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Microbial transglutaminase has a lower deamidation preference than human tissue transglutaminase on a celiac disease relevant wheat gliadin T-cell epitope

Andreas Heil [a], Jürgen Ohsam [a], Christian Büchold [a], Ralf Pasternack [a], Keiichi Yokoyama [b], Yoshiyuki Kumazawa [b], Martin Hils [a, *]

[a] Zedira GmbH, Roesslerstrasse 83, Darmstadt, 64293, Germany
[b] Ajinomoto Co., Inc, Institute of Food Sciences & Technologies, 1-1, Suzuki-Cho, Kawasaki-Ku, Kawasaki-Shi, 210-8681, Japan

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ABSTRACT

Tissue transglutaminase (TG2) catalyzed glutamine deamidation within gluten peptides plays a major role in the pathogenesis of celiac disease. Recent studies reported gliadin deamidation by microbial transglutaminase (MTG) and hypothesize an impact on celiac disease incidence. We therefore investigated deamidation and transamidation activities of MTG and human TG2 based on a wheat gliadin peptide containing an immunodominant epitope for celiac disease. Deamidation activity of MTG was about a magnitude lower than transamidation with a maximum at pH 5 and decreasing values at neutral and basic pH. In contrast, TG2-deamidation reaction rate doubled from pH 6 to pH 7. Transamidation activity of MTG showed minor pH dependence, whereas for TG2 it strongly increased from pH 6 to 7 by a factor of 7.5. Additionally, deamidation by TG2 in the presence of a fivefold excess of amine substrate was observed for reactions at pH 6 at an equal rate to transamidation, while at neutral pH no deamidation occurred. MTG only catalyzed deamidation in addition to transamidation when the substrate ratio was below a 2.5 fold excess of amine. In conclusion, transamidation activity rates of MTG and TG2 were higher compared to deamidation rates. In contrast to TG2, MTG shows a strong and pH-independent transamidation preference.

1. Introduction

Transglutaminases catalyze the formation of covalent bonds between glutamine and lysine side chains of proteins. The active site of transglutaminases contains a reactive cysteine, which initially binds to a protein or peptide borne substrate glutamine, resulting in the formation of an acyl-enzyme complex and the release of ammonia. This complex can be resolved in two different ways: the formation of ε-(γ-glutamyl) lysine bonds with protein bound lysine or primary amine groups of small molecules (transamidation), and the incorporation of H_2O, resulting in the conversion of glutamine to glutamic acid (deamidation).

In celiac disease, deamidation of peptides derived from gluten by human tissue transglutaminase (TG2) is a key process in the pathomechanism. In the field of celiac disease, storage proteins from wheat (gliadins and glutenins), rye (secalins), and barley (hordeins) are called gluten. In more detail, in the course of digestion dietary gluten cannot be completely degraded by gastrointestinal proteases to resorbable small peptides and amino acids due to their high proline content (Shan et al., 2002). The resulting rather long-chain gluten peptides (e.g. the 33mer from alpha gliadin) may reach the lamina propria of the small intestinal mucosa, where TG2 is present. TG2 catalyzes deamidation resulting in so-called deamidated gluten or gliadin peptides, which are able to bind with high affinity to DQ2/DQ8-receptors of the MHC-II complexes on antigen presenting cells. Subsequent inflammation of the mucosa, leading to increased intraepithelial lymphocyte counts and villous atrophy as well as antibody-production characterize the onset of celiac disease (Dieterich et al., 1997; Molberg et al., 1998; Stamnaes et al., 2008; Vader, 2002). So far the only therapy for

Abbreviations: CD, celiac disease; DGP, deamidated gliadin peptide; HPLC, high performance liquid chromatography; MTG, microbial transglutaminase; TG2, tissue transglutaminase.

* Corresponding author.
E-mail address: hils@zedira.com (M. Hils).

