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Solution structure of a FK binding protein: Schistosoma mansoni protein (Smp50) from trematode

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ABSTRACT

Recently a 50 kilodalton FKBP protein (Smp50) from the trematode Schistosoma mansoni was produced in bacteria and shown to have biological activity. We present here in this paper a detailed investigation of the secondary structure of this protein from Circular Dichroism (CD) and Fourier Transform Infra-red (FTIR) spectroscopic techniques. We investigated the effect of temperature, pH and salt and solvent compositions on the secondary structure of Smp50. At room temperature and in a phosphate buffer (pH 7.1) Smp50 has 26% α-helix, 42% β-structure and 31% random coil structure and at 0°C, the amount of α-helix increases to 32% with a 36% β-structure and a 33% random coil. When the pH is lowered to 4.0 with an acetate buffer, the α-helix decreases to 29%. Our results indicate that the protein is not destroyed at high temperatures, retaining as much as 20 % α-helix even at 80°C. From an analysis of the FTIR spectrum, Smp50 has 28% α-helix, 42% β-structure and a 30% random coil structure, which is in excellent agreement with the CD results. Our results indicate that the protein has an highly extended β-structure in solution and gives a high degree of stability to the protein based on our temperature and pH variation studies.

KEYWORDS

FK binding protein;
Smp50;
Protein secondary structure;
CD and FTIR studies.

INTRODUCTION

Immunomodulators are drugs that are used to enhance or suppress the body's immune response. Suppression of the immune response is required in the successful transplantation of organs among genetically different individuals. Enhancement of immune response is needed in the treatment of tumors and in the development of vaccines. The mechanism by which drugs induce these immunomodulatory effects are varied and are yet completely understood.

Cyclosporin A, Rapamycin and FK506 are the drugs of choice for immunosuppression. These drugs are administered to patients who receive organs such as heart, lung, liver and kidney. The ubiquitous receptor for these transplant drugs are called Immunophilins. There are two classes of Immunophilins. One is the Cyclosporin A binding receptor called Cyclophilin and

TABLE 1: Amino acid sequence of schistosoma mansoni protein (Smp50)

MADNDGQESG	AQQRVEDEYL	KDFMDLSPSG	DRGILKKVLR	EGYSDVKPCDGD	T V I V H Y V G
TNYGGEKHGE	VFDSSRARNE	KFEFTIGNGS	VIKAWDVGVA	TMKLGEICEL	IASPDYAYKD
GKTLKFEVEL	FETLGSDVSR	NKDGSIRKSI	IRKGKDDIYH	PVAGAEATIV	FRNLTSTEDV
EVTYCCVGDP	PLTVPEELDQ	CLRHMSTDEF	CRVVVCKDKN	SAAEVADKSR	VVYELTLKSF
EKTKHLSGIS	PFSEQMAYRN	VLKEKANNFL	KDSKFDSA	IELYKRLDDELQ	YIVANGPAEQ
KELSAVIVAV	RLNLGLTYLK	LCKPDKCIEF	CRKVLDVFGN	NEKALFRMGQ	AHLLRNDHDE
ALVYFKKIVA	KNPNNASAVK	LLHMCEEEKA	KDMAKKRFRS	IFERCKDSGL	CRKVLDVFGN
NEKALFRMGQ	AHLLRNDHDE	ALVYFKKIVA	KNPNNASAVK	LLHMCEEEKA	KDMAKKRFRS
IFERCKDSGL	DGGVEEHKDGVALNGEKSAL				

the other is the receptor for the other two drugs, FK506 and Rapamycin, called FK binding protein (FKBP). The ligands for these Immunophilins are highly immunosuppressive drugs which have been used in the treatment of graft rejection. These proteins act as molecular chaperones, aiding in the assembly of subunit protein complexes and folding of target proteins. Structural studies of these aminophylline-ligand complexes are very useful in understanding the mechanisms of action of these immunosuppressive drugs and the mode of binding of these drugs to pockets in these proteins.

