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Short Communication

HEXAFLUOROISOPROPANOL-INDUCED SECONDARY STRUCTURE PERTURBATION OF SOYBEAN AGGLUTININ

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ABSTRACT

Fluoroalcohols like 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) are widely used as cosolvent along with the biological solvent water to perturb the native protein molecules. The non native states obtained are of immense importance in the field of protein structure and folding, since these states may be present in the protein folding pathway or in the off pathway which leads to amyloid formation. In this study, HFIP-induced structure perturbation at secondary level of the tetrameric legume lectin, soybean agglutinin (SBA) is examined by far-UV circular dichroism (CD) spectroscopy. Like other member of the legume lectin family, native SBA is also an all β-sheet protein. Analysis of the far-UV CD spectra shows formation of α-helix rich conformations at the expense of native β-sheet in presence of higher concentration (50% or more) of HFIP. Visible aggregation is noticed at lower HFIP concentration (~10%) which disappears at higher concentration of HFIP with concomitant induction of the α-helical secondary structure. The results confirm about the helix propensity of amino acid sequence of SBA and helical intermediates may be involved in the early stage of its folding process.

Keywords: Solvent perturbation, Hexafluoroisopropanol, Lectin, β-Sheet, α-Helix, Far-UV CD

1. INTRODUCTION

Perturbation of protein native structure is of paramount importance in the study of protein structure and folding. Non native states obtained by structure perturbation may resemble the 'folding intermediates' present in the folding pathway of a nascent polypeptide chain leading to the native functional protein [1] or these states may be representative of off pathway structures leading to protein aggregation and eventually amyloid formation [2, 3]. Alcohols and mostly fluoroalcohols are extensively used as cosolvent along with the biological solvent water to perturb the native proteins [4, 5]. 2,2,2-Trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) are the two fluorinated alcohols used frequently for this purpose. Fluorinated alcohols tend to stabilize conformers with predominantly helix secondary structure as seen in case of various peptides and proteins [6-9]. Even proteins with natively β-sheet structure were also reported for high helical conformation in presence of fluoroalcohol [10-12]. In order to address the reasoning behind alcohol perturbation, the whole problem can be viewed as two parts. First, there occurs perturbation of native protein structure and secondly, formation of a regular secondary structure mainly alpha helix. Disruption of the native structure happens because of decreasing hydrophobic effect in alcoholic medium [13]. But why does it adopt preferentially helical structure? Explicit answer to this question still remains unknown. Theoretical computational study using a two-dimensional lattice model indicates about weakening nonlocal hydrophobic contacts and strengthening local helical interactions [14]. Helix forming ability does not depend on exclusively on the property of added alcohol, but also on the intrinsic properties of a particular peptide or protein. So the amino acid sequence is also a determining factor [15, 16]. Lectins, an important protein family, are comprised of protein molecules which bind carbohydrates specifically and reversibly [17]. Lectins are oligomeric proteins and have been involved in various biological processes. Soybean Agglutinin (SBA) is a member of most extensively studied lectin sub-family – legume lectins. SBA is a GalNAc / Gal-specific tetrameric glycoprotein with one Man-9 oligomannose type chain per monomer [18]. Like all other lectins, SBA is also comprised of β-sheet component as the principal secondary structure

along with some turn and random coil structures (Fig. 1). Each subunit has a ''jelly roll'' motif typical of all legume lectins and this fold comprises a six-stranded back β-sheet, a curved seven-stranded front β -sheet and a five-stranded sheet that forms the roof of the molecule. The tetrameric structure (Fig. 2) of the protein involves back-to-back and nearly parallel association of two 'canonical' dimers that interact through contacts between their two outermost strands, creating a channel between them [19]. In this study we have studied effect of addition of HFIP on secondary structures of the native tetrameric state of SBA using far-UV circular dichroism (CD) spectroscopy.

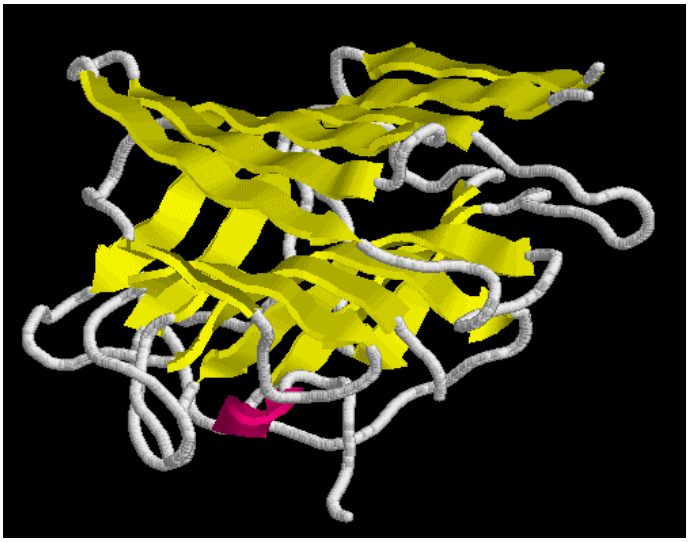

Fig. 1: Structure of SBA monomer (PDB ID: 2SBA). The beta sheet portions are highlighted in yellow color.

