Stability and function of interdomain linker variants of glucoamylase 1 from Aspergillus niger

Several variants of glucoamylase 1 (GA1) from Aspergillus niger were created in which the highly O-glycosylated peptide (aa 468–508) connecting the (alpha/alpha)(6)-barrel catalytic domain and the starch binding domain was substituted at the gene level by equivalent segments of glucoamylases from Hormoconis resinae, Humicola grisea, and Rhizopus oryzae encoding 5, 19, and 36 amino acid residues. Variants were constructed in which the H. resinae linker was elongated by proline-rich sequences as this linker itself apparently was too short to allow formation of the corresponding protein variant. Size and isoelectric point of GA1 variants reflected differences in linker length, posttranslational modification, and net charge. While calculated polypeptide chain molecular masses for wild-type GA1, a nonnatural proline-rich linker variant, H. grisea, and R. oryzae linker variants were 65,784, 63,777, 63,912, and 65,614 Da, respectively, MALDI-TOF-MS gave values of 82,042, 73,800, 73,413, and 90,793 Da, respectively, where the latter value could partly be explained by an N-glycosylation site introduced near the linker C-terminus. The k(cat) and K(m) for hydrolysis of maltoligodextrins and soluble starch, and the rate of hydrolysis of barley starch granules were essentially the same for the variants as for wild-type GA1. beta-Cyclodextrin, acarbose, and two heterobidentate inhibitors were found by isothermal titration calorimetry to bind to the catalytic and starch binding domains of the linker variants, indicating that the function of the active site and the starch binding site was maintained. The stability of GA1 linker variants toward GdnHCl and heat, however, was reduced compared to wild-type.

General information
State: Published
Organisations: Centre de Recherches sur les Macromolécules Végétales, University of Southern Denmark, University of Copenhagen, Carlsberg Research Center
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Pages: 9336-9246
Publication date: 2001
Peer-reviewed: Yes

Publication information
Journal: Biochemistry
Volume: 40
Issue number: 31
ISSN (Print): 0006-2960
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.92
Web of Science (2017): Impact factor 2.997
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.8
Web of Science (2016): Impact factor 2.938
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.02
Web of Science (2015): Impact factor 2.876
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.96
Web of Science (2014): Impact factor 3.015
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.28
Web of Science (2013): Impact factor 3.194
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.33