

Microbial transglutaminase displays broad acyl-acceptor substrate specificity - DTU Orbit (09/11/2017)

Microbial transglutaminase displays broad acyl-acceptor substrate specificity

The great importance of amide bonds in industrial synthesis has encouraged the search for efficient catalysts of amide bond formation. Microbial transglutaminase (MTG) is heavily utilized in crosslinking proteins in the food and textile industries, where the side chain of a glutamine reacts with the side chain of a lysine, forming a secondary amide bond. Long alkylamines carrying diverse chemical entities can substitute for lysine as acyl-acceptor substrates, to link molecules of interest onto peptides or proteins. Here, we explore short and chemically varied acyl-acceptor substrates, to better understand the nature of nonnatural substrates that are tolerated by MTG, with the aim of diversifying biocatalytic applications of MTG. We show, for the first time, that very short-chain alkyl-based amino acids such as glycine can serve as acceptor substrates. The esterified α -amino acids Thr, Ser, Cys, and Trp—but not Ile—also showed reactivity. Extending the search to nonnatural compounds, a ring near the amine group—particularly if aromatic—was beneficial for reactivity, although ring substituents reduced reactivity. Overall, amines attached to a less hindered carbon increased reactivity. Importantly, very small amines carrying either the electron-rich azide or the alkyne groups required for click chemistry were highly reactive as acyl-acceptor substrates, providing a robust route to minimally modified, “clickable” peptides. These results demonstrate that MTG is tolerant to a variety of chemically varied natural and nonnatural acyl-acceptor substrates, which broadens the scope for modification of Gln-containing peptides and proteins.

General information

State: Published

Organisations: Center for Process Engineering and Technology, Department of Chemical and Biochemical Engineering, University of Ottawa, Universite de Montreal

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Pages: 219-230

Publication date: 2013

Main Research Area: Technical/natural sciences

Publication information

Journal: Applied Microbiology and Biotechnology

Volume: 98

Issue number: 1

ISSN (Print): 0175-7598

Ratings:

BFI (2017): BFI-level 1

Web of Science (2017): Indexed yes

BFI (2016): BFI-level 1

Scopus rating (2016): CiteScore 3.57 SJR 1.177 SNIP 1.173

Web of Science (2016): Indexed yes

BFI (2015): BFI-level 1

Scopus rating (2015): SJR 1.254 SNIP 1.217 CiteScore 3.43

Web of Science (2015): Indexed yes

BFI (2014): BFI-level 1

Scopus rating (2014): SJR 1.327 SNIP 1.458 CiteScore 3.71

Web of Science (2014): Indexed yes

BFI (2013): BFI-level 1

Scopus rating (2013): SJR 1.533 SNIP 1.432 CiteScore 4.3

ISI indexed (2013): ISI indexed yes

Web of Science (2013): Indexed yes

BFI (2012): BFI-level 1

Scopus rating (2012): SJR 1.507 SNIP 1.286 CiteScore 4

ISI indexed (2012): ISI indexed yes

Web of Science (2012): Indexed yes

BFI (2011): BFI-level 1

Scopus rating (2011): SJR 1.437 SNIP 1.232 CiteScore 3.72

ISI indexed (2011): ISI indexed yes

Web of Science (2011): Indexed yes

BFI (2010): BFI-level 1

Scopus rating (2010): SJR 1.381 SNIP 1.239

Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.353 SNIP 1.062
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.224 SNIP 0.979
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.036 SNIP 1.021
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.131 SNIP 1.062
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.118 SNIP 1.201
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.169 SNIP 1.162
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 0.969 SNIP 1.24
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.941 SNIP 1.027
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.876 SNIP 1.038
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 0.834 SNIP 1.065
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.13 SNIP 1.253
Original language: English
Amide bond formation, Microbial transformations, Biocatalysis, Peptide modification
DOIs:
10.1007/s00253-013-4886-x
Source: dtu
Source-ID: u::8689
Publication: Research - peer-review › Journal article – Annual report year: 2013