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Original article

Binding sites of retinol and retinoic acid with serum albumins

A. Belatik [a], S. Hotchandani [a], J. Bariyanga [b], H.A. Tajmir-Riahi [a,*]

[a] Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C. P. 500, Trois-Rivières (Québec), G9A 5H7, Canada
[b] Department of Chemistry, University of Hawaii-West O'ahu, 96-129 Ala Ike, Pearl City, HI 96782, USA

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ABSTRACT

Retinoids are effectively transported in the bloodstream *via* serum albumins. We report the complexation of bovine serum albumin (BSA) with retinol and retinoic acid at physiological conditions, using constant protein concentration and various retinoid contents. FTIR, CD and fluorescence spectroscopic methods and molecular modeling were used to analyze retinoid binding site, the binding constant and the effects of complexation on BSA stability and secondary structure. Structural analysis showed that retinoids bind BSA *via* hydrophilic and hydrophobic interactions with overall binding constants of $K_{Ret-BSA} = 5.3\ (\pm 0.8) \times 10^6\ M^{-1}$ and $K_{Retac-BSA} = 2.3\ (\pm 0.4) \times 10^6\ M^{-1}$. The number of bound retinoid molecules (n) was 1.20 (±0.2) for retinol and 1.8 (±0.3) for retinoic acid. Molecular modeling showed the participation of several amino acids in retinoid–BSA complexes stabilized by H-bonding network. The retinoid binding altered BSA conformation with a major reduction of α-helix from 61% (free BSA) to 36% (retinol–BSA) and 26% (retinoic acid–BSA) with an increase in turn and random coil structures indicating a partial protein unfolding. The results indicate that serum albumins are capable of transporting retinoids *in vitro* and *in vivo*.

1. Introduction

13-*cis* Retinoic acid (Scheme 1) is rapidly absorbed into cells and exerts its anti-proliferative effect on human sebocytes by specific isomerization to high levels of all-*trans* retinoic acid, while binding the retinoic acid receptors [1]. Several retinol binding proteins are identified and structurally characterized [2–5]. It has been suggested that the addition of high concentration of bovine serum albumin controls the uptake of 13-*cis* and all *trans*-retinoic acid in cell and reduces significantly the isomerization of all *cis*-retinoic acid to all *trans*-retinoic acid [1]. However, the mechanism by which serum albumins alter the activity of retinol and retinoic acid is not well understood.

Serum albumins are constituents of the circulatory system and have many physiological functions [6]. The most important property of this group of proteins is that they serve as transporters for a variety of compounds including retinoids. BSA (Scheme 1) has been one of the most extensively studied of this group of proteins, particularly because of its structural homology with human serum albumin (HSA). The BSA molecule is made up of three homologous domains (I, II, III) that are divided into nine loops (L1-L9) by 17 disulfide bonds. The loops in each domain are made up of a sequence of large–small–large loops forming a triplet. Each domain in turn is the product of two subdomains (IA, IB, etc.). X-ray-crystallographic data [7,8] show that the albumin structure is predominantly α-helical with the remaining polypeptide, occurring in turns and in extended or flexible regions between subdomains with no β-sheets. BSA has two tryptophan residues that possess intrinsic fluorescence [9,10]. Trp-134 in the first domain and Trp-212 in the second domain. Trp-212 is located within a hydrophobic binding pocket of the protein and Trp-134 is located on the surface of the molecule. While there are marked similarities between BSA and HSA in their compositions, HSA has only one tryptophan residue Trp-214, while BSA contains two tryptophans Trp-212 and Trp-134 as fluorophores capable of fluorescence quenching.

Fluorescence quenching is considered as a technique for measuring binding affinities. Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophores induced by a variety of molecular interactions with quencher molecule [11]. Therefore, it was of interest to use quenching of the intrinsic tryptophan fluorescence of BSA as a tool to study the interaction of retinol and retinoic acid with BSA in an attempt to characterize the nature of retinoid-protein complexation.

We report the spectroscopic analysis of BSA complexes with retinol and retinoic acid in aqueous solution at physiological conditions, using constant protein concentration and various

Abbreviations: BSA, bovine serum albumin; HSA, human serum albumin; ret, retinol; retac, retinoic acid; FTIR, Fourier transform infrared spectroscopy; CD, circular dichroism.