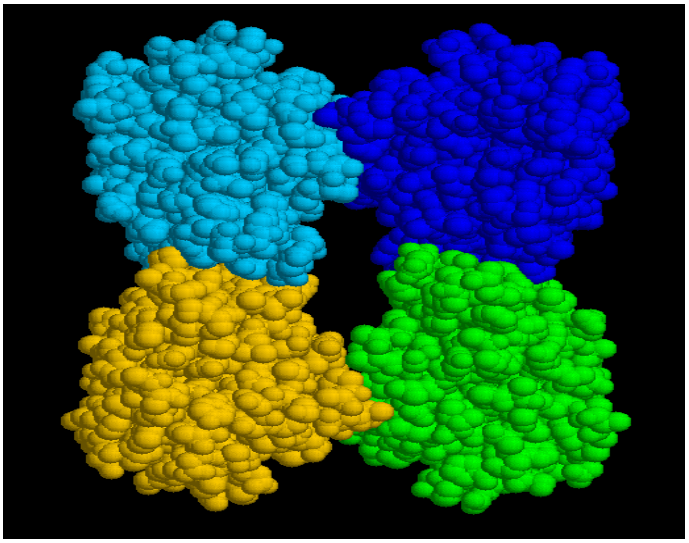

Fig. 2: Structure of SBA tetramer (PDB ID: 2SBA). Four subunits are shown in different color.

2. MATERIAL AND METHODS

2.1. Material

Soybean flour, guar gum, and HFIP were purchased from Sigma-Aldrich. Cross-linked guar gum matrix was prepared using epichlorohydrin as cross-linking agent [20]. All other reagents used were of analytical grade. 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 [21]. Since 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. Protein concentration was determined spectrophotometrically using A1 %, 1 cm = 12.8 at 280 nm and expressed in terms of monomer (M_r = 30 000) [22].

2.3. Circular Dichroism (CD) measurement

CD experiments were performed on Jasco-815 spectropolarimeter equipped with a Peltier temperature-controlled cell jacket. Protein concentration of 10 μM was used for the far-UV CD measurement. The spectra were measured in the wavelength range of 260-190 nm. Scan speed was 50 nm/min with a response time of 2 s, and at least five scans were done to eliminate signal noise. Solution of SBA tetramer was prepared in 10mM phosphate buffer, pH 7.2. All measurements were carried out with systems reaching equilibrium and were corrected by subtraction of appropriate blanks.

3. RESULTS

3.1. Far-UV CD measurement

Native SBA, purified from Soybean flour, remains as tetramer at physiological pH. Crystallographic structure of native SBA (pdb id: 2sba) shows that it consists of predominantly β-sheet secondary structure [19]. Secondary structure of protein samples in solution is widely studied by employing circular dichroism (CD) spectroscopy in the far-UV region (240-190 nm) utilizing peptide amide bonds as chromophore [23, 24]. Far-UV CD measurement of protein solution results in characteristic spectra in terms of shape and peak position depending on specific secondary structure pattern. Beta sheet structure exhibits far-UV CD spectra with single minima, whereas alpha helical structure displays spectra with two negative peaks [25]. Fig. 3 shows far-UV CD spectra of native SBA in different HFIP percentage at

20°C. Native SBA, at pH 7.2, in the absence of any HFIP exhibits atypical β-sheet band shape with negative peak at 226 nm and positive peak at 196 nm which is consistent with the earlier report [26]. This atypical band shape is retained up to 5% HFIP concentration and the spectra overlaps with that of native protein. With slight increment of HFIP concentration, the CD spectra changes distinctly. Though the spectral minimum slightly shifts to 230 nm in presence of 10% HFIP, but the intensity decreases drastically. At this HFIP concentration, visible aggregation appears in the protein solution and this may be the reason for intensity drop. Upon addition of more HFIP (20%), the visible aggregation did not appear and spectral shape remained similar as in 10% HFIP but with enhanced intensity. When the HFIP concentration raised to 50%, far-UV CD spectra changes its nature completely, and now two negative band (at 208 nm and 222 nm) along with a positive band at 192 nm is noticed. This type of far-UV CD spectra is characteristics of α-helical protein conformations [24, 25]. On further increment of HFIP concentration up to 80%, α- helical band shape exists and the intensity (both positive and negative) remains unaltered, indicating no more helix conformation.

