FUSION PROTEINS AS ALTERNATE CRYSTALLIZATION PATHS TO DIFFICULT STRUCTURE PROBLEMS

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Abstract: The three-dimensional structure of a peptide fusion product with glutathione transferase from *Schistosoma japonicum* (*Sj*GST) has been solved by crystallographic methods to 2.5 Å resolution. Peptides or proteins can be fused to *Sj*GST and expressed in a plasmid for rapid synthesis in *Escherichia coli*. Fusion proteins created by this commercial method can be purified rapidly by chromatography on immobilized glutathione. The potential utility of using *Sj*GST fusion proteins as alternate paths to the crystallization and structure determination of proteins is demonstrated.

As recombinant technology continues to make advancements, the number of important structural problems available to structural biologists will expand tremendously placing an increasing emphasis on the crystallization of important proteins, such as those from the viral genome of the human immunodeficiency virus (HIV). Crystallization of the latter has been proven particularly difficult; to date structures of only two of the twenty protein components of HIV-1 have been reported. Additionally, smaller peptide structures from 8 to 20 amino acids in length have been historically difficult to crystallize. Strategies employed to favorably effect the solubilities of such peptides or proteins for crystallization have included the formation of antigen/antibody complexes, the addition of other substances such as detergents to the crystallization milieu, or, more recently, the chemical modification of the protein [1]. The formation of fusion proteins containing the desired peptide or protein of interest represents an alternative approach and one which is convenient within the procedure of producing the recombinant protein. Here we present the serendipitous, but successful application of this structural approach to the solution of a hexapeptide fragment of gp41 of HIV-1 which represents a conservative epitope recognized by the neutralizing human monoclonal antibody 2F5 [2].

Proteins or peptides of interest can be fused with glutathione S-transferase (GST) and expressed in a plasmid vector for rapid synthesis in *Escherichia coli* [3]. Fusion proteins, thus created, can be purified by affinity chromatography on immobilized glutathione. The system utilizes GST from *Schistosoma japonicum* (*Sj*GST), a 26kD protein which as with other GST's, functions as a dimer catalyzing nucleophilic addition of the reduced sulphydryl to a variety of electrophiles [4]. These reactions are important in the metabolism of potentially harmful alkylating agents. Because of the rapidity and purity with which the fusion products could be prepared,

we undertook a series of co-crystallization experiments with antibody 2F5 utilizing a portion of the epitope fused to *Sj*GST as part of an ongoing effort to further elucidate HIV antigen/antibody complexes [5]. Initial crystallization attempts of the antibody/antigen complexes were unsuccessful; however, high quality crystals of the *Sj*GST fusion protein were obtained. The crystals grow in the space group $P4_32_12$ with a = b = 94.7 Å, c = 58.1 Å from solutions of PEG 3350 MW. The structure has been solved by the molecular replacement method using coordinates derived from GST of the μ gene class derived from rat liver (μGST) [6] and represents the first example of an invertebrate GST. *Sj*GST shows 42% sequence identity with μGST. The structure is refined including data to 2.5 Å resolution and is notable among GST structures in containing a monomer in the asymmetric unit and an ordered glutathione in the active site which is providing further insight into GST chemistry.

Electron density consistent with the hexapeptide fusion product, Glu-Leu-Asp-Lys-Trp-Ala (Fig. 1) has been observed and incorporated into the refined structure. The conformation of the antigenic peptide is stabilized by crystal packing interactions with symmetry related molecules (Fig. 2). Although it is possible that the observed conformation of such a small peptide fragment may not assume its normal secondary structure within gp41, larger peptides with more stable secondary structure elements, or proteins, should not suffer from this possible limitation. Still, the tremendous potential of the method is clearly demonstrated. Structure determination of smaller peptides or protein fusion products with *Sj*GST should be straightforward with the aid of the known *Sj*GST structure and conventional molecular replacement methods. Larger fusion products which represent more difficult phasing problem could be overcome by the development of novel iodine or other modified glutathione based substrates (suggested by the protein complex) [7] or by genetically engineered heavy atom sites within the *Sj*GST structure, such as in the case of the structure determination of μGST [6] which can then be utilized for complimentary phasing by isomorphous replacement methods.

Additional importance of *Sj*GST relates to its *Schistosoma* origin. Schistosomiasis, a major parasitic disease, second only to malaria, is estimated to infect over 250 million people worldwide. Since it has been suggested that the specific inhibition of GST could offer the possibility of combining chemotherapy and immunotherapy to combat this disease [8, 9]; the determination of other GST members of this parasitic family may be an attractive approach to the development of a multi-species cross-reactive vaccine against the transmission of schistosomiasis.