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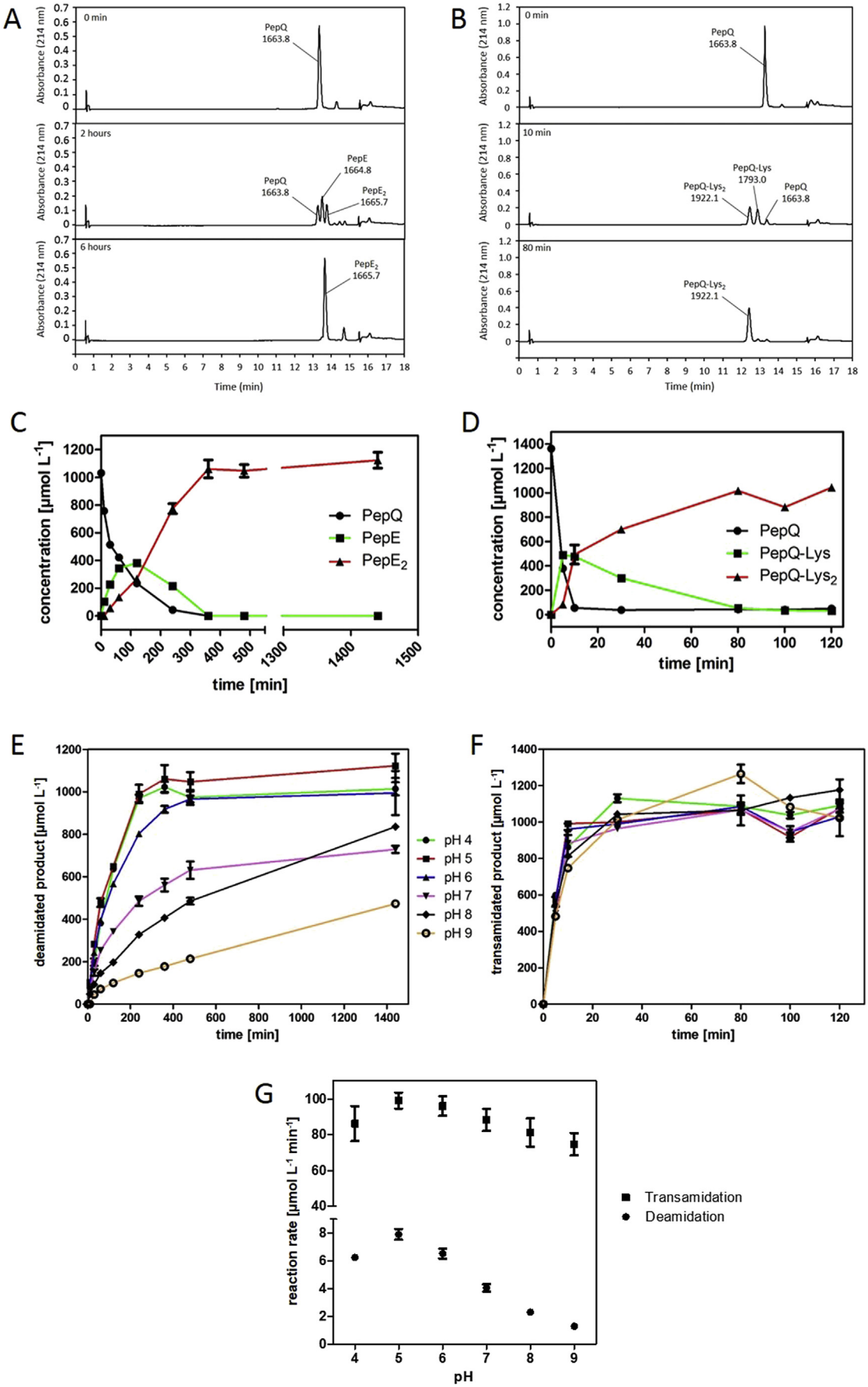
A
0 min
PepQ
1663.8
2 hours
PepE
1664.8
PepQ
1663.8
PepE2
1665.7
6 hours
PepE2
1665.7
Absorbance (214 nm)
Time (min)
B
0 min
PepQ
1663.8
10 min
PepQ-Lys
1793.0
PepQ-Lys2
1922.1
PepQ
1663.8
80 min
PepQ-Lys2
1922.1
C
concentration [µmol L-1]
PepQ
PepE
PepE2
time [min]
D
concentration [µmol L-1]
PepQ
PepQ-Lys
PepQ-Lys2
time [min]
E
deamidated product [µmol L-1]
pH 4
pH 5
pH 6
pH 7
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pH 9
time [min]
F
transamidated product [µmol L-1]
time [min]
G
reaction rate [µmol L-1 min-1]
Transamidation
Deamidation
pH