Supplementary material

Crosslinking mechanism on a novel *Bacillus cereus* transglutaminase-mediated conjugation of food proteins

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Table S1. Primers used in this study.

Primers	Sequences
bamtg-F-NcoI	CATGCCATGGGCATTATCATATCCGGCCAAGT
bamtg-R-HindIII	CCCAAGCTTATGCATGATCTGATAAAGCG
bcetg-F-NcoI	CATGCCATGGGCATTGTAATAGGCCGTTCTATTGTAC
bcetg-R-NotI	AAGGAAAAAAGCGGCCGCATAAACTGTAGTTGTGTTGCCAAC
bsatg-F-NcoI	CATGCCATGGGCATTATCCTTTCAGGAAAGCC
bsatg-R-HindIII	CCCAAGCTTGCTGCCAAACATTTGCC
bartg-F-NcoI	CATGCCATGGGCATTAAAGTTAACCAACAAATTGTG
bartg-R-HindIII	CCCAAGCTTAGCTACATACGTAATCGACCC

Underline indicated restriction recognition sites.

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BSUTG : MTIVSGQLLR PQDIENWQ-I DQNLNPLIKE MIETPVQFD HSIAELAFEL KIMNIVAAI KTLHKSGAKG ATFLKTYGNT TLRVSPEGA LLKYRMPIS KITRDIAENG PFYAHECATA IVITYYL LI DTIGEDKFNA SIDRIILIIW : 149
BAMTG : MTLISGQVLR PQDIANWQ-T EKSLVPYINE LLNSPVQFD GSIAELAFEV RILRHIVEAI RELHGSGYKF ATFAKTYGNI ALLRVTPEGA LLLAYRIPAI KITRDIHENG AFYAHECATA IVITYYLIVI KIIGEERFNI KIRDIILIIW : 149
BCETG : MIVIGRSIVH PYIINEYEPF ANEKQQIISI MAGNQEIYS RTSGELSGDL NILVNIITS LELFQSGFQF RIFQQSFCNP QILKRTSLGG FILLPNIPIS IITQDIFKNG KLYITECATA MIIIFYK LI ALYGEETFNI LIANLLLITW : 150
BSATG : MTILSGKPVT NEQLASFQ-L EGQKRIIIMQ LQASNDTFR RQASDLLFEV TILSNIMNAI RDINKSCASE ILFQRSRAND AILRVSEACA LILRYQVEIS RITKDIFENG SKYAIECATA IVIVFYM VI QTVGEEKFNI RIRSLILIIG : 149
BARTG : MTKVNQQIVK ISDLNNSS-L TKEKADIIKQ MDAYREVYEI ATFDQLGGDV SLILQIIESS VLLRKSCARG ATBARSRCNE KILDKRTDNGG FILLPTVSIH QILDDIFYNG HBYAIECATA VIIIFYK VI NNICKANENI LLADLYLHIW : 149


BSUTG : HYEK-LPIY ETGHHPFLGD GLYFINPEFD GQKAILRGEN VELIGEDKIF AHGIGILNCK QITLDRLNSFR KKCQLISAIL LSQAIRLIVP SIFRIVR--- ---------- ---------- ------          : 245
BAMTG : HYEH-LPIY ETGSHFLRGD GLYFINPDFN GAKAILRGEN VLVIGNDQIF AHGIGILIAE QILLDRLNSLR KKNAVIPAIL LNQAIRLIAP AIYQIMH--- ---------- ---------- ------          : 245
BCETG : DYDQDIKLI KTGGDLVPGD LVYFINPQVN GATIITGGEN THYIGNFFIY GHGVGIKIKE EITYALNERR VPYAFISAIL TDTIIRLISR LISYHASPST PQTSIGFIPI RDDAIVATVG NTTIVY          : 276
BSATG : HYDT-ISIY ERGNDFIYGD GLYFINPEFS YQQSITRGEN VEYIGEDQIY GHGIGILIAA EITDKLNKRR RPGAVISAIL LPQTIRMIVI YIRQMFGS--- ---------- ---------- ------          : 246
BARTG : GYDEDIELHG YKGSDYLPGD GAYFINPDYN GDTPILKGEN TEVIGETLIF GHGIGITIRE RILIEVLNIKR KDDAKISAIL SDEIVRLHTA HISYFAVRYE PVVWYD----- RNSAIISTIG SITYVA          : 271
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Figure S1. Multiple alignments analysis of protein sequences for BAMT, BCETG, BSATG, and BARTG with software DNAMAN. The similar amino acids were labeled in grey and the identical amino acids were highlighted in solid black. The active sites were presented in yellow color.

