Recombinant Expression, Purification and Conformational Studies of Polypeptides Derived from the B13 Antigen of *Trypanosoma cruzi*

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Abstract: *Tandemly repeated regions in pathogenic protozoa proteins have been associated with an immunodominance role. The "smoke screen" hypothesis interprets the occurrence of such repeats as a means developed by the parasites to protect themselves by diverting the immune response away from antigen functional domains. The* Trypanosoma cruzi *B13 protein is an immunodominant antigen exposed on the surface of tripomastigote forms of the parasite. It contains a central domain composed of a number of 12-residue tandemly repeated units. In order to understand the three-dimensional organization of this segment of the protein B13, polypeptides containing 4 and 8 repeat units (B13-4r and B13-8r) were produced in an* E. coli *expression system. Structural studies on these peptides have been performed using circular dichroism (CD), nuclear magnetic resonance (NMR) spectroscopy, and molecular modeling calculations. Experimental data previously obtained for the peptide corresponding to one repeat were used to build theoretical models for B13-4r and B13-8r. These structures were further subjected to molecular dynamics simulations in an explicit solvent environment composed of a box containing water and trifluoroethanol model molecules. Overall, the experimental and theoretical results strongly suggest that the 3D structures of B13-4r and B13-8r do not present canonical secondary structures, probably because of the conformational restraints imposed by their high proline content. In fact, one can envisage that the structurally disordered character of the B13 repetitive domain could allow a greater epitope exposition conferring the ability to present them throughout the molecule surface.*

The frequency and magnitude of tandemly repeated regions in pathogenic protozoa proteins have been extensively described. One of the most noticeable features of these regions is their immunodominance, possibly associated with the epitope conformation and the repetitive character itself. The molecular mechanisms involved in this immunodominance presented by most but not all of such antigens are still not totally understood. Their multivalency has been associated both with the ability to directly activate B cells and to behave as T-cell independent antigens[1], thus preventing the host to elicit protective immune responses against these domains.

The "smoke screen" hypothesis[2] interprets the immunodominance of these tandem repeats as a means developed by the parasites to protect themselves by diverting the immune response away from the antigen functional domains. There is much evidence that the epitope conformation can be relevant to antigen-antibody binding and, in fact, peptide sequences with conformational preferences have shown to be preferentially

recognized by antibodies against native proteins epitopes. The *Trypanosoma cruzi* B13 protein is an immunodominant antigen exposed on the surface of tripomastigote forms of the parasite. It contains a central domain composed of a number of 12-residue tandemly repeated units. A previous study [3] indicated that the antigenic activity of several peptides derived from B13 correlate with the existence of a slight helical preference elicited in trifluoroethanol (TFE). In order to determine the 3D structure of the repetitive domain of B13, polypeptides containing 4 and 8 repeat units (B13-4r and B13-8r, corresponding to 48 and 96 amino acid residues, respectively) were produced in an *E. coli* expression system. The structural characterization of the two B13 derived polypeptides has been performed using circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopic as well as molecular modeling calculations. Initial CD experiments revealed that the two polypeptides assumed a random coil conformation in water, regardless of the ionic strength or pH of the solution. However, the polypeptides presented a certain propensity to assume a partial helical conformation either in the presence of sodium dodecylsulphate (SDS) below and above the critical micellar concentration at low pH, or in 30% TFE. ^{1}H-NMR 2D experiments reproduced the data obtained with CD spectroscopy. ^{1}H-NMR spectra carried out in TFE allowed us to verify that the partially folded state observed in the CD spectra is restricted to the AAA region that belongs to the antigenic epitope, Figure 1, and, interestingly, is the farthest position from the Pro residues in each repeat.

Figure 1. Three-dimensional model structure of B13-4r. The assembly comprises 100 superposed structures taken from the last 2.5 ns of the molecular dynamics simulation. The segments marked in black correspond to the putative epitope core.

The 3D models of the two polypeptides were built using NMR data previously obtained[3] for one repeat. Subsequently, the same NMR restraints have been used for the first part of a 5ns molecular dynamics simulation of B13-4r and B13-8r in 30 % TFE. The obtained data discard the possibility that the ensemble of a number of repeats can favor the induction of new secondary or tertiary structure elements. Overall, our results strongly suggest that the 3D structures of B13-4r and B13-8r do not present canonical secondary structures, probably due to conformational restraints imposed by their high proline content. In fact, one can envisage that the structurally disordered character of the B13 repetitive domain could allow greater epitope exposition, favoring the B cell surface immunoglobulin cross-linking. This hypothesis is in accordance with the experimental evidence that repetitive protein domains can act as T-cell independent antigens.

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