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Biointeractions of C.I. Acid Red 2 and its structural analogues with transporter albumin: Fluorescence, circular dichroism, and ligand docking approaches

Wei Peng [a,b,c], Fei Ding [a,c,d,*], Yong Xie [e]

[a] College of Agriculture and Plant Protection, Qingdao Agricultural University, Qingdao 266109, China
[b] College of Food Science and Engineering, Qingdao Agricultural University, Qingdao 266109, China
[c] Department of Chemistry, China Agricultural University, Beijing 100193, China
[d] Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States
[e] State Key Laboratory of the Discovery and Development of Novel Pesticide, Shenyang Research Institute of Chemical Industry Co. Ltd., Shenyang 110021, China

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ABSTRACT

In this contribution, the toxicological effects of C.I. Acid Red 2 and 1-(2-pyridylazo)-2-naphthol (PAN) have been elucidated by utilizing plasma albumin as a biological model. Fluorescence data indicated that the Trp-214 residue was quenched by both azo compounds, but the quenching degree of C.I. Acid Red 2 is less than PAN. According to the results of time-resolved fluorescence decay, it may be observed that the quenching of Trp-214 residue is controlled by static type; this corroborates the Stern–Volmer analyses and the conformational transition of protein was concurred. The experiments also found that azo colorants are situated within subdomain IIA, several amino acid residues, such as Ser-202, Ala-210, and Trp-214 were believed to be yielded direct interaction with the two chemicals, yet the operating distances between C.I. Acid Red 2 and relevant residues are greater than PAN. Interestingly, we may ascertain that the azo colorants with naphthalene ring possess stronger affinity with protein than those just having benzene ring in their molecular structure. This suggested that the existence of naphthalene ring substituent could hold relatively great risk for the human body due to large hydrophobicity (*c*Log*P*); therefore, the hydrophobicity of azo colorants can probably be a major element of its toxicological activities.

1. Introduction

Food color is an essential part to sensory quality of different foods. One kind of chemical agent, that is, colorant, has generally been used both in commercial food production and in domestic cooking, in order to increase the hobby and stimulate the appetite of humans [1,2]. Artificial food coloring usually dominates the commercial market in many developing countries such as China and India; these chemicals are largely used in many kinds of food processing, e.g. cakes, candies, drinks, ice creams, jellies, liquors, meats, oils, etc. [3,4]. It is estimated that the amount of synthetic food colorants will continue to rise along with the improvement of living standards. Unfortunately, the irregular addition and application of various artificial colorants by the manufacturer during the food production process have brought severe issue of food safety, which could further create enormous potential harm to human health [5]. Among these synthetic colorants, azo compounds account for a considerable proportion of the colorant market. It is worthwhile to note that the acute toxicity of azo colorants in different foods is generally low, and pathological symptoms such as poisoning might not emerge if one just consumes a small amount of food added with azo colorants. Instead, the consumption of artificial azo colorants in the long term may well lead to chronic toxicity to the human body [6].

The accumulative biochemical and toxicological results have clearly shown that part of azo colorants has comparatively large side effects such as carcinogenicity, mutagenicity, and teratogenicity in experimental animals [7]. The International Agency for Research on Cancer (IARC), which is a branch of the World Health Organization, also summarized some azo colorants with potential carcinogenicity for mammalians [8]. Moreover, several azo chemicals have been prohibited for use as food color additives, but regrettably, these illicit substances are still used heavily to color diversely commercial food products. For example, C. I. Solvent Yellow 14 (known as Sudan I) has been proven to be highly carcinogenic/genotoxic to humans (bladder cancer and hepatic tumor);

Abbreviations: Ala, alanine; ANOVA, analysis of variance; ANS, 8-anilino-1-naphthalenesulfonic acid; CD, circular dichroism; GuHCl, guanidine hydrochloride; IARC, International Agency for Research on Cancer; IRF, instrument response function; Leu, leucine; Lys, lysine; PAN, 1-(2-pyridylazo)-2-naphthol; Phe, phenylalanine; R, correlation coefficient; S.D., standard deviation; Ser, serine; Tris, tris(hydroxymethyl)aminomethane; Trp, tryptophan; Tyr, tyrosine; Val, valine.

* Corresponding author at: College of Agriculture and Plant Protection, Qingdao Agricultural University, Qingdao 266109, China.
E-mail addresses: alexf.ting@outlook.com, feiding@cau.edu.cn (F. Ding).

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however, this azo compound is used extensively in duck egg processing so as to make the yolk more attractive [9,10].

Frequently, the differences in ligand structures could arouse the disparities of molecular recognition of azo colorants by the key biomacromolecules in the human body and further lead to the pathological changes and lesion in cells, tissues, and organs. These events would ultimately be represented as the toxic distinctions of these compounds in vivo. With regard to this aspect, certain previous tasks have explored the poisonous differences of several azo compounds possessing different structures for laboratory animals. For instance, the results of carcinogenic evaluation displayed obviously that a few of azo colorants with naphthalene ring, i.e. C.I. Food Red 5, C.I. Food Red 6, C.I. Solvent Orange 2, C.I. Solvent Orange 7, C.I. Solvent Yellow 14, and C.I. Solvent Red 80, will significantly induce cancer in mammalians [11,12]. In two earlier studies, Ashby et al. [13] and Dashwood et al. [14] found that 5-(*p*-dimethylaminophenylazo)indazole and 6-(*p*-dimethylaminophenylazo)benzothiazole (involving benzopyrazole and benzothiazole groups) have stronger mutagenic ability, as compared with 4-dimethylaminoazobenzene (commonly known as C.I. Solvent Yellow 2). This demonstrates that mutagenicity is very strongly implicated in the molecular structures of azo compounds. In view of these beneficial explorations, one could perceive that the scrutinization of structure–activity relationships of azo chemicals, especially the impacts of ligand structural characteristics such as benzene ring and naphthalene ring on the biopolymer-azo colorants recognition processes, is imperative, since these useful efforts help us to greatly understand the potential hazards of synthetic azo colorants to human health.

