

The 2.3Å crystal structure of GAD65, a major autoantigen in type 1 diabetes and neurological diseases

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The 65kDa isoform of glutamic acid decarboxylase (GAD65) is a major autoantigen in type 1 diabetes (T1D), neurological and polyendocrine diseases. In T1D, autoantibodies are early markers of the autoimmune process and predict development of type 1 diabetes. To gain structural insights into the autoantigenic properties of the molecule, an enzymatically active N-terminal truncation of GAD65 was expressed in *Saccharomyces cerevisiae*, purified and successfully crystallized. Data were collected at the IMCA-CAT beamline at the Advanced Photon Source (Chicago, USA), and crystals diffracted to 2.3Å resolution. The data was merged and processed using MOSFLM and SCALA and crystals belong to space group C2221, with unit cell dimensions of $a = 78.25 \text{ \AA}$, $b = 99.06 \text{ \AA}$, $c = 120.1 \text{ \AA}$, consistent with one molecule per asymmetric unit. Although the data sets could be phased using a search model constructed from the crystal structure of the closest structural homologue Pig Dopa Decarboxylase (DDC; PDB identifier 1JS3, 21% sequence identity), determination of the structure by molecular replacement was only possible after solving the crystal structure of the related non-antigenic isoform GAD67. The crystallographic resolution of the structure of an obligate GAD65 dimer revealed that the contrasting autoantigenicity of GAD isoforms correlates to key structural differences between the isoforms and regions of high flexibility. Together with epitope mapping data from 16 human mAb, a critical role of this domain for B-cell recognition is suggested. The data provide the structural basis for understanding the humoral autoimmune response to GAD65.