Molecular Immunology 47 (2009) 493–505

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Structural determinants of GAD antigenicity

Yasir Arafat[a], Gustavo Fenalti[a], James C. Whisstock[a], Ian R. Mackay[a], Maria Garcia de la Banda[b], Merrill J. Rowley[a], Ashley M. Buckle[a,*]

[a] The Department of Biochemistry and Molecular Biology, Monash University, Clayton, Melbourne, VIC 3800, Australia
[b] Clayton School of Information Technology, Monash University, Clayton, Melbourne, VIC 3800, Australia

ARTICLE INFO

Article history:
Received 19 August 2009
Accepted 28 August 2009
Available online 23 September 2009

Keywords:
Glutamic acid decarboxylase
Autoantigen
Epitope
Protein–protein interactions
Type 1 diabetes

ABSTRACT

Our aim was to ascertain structural determinants of autoantigenicity based on the model of the diabetes autoantigen glutamic acid decarboxylase 65 kDa isoform (GAD65) in comparison with that of the non-autoantigenic isoform GAD67. This difference exists despite the two isoforms having the same fold and high sequence identity. Autoantibodies to GAD65 precede the development of type 1 diabetes and are clinical markers of this and certain neural autoimmune diseases. To date, epitope mapping has been based on particular amino acid differences between the two isoforms, and there is no explanation as to why autoantibodies that react with GAD65 only infrequently cross-react with GAD67. To characterize each isoform of the enzyme and gain insights into their contrasting autoantigenic properties, we have used the recently determined crystal structures of GAD65 and GAD67 to compare their structure, hydrophobicity, electrostatics, flexibility and physiochemical properties. The results revealed striking differences which appear almost exclusively at the C-terminal domain of the isoforms. Whereas GAD65 displayed a highly charged and flexible C-terminal domain containing numerous patches of high electrostatic and solvation energies, these characteristics were absent in the GAD67 molecule. Additionally, analysis indicated potential N-terminal and PLP domain binding sites surrounding the C-terminal domain of GAD65, a major region of autoantigenic activity, but not of GAD67. These features agree with good accuracy with published epitope-mapping data. Our analysis suggests that the high flexibility and charge of GAD65 in the C-terminal domain is coupled with the mobility of its catalytic loop, a property that is absolutely required for its enzymatic function. Thus, the structural features that distinguish GAD65 from GAD67 as a B cell autoantigen are related to functional requirements for its enzymatic mechanism. This could well apply to the various other enzyme autoantigens and, if so, these features could be used as the basis of future predictive strategies.

1. Introduction

An opportunity to examine structural features of antigenicity is presented by the contrasting antigenic properties of the two structurally related isoforms of glutamic acid decarboxylase, GAD65 and GAD67. Autoantibodies to GAD65 but not GAD67 are characteristic of type 1 diabetes (T1D) (Baekkeskov et al., 1990). GAD is a pyridoxal 5′-phosphate (PLP) dependent enzyme that synthesizes the inhibitory neurotransmitter γ-amino butyric acid (GABA) (Baekkeskov et al., 1990). Members of this superfamily catalyze a range of important reactions including α-decarboxylation, transamination, racemisation and aldol cleavage, with many of these enzymes catalyzing more than one reaction (Jansonius, 1998; Percudani and Peracchi, 2003). Whereas most neurotransmitters are synthesized by a single enzyme, GABA's biosynthesis is catalyzed by two related enzymes, a 67 kDa isoform (GAD67) and a 65 kDa isoform (GAD65). While GAD67 is responsible for basal production of GABA and is constantly active, the major pool of GAD65 exists as autoinactivated apoenzyme (Battaglioli et al., 2003; Fenalti et al., 2007).

Insights into the molecular mechanisms underlying this activity and a rationalisation for the contrasting enzymatic characteristics of the GAD isoforms have been provided recently by the determination of the crystal structures of GAD65 and GAD67. Both GAD isoforms form obligate functional dimers. The monomeric units comprise three domains initially defined from the linear sequence according to functional properties, N-terminal, PLP-binding, and C-terminal (Fenalti et al., 2007) (Fig. 1a and b). Structurally the N-terminal domain includes two parallel α-helices that pack against the N-terminal and PLP-binding domain of the other monomer (Fenalti et al., 2007). The PLP-binding domain adopts the type I PLP-dependent transferase-like fold and comprises nine α-helices surrounding a 7-stranded mainly parallel β-sheet (Fig. 1a). The C-terminal domain contains three α-helices, together with a 2-

* Corresponding author. Tel.: +61 3 9902 0269; fax: +61 3 9905 4699.
E-mail address: Ashley.Buckle@med.monash.edu.au (A.M. Buckle).