As the most abundant protein in the circulatory system and with typical blood concentration of 45 g L^{-1}, albumin contributes 80% to colloid osmotic blood pressure [15]. In mammalians, albumin is synthesized by the liver and possesses a half-life in circulation of 19 days. It has now been determined that albumin is a multifunctional protein, and probably the most remarkable property of albumin is its transport function [16]. This characteristic enables albumin to bind a striking variety of endogenous and exogenous substances, e.g. agrochemicals, bile acids, bilirubin, colorants, drugs, fatty acids, hormones, metal ions, and form reversibly noncovalent adduct [15–18], which is the major transportation mode of these ligands in circulating plasma. Consequently, albumin belongs to nonspecific transport protein; the bioactive ligand will temporary lose its activity and deposit in the macromolecular depot when the protein–ligand recognition occurs. Due to the homeostasis and reversibility of the biointeractions, the transport function of albumin is vitally important to maintain human health, particularly to regulate the pharmacokinetics/toxicokinetics (absorption, distribution, metabolism, and excretion) of these active substances in the human body [19,20]. Moreover, the remediation of hypoproteinemia may be found to associate with the interesting function of this protein. For this reason, a systematic study of the molecular recognition of azo compounds having different structures with albumin, and subsequently a discussion of the structure–activity relationships, and ultimately a rational assessment of the potential hazard of these compounds are extremely practicable.

This story plans to excavate the recognition processes between the crucial albumin and the typical azo chemicals in great detail and further explore the effects of structural discrepancies (benzene ring *versus* naphthalene ring) on the albumin–azo compounds recognition and its immanent structure–activity relationships. The chemicals, that is C.I. Acid Red 2 and 1-(2-pyridylazo)-2-naphthol (PAN), have been selected as the model azo compounds (structure shown in Fig. 1). Different experimental methods, i.e. fluorescence, circular dichroism, and ligand docking, have been adopted to probe the recognition reaction. Nonetheless, we chose other four representative azo compounds, namely, azobenzene, C.I. Acid Orange 52, C.I. Acid Orange 7, and C.I. Solvent Yellow 14, which have analogous skeleton structures to C.I. Acid Red 2 and PAN, respectively, so as to decipher comprehensively the changes of molecular recognition as a result of structural transformation in azo compounds.

2. Experimental

2.1. Materials

Albumin from human serum (A3782, lyophilized powder, fatty acid free, globulin free, ≥99%, CAS number: 70024–90-7), C.I. Acid Red 2 (32654, CAS number: 493-52-7), 1-(2-pyridylazo)-2-naphthol (82960, ≥97%, CAS number: 85-85-8) and 8-anilino-1-naphthalenesulfonic acid (A1028, ≥97%, CAS number: 82-76-8) used in this study were purchased from Sigma–Aldrich (St. Louis, MO) and used without further

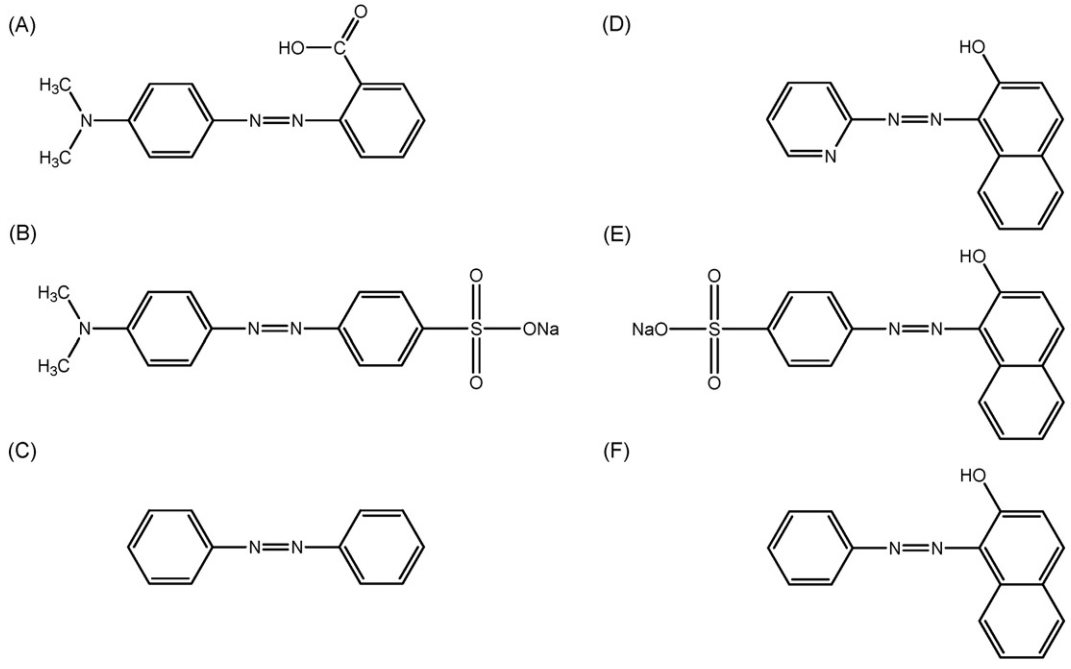

Fig. 1. Molecular structures of C.I. Acid Red 2 (A), C.I. Acid Orange 52 (B), azobenzene (C), 1-(2-pyridylazo)-2-naphthol (D), C.I. Acid Orange 7 (E), and C.I. Solvent Yellow 14 (F).