Biochimie 95 (2013) 204–214

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

# Trifluoroethanol-induced conformational change of tetrameric and monomeric soybean agglutinin: Role of structural organization and implication for protein folding and stability

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ARTICLE INFO

*Article history:*
Received 18 April 2012
Accepted 13 September 2012
Available online 26 September 2012

*Keywords:*
Soybean agglutinin
β-Sheet lectin
Protein stability
Solvent perturbation

ABSTRACT

2,2,2-Trifuoroethanol (TFE)-induced conformational structure change of a β-sheet legume lectin, soybean agglutinin (SBA) has been investigated employing its exclusive structural forms in quaternary (tetramer) and tertiary (monomer) states, by far- and near-UV CD, FTIR, fluorescence, low temperature phosphorescence and chemical modification. Far-UV CD results show that, for SBA tetramer, native atypical β-conformation transforms to a highly α-helical structure, with the helical content reaching 57% in 95% TFE. For SBA monomer, atypical β-sheet first converts to typical β-sheet at low TFE concentration (10%), which then leads to a nonnative α-helix at higher TFE concentration. From temperature-dependent studies (5–60 °C) of TFE perturbation, typical β-sheet structure appears to be less stable than atypical β-sheet and the induced helix entails reduced thermal stability. The heat induced transitions are reversible except for atypical to typical β-sheet conversion. FTIR results reveal a partial α-helix conversion at high protein concentration but with quantitative yield. However, aggregation is detected with FTIR at lower TFE concentration, which disappears in more TFE. Near-UV CD, fluorescence and phosphorescence studies imply the existence of an intermediate with native-like secondary and tertiary structure, which could be related to the dissociation of tetramer to monomer. This has been further supported by concentration dependent far-UV CD studies. Chemical modification with N-bromosuccinimide (NBS) shows that all six tryptophans per monomer are solvent-exposed in the induced α-helical conformation. These results may provide novel and important insights into the perturbed folding problem of SBA in particular, and β-sheet oligomeric proteins in general.



## 1. Introduction

The problem of protein folding, in principle, envisions folding as a stochastic search of many conformations available to a polypeptide chain [1,2]. Thus, considerable experimental research has been directed towards detection of folding intermediates and their characterization including the factors governing their stability to gain insights into the interactions responsible for their formation as well as their role in protein folding [3–5]. Besides conformers having residual native-like secondary structures, there has been emphasis on the importance of nonnative secondary conformations in protein folding pathways. It has been hypothesized that all globular proteins, regardless of their native structure, fold via highly α-helical intermediate structures [6]. Recently, Chen et al. [7] has reported that formation of an α-helix can be a rate-limiting step during folding of a predominantly β-sheet protein. Nonnative states are easily obtained by solvent perturbation. An extensively studied cosolvent is 2,2,2-trifluoroethanol (TFE) which has been widely used to characterize secondary structure states in protein folding [8–10]. TFE tends to stabilize α-helical structures of proteins and peptides, and causes disruption of their native structures. Theoretical computational studies, using a two-dimensional lattice model, suggest that TFE mainly weakens nonlocal hydrophobic interactions and slightly favors local helical interactions [11]. Shiraki et al. [9] has proposed that helical propensity of the TFE-induced state is determined by local interaction based on the amino acid sequence. It has been shown that extensive helix formation in TFE does not occur when a protein has a very low intrinsic helix forming propensity [12]. However, the detailed mechanism of TFE action still remains unclear.

*Abbreviations:* SBA, soybean agglutinin, lectin from soybean (*Glycine max*); TFE, 2,2,2-trifluoroethanol; CD, circular dichroism; FTIR, Fourier transform infrared; NBS, N-bromosuccinimide.

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http://dx.doi.org/10.1016/j.biochi.2012.09.011

Soybean agglutinin (SBA) is an oligomeric β-sheet protein, and belongs to the family of legume lectins. Lectins are carbohydrate-binding proteins that are involved in various biological recognition processes [13]. Further, many lectins are now important tools in biomedical research. The richest source of lectins is, however, the seeds of leguminous plants, and legume lectins are among the most extensively studied families of proteins. The members of this family show a high degree of homology in their tertiary structures, and display an oligomeric state as dimer or tetramer. SBA, which is specific for GalNAc/Gal, exists as a tetramer in the native state. Its three-dimensional structure has been determined by X-ray diffraction analysis [14,15]. The tertiary structure of SBA subunit is similar to that of other legume lectins such as concanavalin A [16], and describes a jelly roll motif that comprises three antiparallel β-sheets: a six-stranded flat 'back', a seven-stranded curved 'front', and a five-stranded 'top' that forms a roof-like structure above the other two (Fig. 1A). Two subunits of SBA dimerize by a side-by-side 'canonical' mode forming a continuous 12-stranded β-sheet. The tetrameric structure of the protein involves nearly parallel, back-to-back association of two such 'canonical' dimers that interact through contacts between their two outermost strands, creating a channel between them (Fig. 1B).

