fluorescence quenching during step-by-step saturation of the BSA solution by Xe gas (the quenching equal to zero corresponds to the solution without Xe). The experimental scheme: measurement-(20 min saturation)-measurement-(20 min saturation)-measurement-..., etc.