0161-5890/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2009.08.022

stranded antiparallel β-sheet. The two active catalytic sites of GAD dimers are located in the centre of the PLP-binding domain at the dimer interface (Fig. 1c). In GAD67 the active sites are covered by an extended catalytic loop, positioning a conserved catalytic tyrosine that is essential for continuous GABA synthesis (Fenalti et al., 2007). In GAD65 however, there is high mobility in the same catalytic loop, permiting a PLP cofactor-controlled activation and subsequent auto-inactivation. Two structurally independent clusters of autoantibody epitopes, referred hereafter as C-terminal cluster 1 (ctc1) and C-terminal cluster 2 (ctc2), were also identified and are located on opposing faces of the C-terminal domain (Fig. 1d) and, according to their reactivities with a panel of human

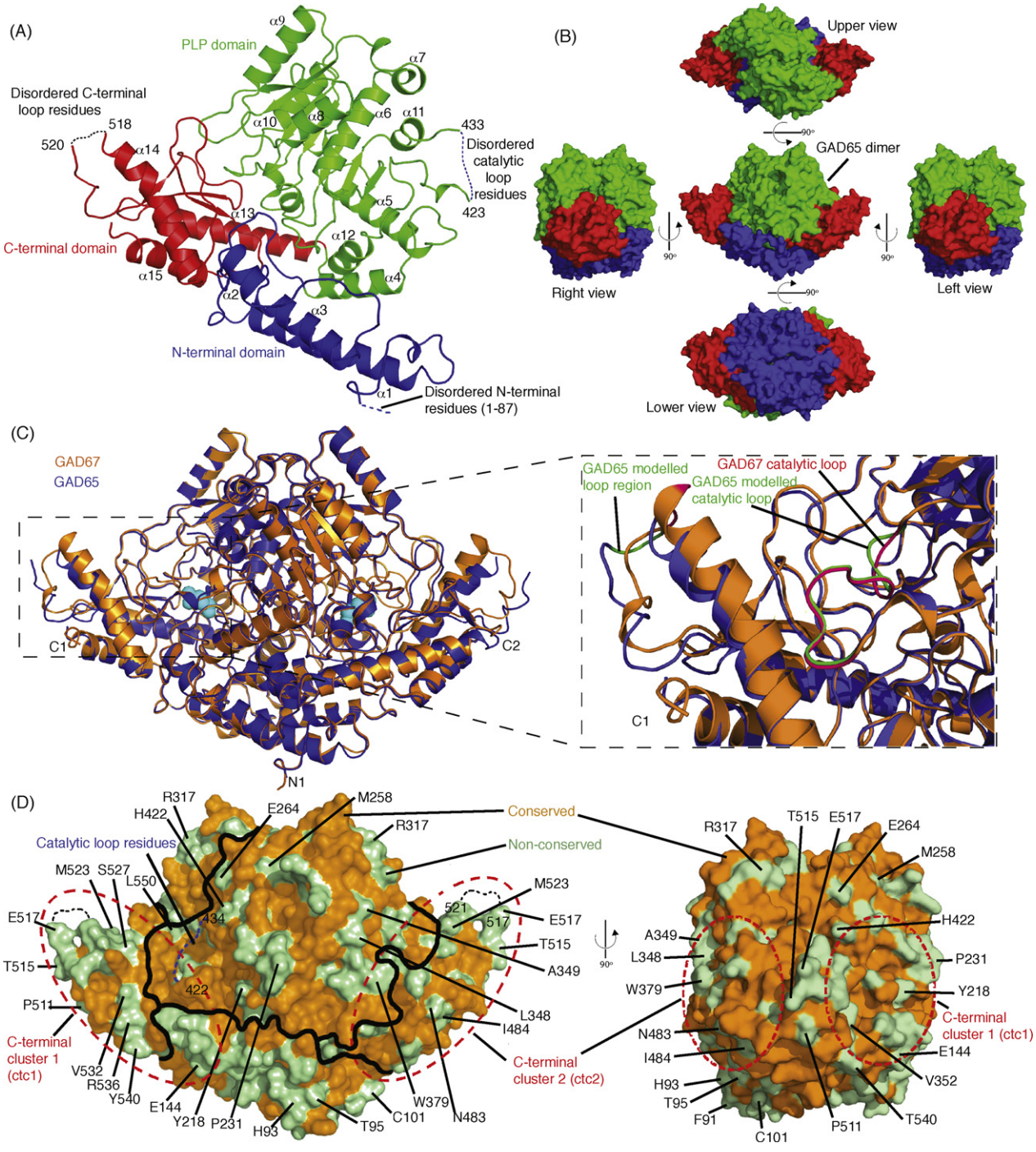

Fig. 1. (a) Cartoon representation of GAD65 monomer coloured by domain: N-terminal (blue), C-terminal (red), and PLP (green). Helices are labeled α1–α15. The unstructured C-terminal flexible loop residues 518–520 and the catalytic loop residues 423–433 are shown by black and blue dotted lines, respectively. (b) Orthogonal surface representations of GAD65 dimer with domains coloured according by colour scheme used in (a). (c) Cartoon representations of the superimposition of GAD65 (blue) and GAD67 (orange) structures, showing the greater structural differences in the C-terminal domain. Bound GABA molecules are shown in cyan. The dotted lines show a zoom-in of the superposition showing C-terminal region and catalytic loop. The GAD67 catalytic loop is shown in red and the modeled loop regions of GAD65 are shown in green. (d) Amino acid conservation between the GAD isoforms mapped on the molecular surface of GAD65. Conserved and non-conserved residues are coloured orange and pale green respectively. C-terminal clusters ctc1 and ctc2 are shown by red dotted ellipses. In this and subsequent figures, solid black lines depict domain boundaries. Domains are indicated by the surface representation shown in (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)