* Corresponding author. Tel.: +1 819 376 5011x3310; fax: +1 819 376 5084.
E-mail address: tajmirri@uqtr.ca (H.A. Tajmir-Riahi).

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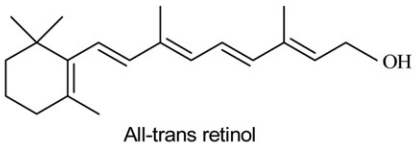
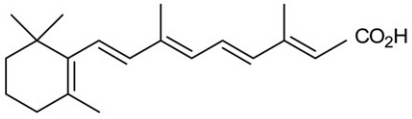
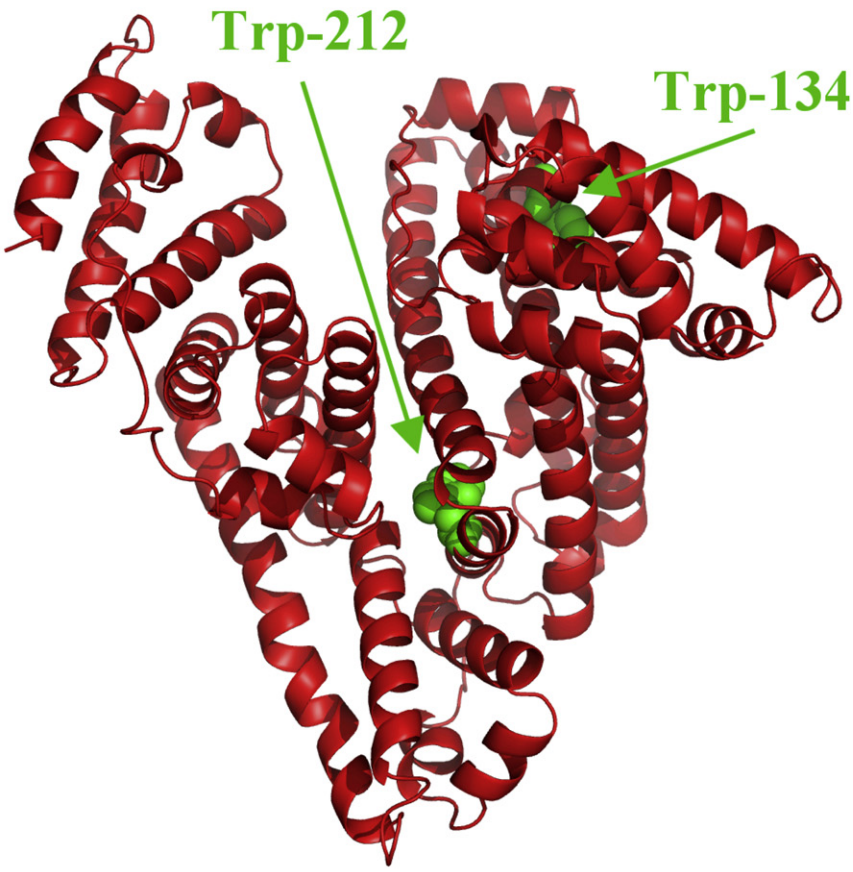


All-trans retinol



All-trans retinoic acid

Chemical structures of retinol and retinoic acid



3D structure of bovine serum albumin showing Trp-212 and Trp-134 in green color

Scheme 1.

retinoid contents. Structural information regarding retinoid binding sites and the effect of retinoid–BSA complexation on the protein stability and secondary structure is reported. Furthermore, a comparison between retinol and retinoic acid complexes with BSA and HSA was made here.

2. Experimental section

2.1. Materials

BSA fraction V and all *trans*-retinol and all *trans*-retinoic acid were purchased from Sigma Chemical Company and used as supplied. Other chemicals were of reagent grade and used without further purification.

2.2. Preparation of stock solutions

Bovine serum albumin was dissolved in aqueous solution (40 mg/ml or 0.5 mM) containing 10 mM Tris–HCl buffer (pH 7.4). The protein concentration was determined spectrophotometrically using the extinction coefficient of 36 500 M^{-1} cm^{-1} at 280 nm [12]. Retinoid solution (1 mM) was first prepared in Tris–HCl/ethanol 50% and then diluted by serial dilution in Tris–HCl/ethanol 50%. After addition of equal volume of retinoid solution to protein