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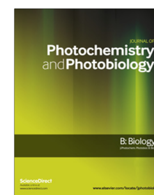

Realizing the recognition features of model antipsychotic compounds by important protein: Photochemical and computational studies

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ABSTRACT

Phenothiazine and its derivatives are the most effective antipsychotic drugs. They have been used in the treatment of serious mental and emotional symptoms including bipolar disorder, organic psychoses, psychotic depression and schizophrenia. However, these drugs cause serious side effects such as akathisia, hyperprolactinaemia and neuroleptic malignant syndrome. In this work we investigated the molecular recognition of two typical phenothiazine compounds, phenosafranin and safranin O by the most pivotal heme protein hemoglobin using steady state and time-resolved fluorescence, extrinsic 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescent probe, circular dichroism (CD) along with computational modeling. Results show phenothiazines complex with protein via formation of adducts at 298 K with moderate strengths of $3.555 \times 10^4\,M^{-1}$ and $2.567 \times 10^4\,M^{-1}$ for the hemoglobin–phenosafranin and hemoglobin–safranin O, respectively. We also found phenothiazines were effectors at the protein allosteric site, which affects the allosteric equilibrium. Further, time-resolved fluorescence and hydrophobic ANS experiments showed the static mechanism is dominated for the shrinkage in the fluorescence intensity of β-37 Trp residue at the $\alpha_1\beta_2$ interface. The stoichiometric proportion of the protein–drug adduct is 1:1, as derived from Job's plot. Several crucial noncovalent bonds, including hydrogen bonds, π–π stacking and hydrophobic interactions played a major role in stabilizing the noncovalent conjugates. Based on three-dimensional fluorescence, we concluded that the conformation of hemoglobin is partially destabilized after recognition with phenothiazines. These alterations were confirmed by far-UV CD spectra that showed the α-helix of protein decreased from 78.3% in free hemoglobin to 62.8% and 64.8% in hemoglobin–phenosafranin and hemoglobin–safranin O, respectively. Computer-aided molecular docking was consistent, indicating that both phenothiazines are situated within the pocket composed of α_1 and β_2 subunits. Affinity of hemoglobin to phenosafranin is superior compared with safranin O. This difference may be explained by the methyl group substituent on A- and C-rings, and by the different molecular volume between phenosafranin and safranin O. Our data provides further explanation of the overall pharmacokinetics of phenothiazines and sheds light on the allosteric regulation of heme proteins.

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1. Introduction

Molecular recognition is a basic procedure in fundamentally any biological process and, on the whole, it occurs chiefly by specific interaction between macromolecules and biological ligands [1]. Realizing how two molecules recognize each other is thereby one of the essential issues in biochemistry, and particularly an integral part of medicinal chemistry, because molecular recognition decides whether a ligand holds useful clinical properties [2]. For the purpose of investigating these molecular recognitions, several biological targets, such as enzymes, proteins and nucleic acids have

Abbreviations: Ala, alanine; ANS, 8-anilino-1-naphthalenesulfonic acid; Asn, asparagine; CD, circular dichroism; IRF, instrument response function; Leu, leucine; Lys, lysine; Phe, phenylalanine; R, correlation coefficient; S.D., standard deviation; Tris, tris(hydroxymethyl)aminomethane; Trp, tryptophan; Tyr, tyrosine; UV/vis, ultraviolet–visible spectroscopy; Val, valine.

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been utilized as receptor macromolecules. Of all these biopolymers, one biomacromolecule that has been rarely used in such a case is hemoglobin. The heme protein, hemoglobin, is the iron-containing oxygen transport metalloprotein in the red blood cells of all vertebrates as well as the tissues of some invertebrates. In mammals, the protein comprises about 97% of the red blood cells' dry content and around 35% of the total content [3]. The foremost physiological function of hemoglobin is to transport oxygen from lungs to different tissues, to yield hydrogen peroxide dispersion, and electron transfer in all organs and parts of the body. It also plays a vital role in the transfer of H^+, CO_2 and 2,3-bisphosphoglycerate from tissues to lungs and kidneys, from which they are excreted [4]. The concentration of hemoglobin in red blood cells is 330 mg mL^{-1}, and hemoglobin is also found outside red blood cells and their progenitor lines. Additionally, the high-resolution X-ray diffraction atomic structure of hemoglobin has been resolved by Perutz and co-workers [5] in 1960s, which is one of the major breakthroughs in the history of molecular biology. Hemoglobin is roughly spherical molecule $6.4 \times 5.5 \times 5.0$ nm, it is a tetrameric protein involving four heme groups, each one associated with each polypeptide chain to which oxygen and several other small molecules can bind reversibly [4,5]. In adult human hemoglobin, there are two identical chains of 141 amino acid residues, the α-chains, and two identical β-chains, each of 146 residues. The α-chains contain seven and the β-chains eight helices, and the four chains are held together by noncovalent bonds [4–6]. Further, the α- and β-chains have different sequences of amino acids, but fold up to shape similar three-dimensional structures. The tetrameric structure of hemoglobin permits cooperative interactions that are essential to its biological function [7].