There are several investigations of the structure of these protein-drug interactions[1-7]. These studies yield very valuable models for the interaction of these protein -drug complexes. An x-ray structural analysis of the human FKBP with the FK506 reveals a large sheet composed of five anti-parallel β-strands, a short amphipathic α-helix with the ligand FK506 tightly binding in a hydrophobic pocket[8-9]. Several FKBPs from different organisms have been isolated, purified and characterized. Smp50 is a 50 kilodalton protein from the trematode, Schistosoma mansoni, isolated and, identified and characterized recently[10]. TABLE 1 gives the amino acid sequence for Smp50 along with the predicted secondary structure and details of homology with some FK binding proteins. A comparison of the sequences of FKBP from several different sources reveals varying degrees of homology from 30% to 49% and most of them exhibit peptidyl-prolyl-cis-transisomerase (PPIase activity), which is typical of immunophilins. There are a minimal number of insertions and deletions in these sequences, ranging from 2 to 7 residues. There is a 42% homology between Smp50 and the yeast FKBP1 and a 44% homology between Smp50 and human FKBP13 proteins. The amino acids in human FKBP which are important for Interaction with FK506 are conserved in Smp50.

Prediction of secondary structure[16] α-helix 40.6% β-structure 18.3% Loops 41.1%				
Comparison with other FK binding proteins				
Protein %	of pairwise sequence homology	% of weighted homology	Number of residues aligned	Number of insertions & deletions
FKB2 drome	49	53	96	2
FKBP mouse	45	48	96	2
FKBP human	44	48	96	2
FKB3 mouse	40	44	89	2
FKB3 yeast	39	43	103	2
Fkb2 bovine	30	32	120	3
FKBB E.coli	35	31	120	4
FKB3 yeast	30	31	123	4

There are several different algorithms and methodologies available currently for predicting and evaluating the secondary structure of a protein from its aminoacid sequence[11-14]. Some of these programs use the amino acid sequence homologies and develops an algorithm to predict the secondary structure[15,16]. Application of Rost and Sander methodology, predicts a 41% α-helix, 18% β-structure and 41% random coil for Smp50.

Circular dichroism and fourier transform infra-red spectroscopy are very powerful tools for studying the structure of macromolecules in the solution. These techniques yield very valuable information on the dynamics of the macromolecules and complement the details obtained from single crystal x-ray diffraction and NMR studies. Some of the most recent application of these were in the elucidation of the secondary structures of isomeric forms of human serum albumin[17], study of the peptide fragments reproducing processing site of oxcytocin-neurophysin precursor[18], study of the sec-

ondary structure of insulinotropin in the solid state[19], study of the tertiary fold of staphylococcal protein A to explore its engineering application[20], study of the orientation in protein-lipid complexes[21] and study of bovine growth hormone[22].

We have undertaken a structure-function study of Smp50 with a view to understand the aminophylline-ligand interactions. As a first step in this line of study, we present in this paper a detailed analyses of the secondary structure of Smp50 using Circular Dichroism (CD) and Fourier Transform Infra-red (FTIR) spectroscopic techniques[23].

MATERIALS AND METHODS

Circular dichroism spectroscopy is a very powerful technique employed for determining changes in the secondary and tertiary structures of proteins and evaluating their dependence on pH, salt content and temperature[24-25]. The CD studies were carried out on a JASCO 500A spectrometer. The secondary structural content were determined using a program VARSLC1 [26-27].

FTIR has become a powerful technique, in addition to CD, to study the secondary structure of proteins[28-31]. FTIR yields vibrational frequencies corresponding to the helical, extended and turn regions in the protein[32]. The FTIR spectra were recorded on a Perkin-Elmer Spectrum 2000 spectrometer.

