

Production of sialic acid rich glycopeptide from bovine κ -casein glycomacropeptide by hydrolyzing with papain

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SUPPLEMENTARY MATERIAL

Supplementary figure legends

Supplementary Figure S1. Primary structure of bovine GMP genetic variant A (C-terminal residues 106-169 of κ -casein). In GMP genetic variant B, Thr-136 is replaced by Ile and Asp-148 by Ala.

Supplementary Figure S2. Chromatography of papain hydrolysate of GMP on a column of DEAE-Sephacel. An arrow shows the position of application of 1 M NaCl. A bar denotes fractions pooled for further study. See text for other details.

Supplementary Figure S3. Size exclusion HPLC on Superdex-75 of GMP and GPF.

Chromatograms A and B show elution patterns of GMP and GPF, respectively. Chromatogram C gives elution patterns of molecular weight markers including: (1) bovine serum albumin (67 kDa); (2) dimeric β -lactoglobulin (36.6 kDa); (3) α -lactalbumin (14.2 kDa); and (4) vitamin B₁₂ (1.355 kDa).

Supplementary Figure S4. Cellulose acetate electrophoresis of GMP and GPF in 0.1 M pyridine-1.2 M acetic acid, pH 3.5. A 5 μ l sample containing 50 μ g GMP or GPF was applied in duplicate to a 2.5 cm \times 15.2 cm cellulose acetate strip having two lanes (each with equal width). After electrophoresis, the first lane was monitored for sialic acid and the second one for phosphorus as described (Nakano et al. 2007). Sialic acid and phosphorus peaks for GMP (A) and GPF (B) are shown.

24 **Supplementary Figure S1.** Primary structure of bovine GMP genetic variant A (C-terminal
 25 residues 106-169 of κ -casein).

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 27 106 110 115 120 125
 28 Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys-Thr-Glu-Ile-Pro-Thr-Ile-Asn-Thr-Ile-Ala-
 29 130 135 140 145
 30 Ser-Gly-Glu-Pro-Thr-Ser-Thr-Pro-Thr-Thr*-Glu-Ala-Val-Glu-Ser-Thr-Val-Ala-Thr-Leu-Glu-
 31 150 155 160 165 169
 32 Asp*-Ser-Pro-Glu-Val-Ile-Glu-Ser-Pro-Pro-Glu-Ile-Asn-Thr-Val-Gln-Val-Thr-Ser-Thr-Ala-Val
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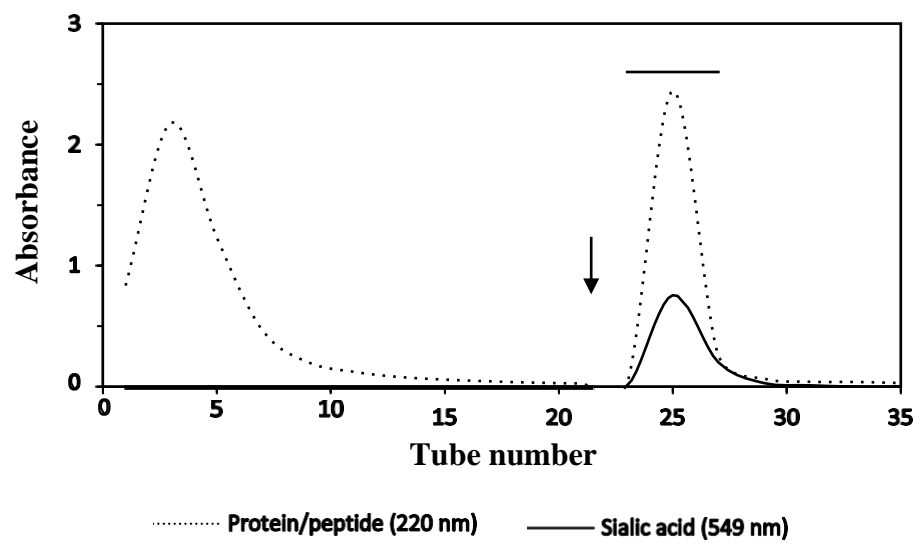
34 * In GMP genetic variant B, Thr 136 is replaced by Ile and Asp 148 by Ala.