The TFE-induced secondary structure changes of this β-sheet lectin at the level of tetramer and monomer, and their implications in protein folding and stability would, therefore, constitute an interesting theme of study. A β-sheet to α-helix transition in presence of TFE for some legume lectins has been reported in the recent past [17–19]. For SBA, experimental studies on its unfolding and stability have been published in the last few years [20–22]. We have previously demonstrated that structured SBA monomer develops and accumulates during refolding from the completely unfolded state, and reconstitutes the tetramer with a kinetic mechanism involving monomer-to-dimer association as the rate-determining step [23]. Recently, we have shown that the tryptophan environment of SBA is characterized by unique structural and dynamic features that are maintained in specific quaternary and tertiary states [24]. In this article, we have explored the effects of TFE on the secondary structure perturbation of SBA tetramer and monomer by far-UV CD and FTIR. Further, tertiary/quaternary structure changes of TFE-induced states, using tryptophan as intrinsic probe, have been investigated by near-UV CD, fluorescence, low temperature phosphorescence and chemical modification. For the first time to our knowledge, this work presents TFE perturbation study on both the native oligomeric state, as well as its building block, namely, monomeric unit of a β-sheet lectin, which could provide new insight into this perturbed folding problem.

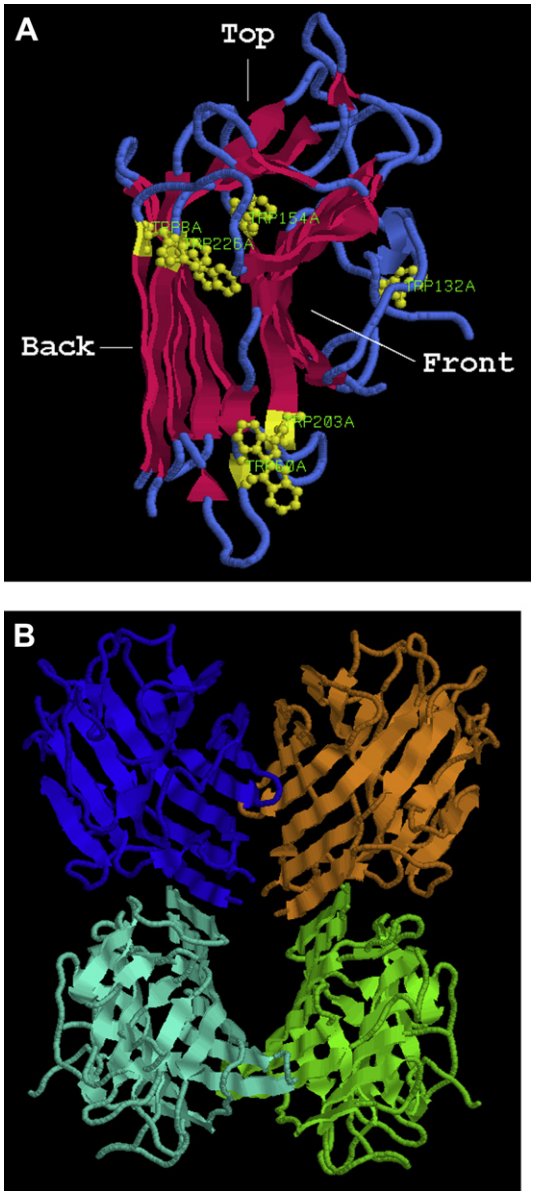


**Fig. 1.** (A) Structure of SBA monomer (PDB entry 2SBA) consisting of three β-sheets (a six-stranded 'back', a seven-stranded 'front' and a five-stranded 'top'). The six Trp residues are highlighted in yellow ball-and-stick. (B) Tetrameric structure of SBA, which involves nearly parallel back-to-back association of two 'canonical' dimers, each of which represents a continuous 12-stranded β-sheet formed by side-by-side alignment of six-stranded back β-sheets of two monomers. [For interpretation of color referred in this figure legend, the reader is referred to web version of the article.]

## 2. Materials and methods

### 2.1. Materials

Soybean flour, guar gum, deuterium oxide ($D_2O$) and 2,2,2-trifluoroethanol (TFE) were purchased from Sigma. Cross-linked guar gum matrix was prepared as described [25]. DCl was prepared by reaction of benzoyl chloride with $D_2O$ and collected in $D_2O$. TFE-OD was obtained by distilling TFE with $D_2O$. All other reagents used were of analytical grade. All pH values reported in this paper denote apparent pH meter readings for solutions prepared in $D_2O$ or in presence of TFE. Double distilled water was used throughout.

### 2.2. Protein purification

SBA was purified from the crude extract of soybean flour by affinity chromatography on cross-linked guar gum matrix [26]. As aggregation of SBA occurs on storage in the lyophilized state, affinity-purified SBA was precipitated by ammonium sulfate (80% saturation) and dialyzed against appropriate buffer before use in different experiments. However, for FTIR measurements, lyophilized SBA was used immediately. The integrity of quaternary structure of SBA was confirmed by size-exclusion chromatography on Sephadex G-100 in 10 mM sodium phosphate buffer, pH 7.2 when the protein was eluted as a single peak corresponding to its tetrameric molecular mass. The purity of the sample was also checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [27]. Monomeric form of SBA was generated exclusively in 10 mM glycine–HCl buffer, pH 2.0 as described [24].