Data collection

Schistosoma Mansoni protein 50(Smp50) was isolated, identified and characterized by us earlier[10]. This protein was produced in bacteria and shown to have biological activity. The original protein (0.3mg/ml) was obtained in PBS buffer. The protein was centrifuged and lyophilized to a powder. Most of the CD spectra were run with the protein dissolved in a 5mM phosphate buffer (pH 7.1) at a concentration of 40μg/mL. For pH studies, different buffers were used-a 5mM sodium borate buffer for high pH (9.14) and 5mM sodium acetate buffer for low pH (3.9). The first CD spectrum was taken at room temperature (27°C) in phosphate buffer. The temperature was either lowered or increased in 10°C increments from 10 to 80°C in an effort to investigate the relationship of the helical content of the protein with temperature.

Smp50 was dissolved in D_2O at a concentration of 5mM. A cell with CaF_2 windows was used with a path length of 100μm. The instrument was continuously purged with dry nitrogen and for each spectrum several interferograms were recorded at very close intervals. For the analysis of protein secondary structure, the amide I band in the IR region, 1600-1710cm^{-1} was utilized. This band consists of several overlapping bands corresponding to the various conformations present. The infra red spectra were resolved into gaussian components. Spectra were collected by subtracting solvent absorption. The resolution enhancement procedures, second derivative and SSRes (a maximum likelihood

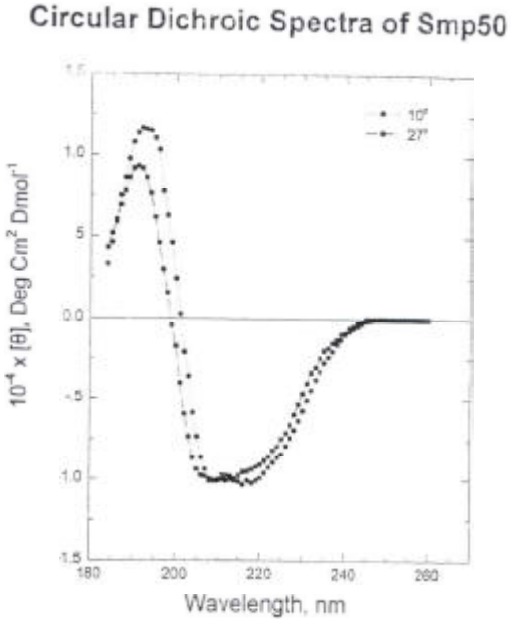

Figure 1: Circular dichroism spectra for Smp50 at 10 and 27°C

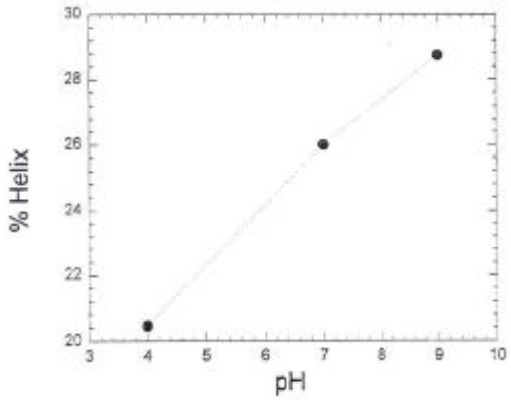

Figure 2(a) : Variation of the percentage of helicity of Smp50 with pH

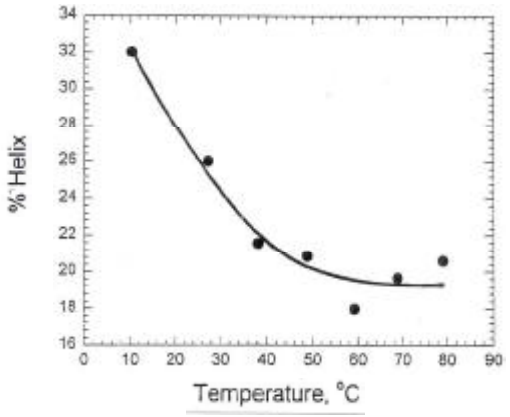

Figure 2(b) : Plot of the variation of the helical content of Smp50 with temperature