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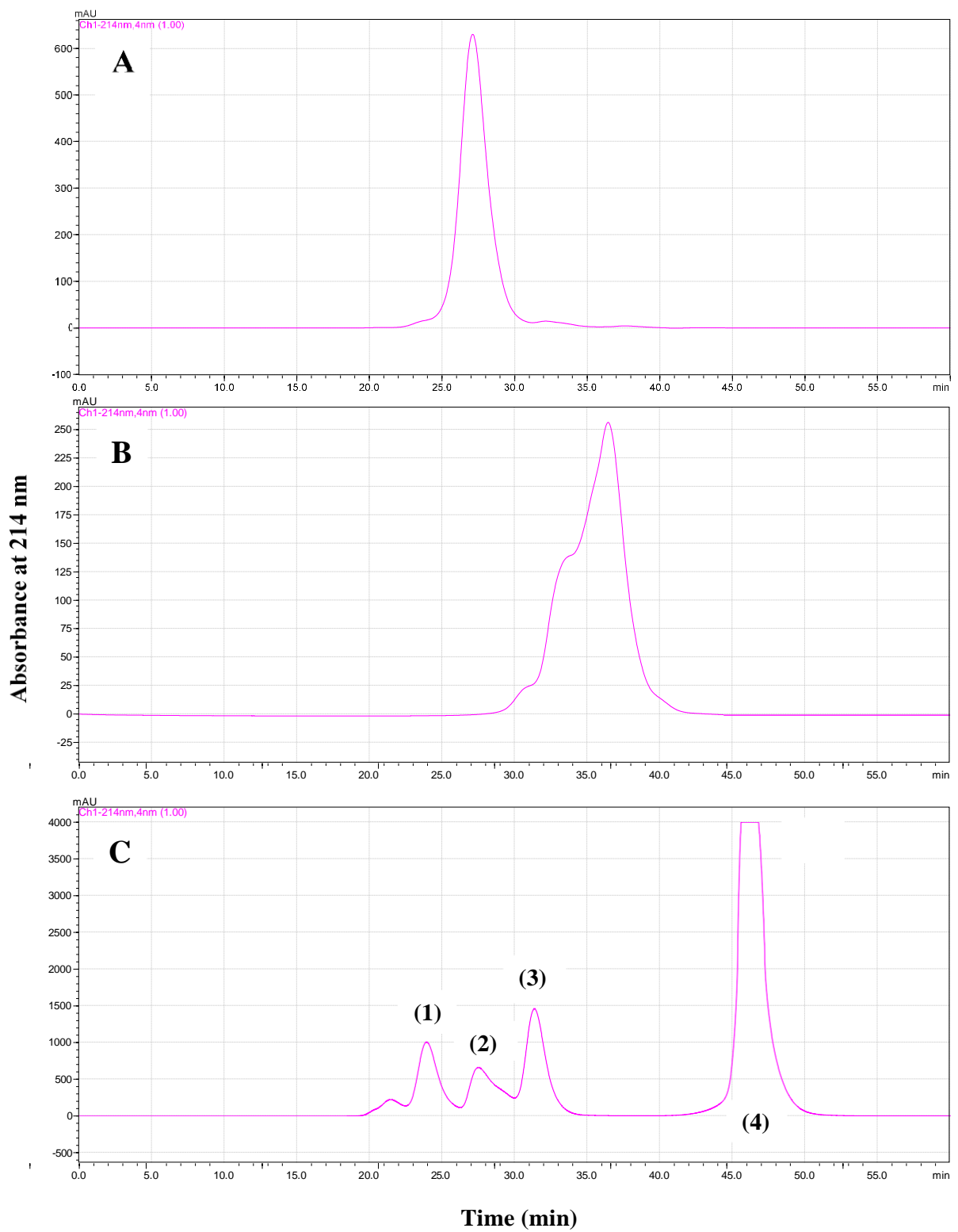
53 **Supplementary Figure S2.**

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Supplementary Figure S3.