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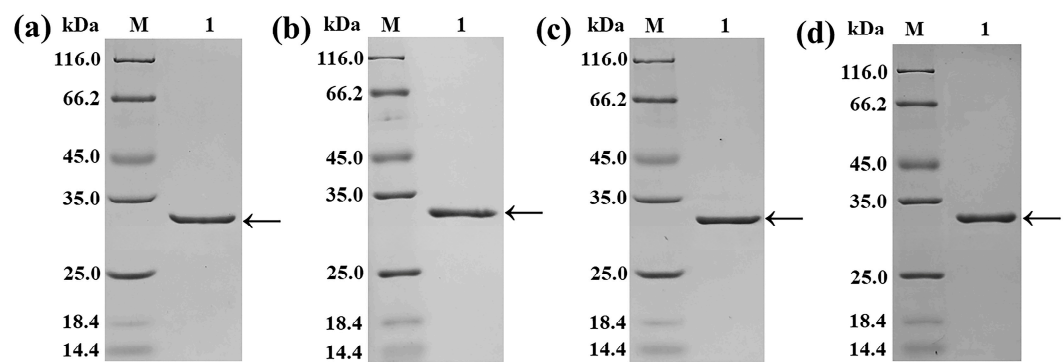
Figure S2. The purified rTGs analyzed using SDS-PAGE. (a) Purified BAMTG. (b) Purified BCETG. (c) Purified BSATG. (d) Purified BARTG. Lane M: Standard protein ladder; Lane 1: purified BAMTG, BCETG, BSATG, and BARTG.
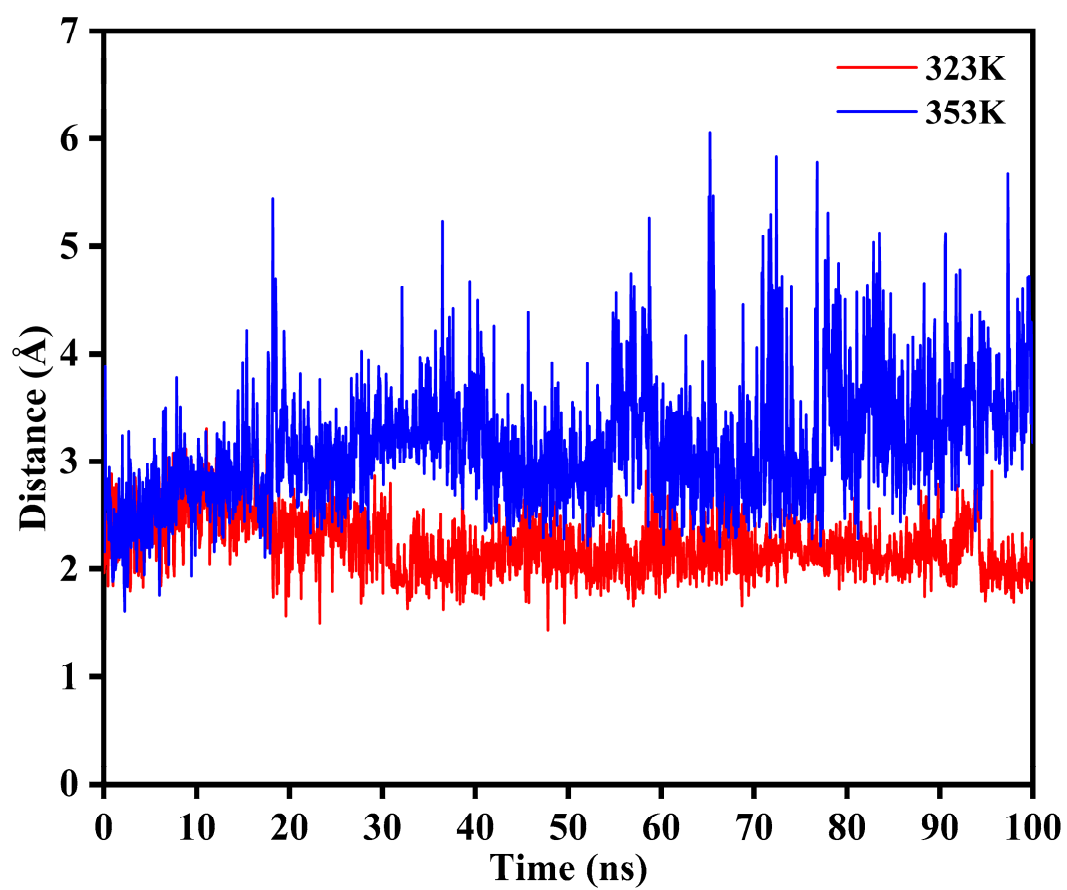