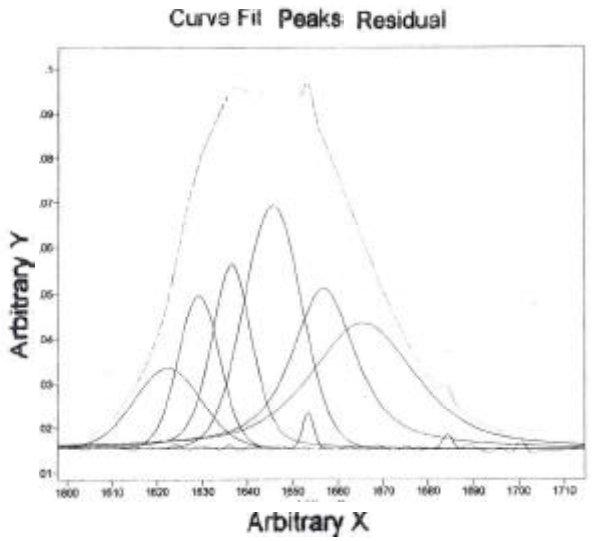

Figure 3: FTIR spectrum of Smp50 in the frequency range 1600-1710cm^{-1}

TABLE 2 : Details of the secondary structure of Smp50 protein

Details of secondary structure						
Temp.	% helix	%↑↓β	%↑↓β	%β-turn	%Random coil	Total
10	32	5	13	18	33	101
27	26	3	18	21	31	99

Effect of pH on secondary structure	
pH	%α-helix
4	20
7	26
9	29

Effect of temperature on secondary structure	
Temp.	%α-helix
10.3	32
27.2	26
38.2	22
48.8	21
59.2	18
68.8	20
79.1	21

restoration program) were used to resolve the overlapping bands, giving their numbers and appropriate frequencies. Curve fitting was performed by several iteration steps, utilizing the Fit procedure. An initial cycle was performed with fixed center positions and widths, so that the program could vary only peak heights. The cycles were repeated for a limited number of variables until a satisfactory fit was obtained.

RESULTS AND DISCUSSION

Figure 1 shows the CD spectrum of Smp50 at room temperature 27°C and at 10°C. At room temperature, the protein has a higher β-structure than α-helix (42% β-sheet and 26% α-helix). Upon cooling to 10°C, there is an increase in the helical content of the protein to 32%. Figure 2(a) shows the variation of the helical content of the protein as a function of pH. There is a linear increase in the helical content of the protein with increasing pH. At a pH of 4, the helical content reduces to 20% and at a pH of 9, it increases to 29%. Figure 2(b) shows the variation of the helical content of the protein with temperature. The protein is very stable at high temperatures. The secondary structural content of the protein as a function of temperature reveals that the protein structure is not completely destroyed at high temperatures. Even at temperatures as high as 80°C, the protein retains 21% helical content. TABLE 2 gives the complete secondary structural details of Smp50 obtained using the program VARSLC1. In solution, Smp50 has 26% helical content, 42% β-structure and 31% random coil at room temperature. At low temperature, the helical content increases to 32% with 36% β-structure and 33% random coil.

Figure 3 gives the Smp50 FTIR spectrum in the range 1600-1710cm^{-1}. The figure gives both the original FTIR band and the individual calculated components bands as well. FTIR spectrum of Smp50 gives 28% α-helix, 42% β-structure and 29% random coil, which is in good agreement with the results from CD studies. We are currently attempting to grow crystals of Smp50 suitable for single crystal x-ray diffraction analysis.

CONCLUSION

Our results from CD and FTIR studies reveal that Smp50 has an extended structure in solution, with a higher β-structure than α-helix (42% β-sheet and 26% α-helix). The protein is very stable at high temperatures, retaining as much as 21% helical structure at 80°C. These secondary structural features of Schistosoma mansoni protein are very similar to the human FKBPs. We are currently studying the complex of Smp50 with FK506 to understand the dynamics of their interactions. We have obtained small microcrystals of Smp50 which diffract only upto 4Å resolution. We are trying to improve the quality of the crystals so that we can study the structure by x-ray methods and compare it with the structure obtained by solution techniques.

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