Conformation of an immunoreactive undecapeptide fragment (10–20) of Asp f 1 by NMR and molecular modeling

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Summary
Allergic bronchopulmonary aspergillosis (ABPA), caused by Aspergillus fumigatus, is a complication of allergic asthma. Asp f 1 secreted by A. fumigatus is reported to be a major allergen/antigen involved in pathogenesis of aspergillosis. A 11-mer immunodominant epitope (Leu-Asn-Pro-Lys-Thr6-Asn-Lys-Trp-Glu-Asp10-Lys) of Asp f 1 has shown immunoreactivity with specific IgG and IgE antibodies in the sera of patients with ABPA in ELISA inhibition assay. Various studies have suggested that the peptide has a potential use in the development of ELISA based diagnostic kit for early diagnosis of infections caused by A. fumigatus. In view of these interesting properties of the undecapeptide we have embarked on an investigation of its conformation to understand the relationship between structure and immunoreactivity. NMR and molecular modeling studies of the peptide suggest a structure with a β-turn spanning residues Asn6 – Glu9 in water at pH 4.0, a β-pleated sheet in DMSO and a α-helix in 40% HFA.

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; Boc, butyloxycarbonyl; CSI, chemical shift index; DDS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; DIC, 1,3-diisopropyl carbodiimide; DMAP, dimethylaminopyridine; DMF, dimethylformamide; DQF-COSY, double quantum filtered correlated spectroscopy; ELISA, enzyme-linked immunosorbent assay; FAB-MS, fast atom bombardment-mass spectrometry; Fmoc, fluorenyl-methoxycarbonyl; HFA, hexafluoroacetone; HoBt, hydroxy benzotriazole; HSQC, hetero nuclear single quantum coherence; IRMA, iterative relaxation matrix analysis; MD, molecular dynamics; DMSO, dimethyl sulfoxide-d6; NOESY, nuclear Overhauser effect spectroscopy; nOe, nuclear Overhauser effect; OBu, oxybutyl; RMSD, root mean squared deviation; ROESY, rotating frame nuclear Overhauser effect spectroscopy; TFE, trifluoroethanol; TOCSY, total correlated spectroscopy.

Introduction
Aspergillus fumigatus, a ubiquitous fungus present in nature, causes a variety of respiratory disorders including ABPA. Invasive aspergillosis is generally observed in immunocompromised patients and is usually fatal in these cases [1]. ABPA is a complication of allergic asthma. It is a progressively disabling lung disease characterized by elevated total and anti-Aspergillus IgE, pulmonary and peripheral blood eosinophilia, central bronchiectasis, and an obstructive airway dis-ease [2, 3]. Early diagnosis of aspergillosis can lead to more effective therapy and an increase in survival rate.

Asp f 1 is an 18 kDa protein secreted by A. fumigatus that is reported to be a major allergen/antigen involved in pathogenesis of Aspergillosis [4]. Several epitopes of Asp f 1 have been identified and their immunological properties studied [5]. These epitopes have the potential for development as synthetic peptide based diagnostics, which hitherto has been dependent on the crude antigen.

Recently, an eleven amino acid immunodomin-
ant epitope (Leu-Asn-Pro-Lys-Thr-Asn-Lys-Trp-Glu-Asp-Lys) present in the N-terminal region of Asp f 1 (10–20) has been identified based on algorithmic predictions [6]. The synthetic peptide showed immunoreactivity with specific IgG and IgE antibodies in the sera of patients of ABPA in ELISA inhibition assay. The ELISA absorbance values obtained with synthetic peptide are comparable to those obtained with mixture of potent antigens, indicating that this peptide can be used for diagnosis of *A. fumigatus* specific antibodies in sera of APBA patients. This suggests a potential for the peptide to be used in the development of ELISA based diagnostic kit, for early diagnosis of *A. fumigatus* [7].