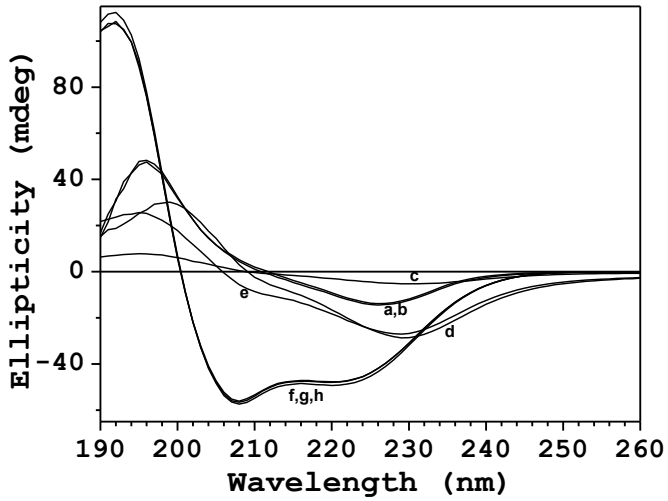

Fig. 3: Far-UV CD spectra of SBA tetramer at pH 7.2 in presence of (a) 0% (b) 5% (c) 10% (d) 20% (e) 40% (f) 50% (g) 70% and (h) 80% HFIP. Protein concentration was 0.3 mg/mL. Corresponding buffer spectra were subtracted in each case.

3.2. Deconvolution of CD spectral data

Although the far-UV CD spectral band shape indicates about the dominant secondary structure, it is qualitative evidence. Quantitative information about the protein secondary structure can also be obtained from computational analysis of the spectra by various programs [27]. In this study, secondary structure components are estimated employing CDNN software, version 2.1 [28]. Estimation of the native protein in absence any alcohol reveals about ~49 % of total β-sheet component including ~43 % of antiparallel sheet. This result is fairly consistent with crystallographic data and earlier study [19, 26]. Although according to crystallographic structure native SBA has no helical component, CD spectra deconvolution shows ~16% helical component. Again FTIR analysis, another method for protein secondary structure examination in solution, also showed no helical part [29]. Since we are considering relative change of secondary structures, it was assumed as background data and this ~16% helical component was considered as zero percentage. Relative increase in the helix percentage is considered as absolute value in order to focus on the gradual conversion of sheet to helix structure. Fig. 4 shows the plot of change of secondary structure components (β-sheet, α-helixl) with gradual increase of HFIP concentration. At 10% HFIP concentration, a small increment of sheet structure is noticed which may be due to the formation of intermolecular β-sheet structure responsible for the visible aggregation. With addition of 20 % HFIP, loss of the original β-sheet structure happens without significant gain of helix structure, rather the random coil part marginally enhanced. In presence of 50 % HFIP, native β-sheet structure is almost completely lost and α-helical structure rises sharply (~50 %). Upon further increase of HFIP concentration (up to 80%), the analysis results show similar output.

4. DISCUSSION

Biological relevance has also played role in choosing alcohols for structure perturbation of proteins since alcohols make the environments similar to that in cell membrane or near the membrane [30, 31]. Alcohol-induced perturbation of proteins has unique importance in the study of protein folding process as protein conformers with non native secondary structures can be obtained by this method. According to non-hierarchical model proposed by Lim V.I. [32], regardless the final protein structure, protein folding process from the polypeptide chain is always associated with initial formation of alpha helical intermediate because it is dependent only on the local interactions. An experimental study by Chen et al detected short lived helical intermediates in the folding of a β-sheet protein

[33]. So, the helical states obtained from HFIP perturbation may be involved in the folding pathway of SBA. Structure perturbation of SBA by another fluoroalcohol, TFE, was studied earlier by us and we had shown about 57 % helix formation in presence of 95 % TFE [26]. Here, HFIP-induced denaturation results in ~50 % helix formation in presence of 50 % HFIP concentration and unlike TFE-induced perturbation of SBA, further addition of HFIP (up to 80 %) cause no more structural change (Fig. 4). Though HFIP is believed to be stronger helix-inducing solvent than TFE [13], we have found little less amount of helix percentage in HFIP. Again, no aggregation was noticed during TFE mediated denaturation SBA at low protein concentration, but low HFIP concentration (10%) triggers visible aggregation. Analysis by TANGO software [34, 35] reveals few hotspots for β-aggregation in amino acid sequence of SBA, comprising mainly hydrophobic residues- VASFAASFNFTFY (67-69), LAFFL (90-94), VLITY (164-168) and LLVASLV (174-180). Thus, native β-sheet rich SBA tetramer is converted to α-helical non native conformation by HFIP which may be present in the folding pathway.

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