

Supplementary information;

Limited lactosylation of beta-lactoglobulin from cow's milk exerts strong influence on antigenicity and allergenicity

Gerlof P. Bosman¹; Sergio Oliveira²; Peter J. Simons³; Javier Sastre Torano¹; Govert Somsen⁴; Leon Knippels^{2,5}; Rob Haselberg⁴; Roland J. Pieters¹, Johan Garssen^{2,5}, Karen Knipping^{2,5*}.

Table S1 - Observed masses vs theoretical masses as shown in figure 2

Protein	Theoretical mass (Da)	Observed mass (Da)
BLGA*	18363.2	18364.8
BLGB**	18277.1	18277.0
BLGWT	BLGA	18363.6
	BLGB	18278.0

*BLGA

*LIVTQTMKGL DIQKVAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP
 EGDLEILLQK WENDECAQKK IIAEKTKIPA VFKIDALNEN KVLVLDTDYK
 KYLLFCMENS AEPEQSLVCQ CLVRTPEVDD EALEKFDKAL KALPMHIRLS
 FNPTQLEEQC HI*

Disulfide bridge formation; 66-160, 106-119

**BLGB

*LIVTQTMKGL DIQKVAGTWY SLAMAASDIS LLDAQSAPLR VYVEELKPTP
 EGDLEILLQK WENGECAQKK IIAEKTKIPA VFKIDALNEN KVLVLDTDYK
 KYLLFCMENS AEPEQSLACQ CLVRTPEVDD EALEKFDKAL KALPMHIRLS
 FNPTQLEEQC HI*

Disulfide bridge formation; 66-160, 106-119

Theoretical mass (Da) was calculated using PerkinElmer ChemDraw Professional 17.1 based on the sequences above which were deduced from Uniprot P02754.

Table S2 - Protocol for applying a triple coating on a bare fused silica capillary

#	Pressure (psi)	Duration (min)	Component	Event
1	30	30	NaOH	Activation silanol groups
2	30	20	MQ	Rinse
3	20	20	BGE	Rinse
4	10	30	PB	Application 1 st layer
5	30	10	MQ	Rinse
6	10	30	DS	Application 2 nd layer
7	30	10	MQ	Rinse
8	10	30	PB	Application 3 rd layer
9	30	45	MQ	Rinse
10	20	45	BGE	Rinse

MQ = MilliQ water, BGE = background electrolyte, PB = polybrene, DS = dextran sulphate

Proteins may adsorb to the negatively charged inner wall of BFS capillaries, complicating CE separation and reproducibility. Hence, to avoid adsorption, a 90-110 cm capillary was coated with a triple layer of PB, DS and PB. The separation of BLGA and BLGB was optimized using different BGE compositions (0.25, 0.5, 1 and 2 M acetic and formic acid) and by varying other parameters like capillary temperature, separation voltage and injection volume. A 90 cm triple layer coated capillary, maintained at 25°C, using a BGE of 1 M formic acid, 30 kV separation voltage and an injection volume of 0.86% of the total capillary volume were found to be optimal for the analysis of BLGA and BLGB.

Table S3 - Determination optimal resolution BLGWT separation

BGE	M	Resolution (90cm)	Resolution (110cm)	Commentary (90cm)	Commentary (110cm)
AA	0.25	n.c.	0.39	No separation	
	0.5	n.c.	0.84	No separation	
	1	n.c.	0.82	No separation	
	2	0.59	n.c.		Very low intensity
FA	0.25	0.67	0.63		
	0.5	0.78	n.c.		Retention time > 60 min
	1	0.88	n.c.		Retention time > 60 min
	2	n.c.	n.c.	Very low intensity	Retention time > 60 min

n.c. = not calculated

Resolution was calculated using formula: $2(t_{r2}-t_{r1})/(W_2+W_1)$, in which t_r = retention time, W = elution peak width of first eluting peak (1) or second eluting peak (2). Minimal capillary length of ~85 cm is necessary to able to connect the CE to the MS equipment. AA = acetic acid, FA = formic acid.

Table S4 - Observed masses of intact BLG and conjugates

Theoretical masses intact protein			Observed masses intact protein					
Lactose units	BLGWT		<i>t</i> =0		<i>t</i> =3			
	BLGA	BLGB	BLGA	BLGB	BLGA	BLGB	BLGA	BLGB
0	18367.3	18281.2	18363.4	18276.2	18362.2	18275.4		
1	18691.6	18605.5			18687.4	18604.5		
2	19015.9	18929.8			19010.4	18925.5		
3	19340.2	19254.1					19335.6	19249.8
4	19664.5	19578.4					19659.7	19573.7
5	19988.8	19902.7					19983.8	19897.7
6	20313.1	20227.0					20308.0	20222.9
7	20637.4	20551.3					20632.0	20547.9
8	20961.7	20875.6					20955.2	20872.3
9	21286.0	21199.9					21279.6	21196.7
10	21610.3	21524.2						
11	21934.6	21848.5						
12	22258.9	22172.8						
13	22583.2	22497.1						
14	22907.5	22821.4						
15	23231.7	23145.6						
16	23556.0	23469.9						
17	23880.3	23794.2						
18	24204.6	24118.5						
19	24528.9	24442.8						
<i>Range lactose units / protein:</i>			0		0-2		3-9	
Lactose units			<i>t</i> =8		<i>t</i> =16		<i>t</i> =24	
	BLGA	BLGB	BLGA	BLGB	BLGA	BLGB	BLGA	BLGB
0	18367.3	18281.2						
1	18691.6	18605.5						
2	19015.9	18929.8						
3	19340.2	19254.1						
4	19664.5	19578.4						
5	19988.8	19902.7						
6	20313.1	20227.0						
7	20637.4	20551.3						
8	20961.7	20875.6	20958.7					
9	21286.0	21199.9	21282.0	21194.3				
10	21610.3	21524.2	21605.3	21517.6				
11	21934.6	21848.5	21930.3	21842.5				
12	22258.9	22172.8	22254.4	22167.7	22255.4	22168.7		
13	22583.2	22497.1	22578.6	22492.4	22578.6	22494.2	22576.5	22490.3
14	22907.5	22821.4	22902.7	22817.3	22901.8	22817.4	22901.7	22817.9
15	23231.7	23145.6	23228.3	23142.6	23228.0	23140.4	23227.0	23140.4
16	23556.0	23469.9			23551.0	23464.3	23551.4	23463.5
17	23880.3	23794.2						
18	24204.6	24118.5						
19	24528.9	24442.8						
<i>Range lactose units / protein:</i>			8-15		12-16		13-16	