Detailed comparisons of the structure with other members of the GST family will be published [10]. Coordinates of *Sj*GST are available from the authors and have been deposited with the Brookhaven Protein Data Bank.

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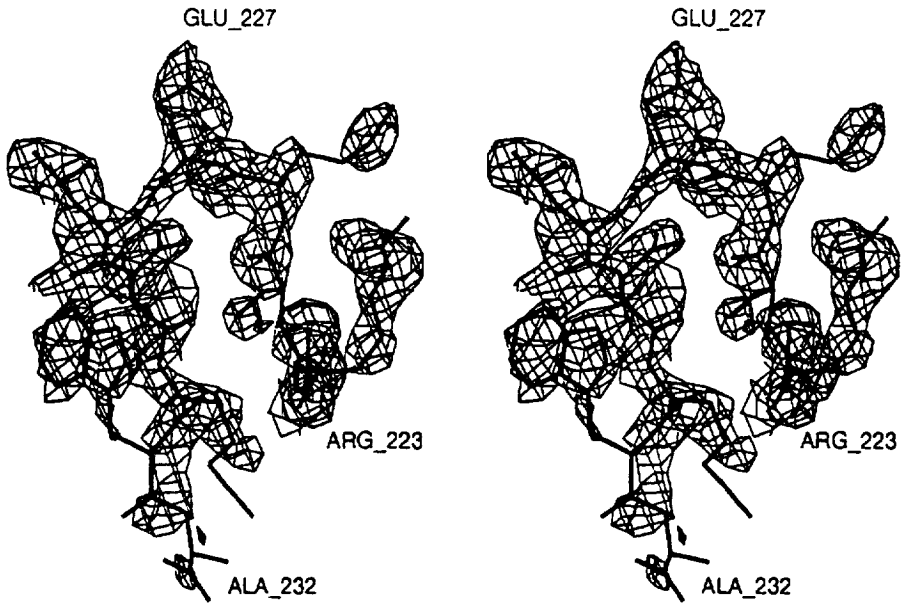

Figure 1. Stereo view of 2.3 Å omit map of the gp41 fusion peptide contoured at 2.5 σ level.

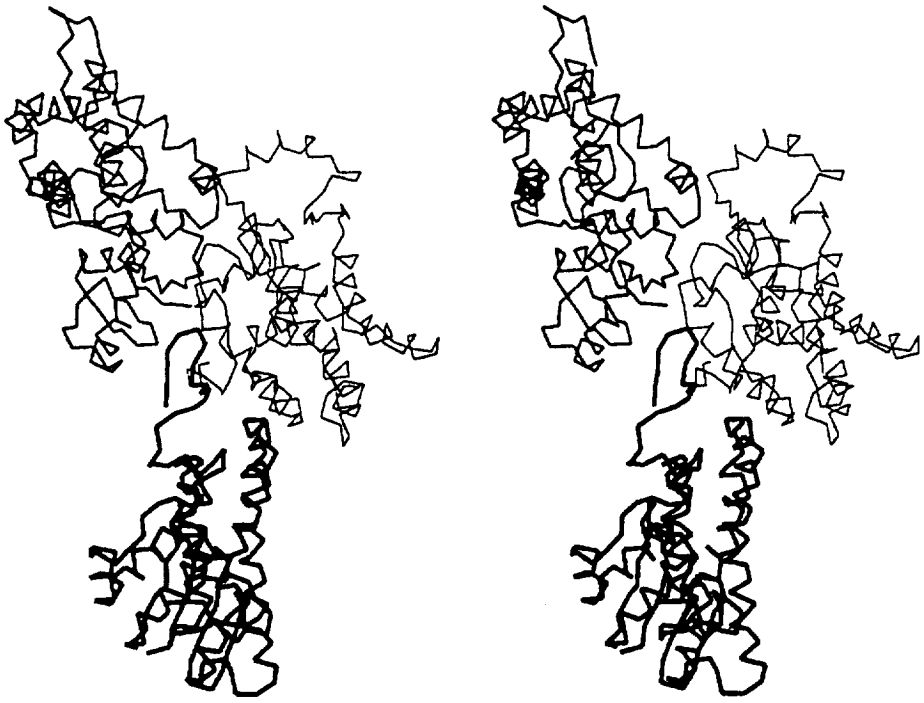

Figure 2. Stereo view of Cα tracing of *Sj*GST with its fusion peptide interacting with symmetry-related molecules.