Several epitopes corresponding to the N- or C-terminal regions of Asp f 1 and other antigenic/allergenic proteins have been reported to bind IgG and IgE immunoglobulin [5]. However, no sequence homology has been observed among the epitopes. The antibody binding affinity and specificity of these epitopes have been attributed to their conformational properties [8]. In view of the interesting immunological properties of the undecapeptide [7] we have embarked on a study of its conformation in diverse media that could be used in structure activity relationship studies.

Methods

Synthesis of peptide

The peptide was synthesized by solid phase synthesis using Fmoc chemistry on Wang resin [9]. Fmoc-Lys(Boc)-OH was immobilised on Wang resin (Sigma) by treatment with DIC and DMAP. The loading was found to be 0.51 meq. g⁻¹ [10]. Fmoc group from the resin (500 mg) was removed by 50% piperidine in DMF and Fmoc-Asp(OBu)-OH was coupled by DIC/HOBt method. The coupling was monitored by Kaiser’s test. Subsequently, remaining amino acids, appropriately protected, were coupled on the growing sequence. After all couplings the Fmoc group was removed and the resin dried. The peptide was cleaved from the resin by treatment with 95% trifluoroacetic acid and 5% thioanisol in presence of suitable scavengers for 1.5 hr. The reaction mixture was filtered and the solvent removed under reduced pressure and the peptide precipitated by addition of anhydrous ether. The hygroscopic precipitate was precipitated twice from absolute methanol/ether. The crude peptide was purified by reversed phase HPLC on a C₁₈ column using gradient elution (0–90% B in 90 min; A = 0.1% trifluoroacetic acid in water, B = 0.1% acetonitrile). The pure fractions were pooled and lyophilized to get the undecapeptide as a white powder (370 mg). The peptide was characterized by FAB-MS (M + 1 1373).

DMSO (99.9%) was purchased from Sigma Chemical Co., (USA), D₂O and DSS from Merck (Germany), and HFA from Aldrich Chemical Co., (USA).

The peptide (3 mg) was dissolved in 0.6 ml DMSO (4 mM solution) in a nitrogen atmosphere. For studies in water, the same amount of peptide was dissolved in a 0.6 ml solution of 95% H₂O:5% D₂O mixture. It is now well established that besides the solvents mentioned above, TFE and HFA are invaluable media for probing biomolecular structure, function, dynamics and protein folding. There is strong evidence that structures seen in such solvents are similar to those adopted by the peptide in their active conformation. The study of the conformation and dynamics of membrane bound peptides is a challenging one. A number of model systems have been suggested to mimic features of the membrane. It is now emerging that TFE and HFA are suitable model systems for the membrane [11, 12]. To study the effect of HFA, a mixture of 55%H₂O:40% HFA:5%D₂O was used. About 10 μL of a 0.1% solution of DSS in appropriate solvent was used as internal standard for the three solvents. The pH of aqueous samples was adjusted to 4.0 using dilute NaOH. The solutions were filtered and transferred to 5 mm NMR tubes.

NMR

All NMR experiments were performed on a Varian Unity Plus 600 MHz FT NMR spectrometer using a 5 mm ID probe with 90° proton pulse length of 7.5 μs at a transmission power of 60 db. Data were collected using VNMR (v. 6.1b) on a Sun Sparc workstation. The length of the 90° pulse was checked at the beginning of the session and the temperature was controlled throughout the experiments to ± 0.1 K. The data was processed using FELIX software (v. 97, MSI, USA) running on a Silicon Graphics O2 workstation. All 2D spectra were acquired without spinning, whereas samples were spun (at 20 rpm) for all 1D experiments. 1D-NMR spectra of serially diluted samples were recorded to confirm that the peptide does not aggregate under the conditions of study.

All experiments were recorded with the States-Haberkorn [13] method of quadrature detection. A sweep width of 9500 Hz was used in both dimensions. Typically, 2K data points in D2 and 512 experiments in