Phenothiazine is an organic chemical that arises in various antipsychotic and antihistaminic drugs. The compound is connected with the thiazine group of heterocyclic agents, and derivatives of the parent compound also find vastly utilize as drugs [8]. Historically, the chemical was originally synthesized by Bernthsen [9] in 1883 through the reaction of diphenylamine with sulfur, but soon afterwards syntheses depend on the cyclization of 2-substituted diphenyl sulfide. Phenothiazine itself was introduced by DuPont as an insecticide in 1935, and it is sometimes applied as an anthelmintic in livestock [10]. Currently, the largest widely used neuroleptic antipsychotic drugs are principally derived from the phenothiazine structure, e.g. chlorpromazine, promethazine and thioridazine. These derivatives have also excellent antipsychotic and antiemetic activities, although they may possibly give rise to serious side effects, such as extrapyramidal symptoms, including akathisia and tardive dyskinesia, hyperprolactinaemia, and the scarce but potentially mortal neuroleptic malignant syndrome as well as substantial weight gain [11–13]. Moreover, phenothiazine derivatives are employed as inodilator in congestive heart failure, acting upon the phosphodiesterase I; and chlorpromazine and prochlorperazine are especially exploited in emergency rooms to cure migraine and other intractable headaches [14,15].

As noted earlier, in arousing pharmacological and toxicological responses, drugs commonly unite either reversibly or irreversibly with action sites on intrastitial biomacromolecules or organelles, and thus induce changes of physicochemical or biochemical processes in the human body [16,17]. Furthermore, the dispersion of most drugs is reigned by their reversible conjunctures with proteins and other ingredients in blood and tissues. As a result, perhaps one can deliberate the identification of the molecular recognition of various drugs by biopolymers in most mammals (especially humans) frequently gives an indicative pattern of the pharmacokinetics of drugs, because almost any drugs are bound to both tissue and plasma proteins [18,19]. In spite of a large volume of literature, there is little consolidated information available on the kinetic and physiological cues of recognition properties of phenothiazine derivatives with heme protein at the molecular level. It is therefore proper to contemplate the role of hemoglobin in providing phenothiazines binding and pharmacological data.

Up to now plentiful experimental and to a lesser extent theoretical approaches have been reported to decipher the recognition process and the structure of the complexes, including calorimetry, chromatography, circular dichroism, crystallology, electrophoresis, equilibrium dialysis, fluorescence, Fourier transform infrared spectroscopy, light scattering, nuclear magnetic resonance, rheology, small-angle neutron scattering, surface enhanced Raman scattering, surface tension, turbidity, ultracentrifugation and ultrafiltration [20–28]. Within these techniques, fluorescence spectroscopy is just appropriate tool to measure the most comprehensively qualitative and quantitative parameters on the ligand–protein molecular recognition, as already demonstrated by MacManus-Spencer et al. [29] recently. The scope of the present scenario was to characterize the recognition reaction as well as the structure of the complexes formed between the phenothiazine derivatives, phenosafranin (3,7-diamino-5-phenylphenazinium chloride) and safranin O (3,7-diamino-2,8-dimethyl-5-phenylphenazinium chloride) (structure shown in Fig. 1), and the protein hemoglobin by means of steady state and time-resolved fluorescence, extrinsic 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence and molecular docking. Also, the conformation of hemoglobin after complexation with phenothiazine derivatives was monitored by circular dichroism (CD) and three-dimensional fluorescence measurements.

Phenosafranin and safranin O are both phenothiazine derivatives and generally used in biology as a sensitizer in electron transfer reactions in homogeneous media, in semiconductors and in polymeric media [30]. They were also employed as a probe in micellar systems, covalently bounded to macromolecules, and determination of nucleic acid [31–33]. Nevertheless, the antimalarial potency of these compounds has been shown [34] and in a more recent study, Zucca et al. [35] deemed that

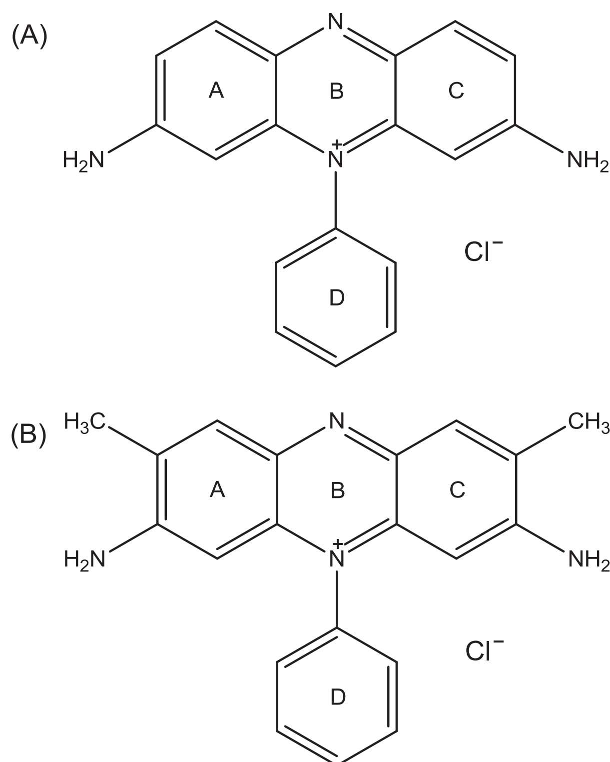

Fig. 1. Molecular structures of phenosafranin (A) and safranin O (B).

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