Figure S3. Distance between the active center of BCETG and MDC as a function of time (100 ns) at 323 K and 353 K.
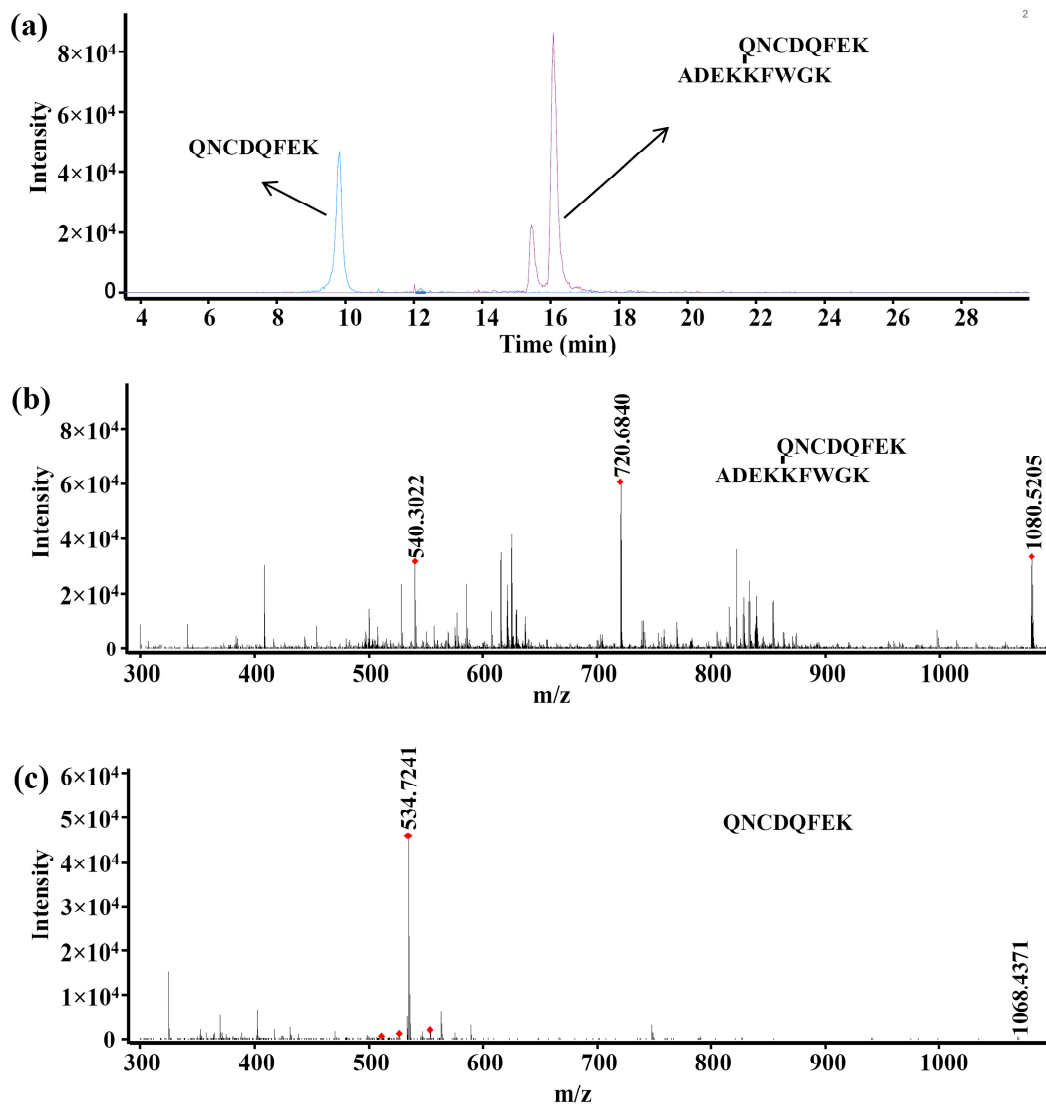
Figure S4. LC-MS evaluation of the cross-linked peptides of ADEKKFWGK and QNCDQFEK from cross-linked BSA. (a) EIC of the cross-linked peptides ADEKKFWGK-QNCDQFEK and QNCDQFEK. (b) Mass spectrum of cross-linked peptide ADEKKFWGK-QNCDQFEK. (c) Mass spectrum of peptide QNCDQFEK.