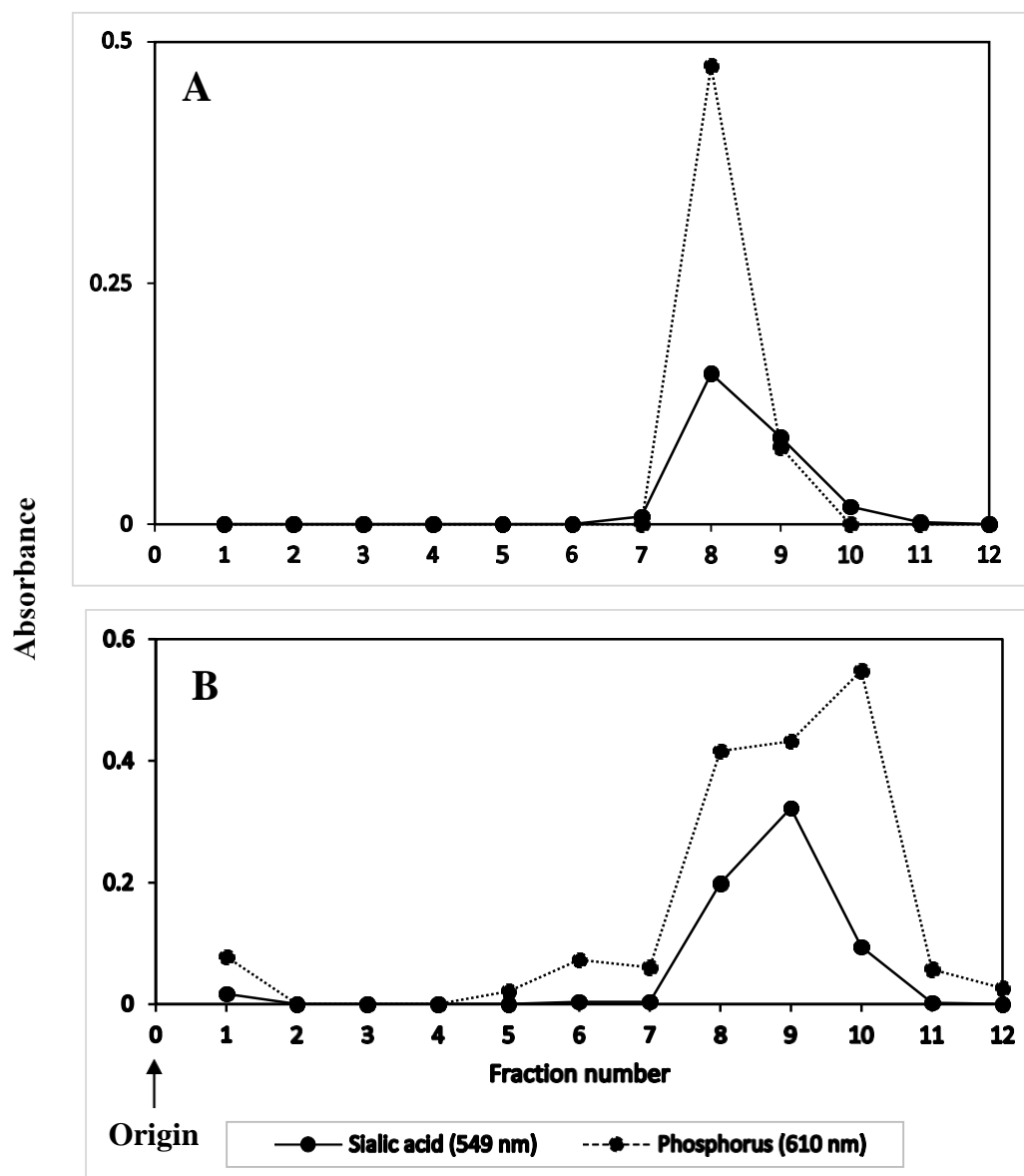


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75 **Supplementary Figure S4.**

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