Table S5 - Percental increase glycation peptides during first 8 hours

Percental increase of identified glycated peptides at t = 3 relative to t = 0;

1 L 22.5	2 I 22.5	3 V 22.5	4 T 22.5	5 Q 22.5	6 T 22.5	7 M 22.5	8 K 22.5	9 G 22.5	10 L 8.5	11 D 8.5	12 I 8.5	13 Q 8.5	14 K 8.5	15 V 8.5	16 A 8.5	17 G 8.5	18 T 8.5	19 W 8.5	20 Y 8.5	21 S 8.5	22 L 8.5	23 A 8.5	24 M 8.5	25 A 8.5	26 A 8.5	27 S 8.5	28 D 8.5	29 I 8.5	30 S 8.5	
31 L L	32 D A	33 A Q	34 S P	35 S L	36 A R	37 A R	38 P R	39 L R	40 V 8.5	41 Y 8.5	42 V 8.5	43 E 8.5	44 E 8.5	45 L 8.5	46 K 8.5	47 K 8.5	48 P 8.5	49 T 8.5	50 P 8.5	51 E 8.5	52 G 8.5	53 D 8.5	54 L 8.5	55 E 8.5	56 I 8.5	57 L 8.5	58 Q 8.5	59 K 8.5	60 K 8.5	
61 W E	62 N D	63 D E	64 C A	65 C A	66 K Q	67 K Q	68 K A	69 K A	70 K I	71 I I	72 I A	73 A E	74 E K	75 K 14.4	76 T 14.4	77 K 14.4	78 I 14.4	79 P 14.4	80 A 14.4	81 V 14.4	82 F 14.4	83 K 14.4	84 I 14.4	85 D 14.4	86 A 14.4	87 L 14.4	88 N 14.4	89 E 14.4	90 N 14.4	
91 K 17.0	92 V L	93 V L	94 L D	95 D T	96 D T	97 Y D	98 Y D	99 Y D	100 K 2.0	101 K 4.4	102 Y 4.4	103 L 4.4	104 L 4.4	105 F 4.4	106 C 4.4	107 M 4.4	108 E 4.4	109 N 4.4	110 S 4.4	111 A 4.4	112 E 4.4	113 P 4.4	114 E 4.4	115 Q 4.4	116 S 4.4	117 L 4.4	118 V 4.4	119 C 4.4	120 Q 4.4	
121 C 4.4	122 L 4.4	123 V 4.4	124 R 4.4	125 T 4.4	126 P 4.4	127 E 4.4	128 V 4.4	129 D 4.4	130 D 4.4	131 E 4.4	132 A 4.4	133 L 4.4	134 E 4.4	135 K 4.4	136 F 4.4	137 D 4.4	138 K 62.4	139 A 62.4	140 L 62.4	141 K 62.4	142 A 118.9	143 L 118.9	144 P 118.9	145 M 118.9	146 H 118.9	147 I 118.9	148 R 118.9	149 L 118.9	150 S 118.9	
151 F 29.9	152 N 29.9	153 P 29.9	154 T 29.9	155 Q 29.9	156 L 29.9	157 E 8.9	158 E 8.9	159 Q 8.9	160 C 8.9	161 H 8.9	162 I 8.9																			

Percental increase of identified glycopeptides at t = 8 relative to t = 3;

1 L 97.4	2 I 97.4	3 V 97.4	4 T 97.4	5 Q 97.4	6 T 97.4	7 M 97.4	8 K 97.4	9 G 97.4	10 L 196.6	11 D 196.6	12 I 196.6	13 Q 196.6	14 K 196.6	15 V 196.6	16 A 196.6	17 G 196.6	18 T 196.6	19 W 196.6	20 Y 196.6	21 S 196.6	22 L 196.6	23 A 196.6	24 M 196.6	25 A 196.6	26 A 196.6	27 S 196.6	28 D 196.6	29 I 196.6	30 S 196.6	
31 L L	32 D A	33 A Q	34 S P	35 S L	36 A R	37 A R	38 P R	39 L R	40 V 196.6	41 Y 196.6	42 V 196.6	43 E 196.6	44 E 196.6	45 L 196.6	46 K 196.6	47 K 196.6	48 P 196.6	49 T 196.6	50 P 196.6	51 E 196.6	52 G 196.6	53 D 196.6	54 L 196.6	55 E 196.6	56 I 196.6	57 L 196.6	58 Q 196.6	59 K 196.6	60 K 196.6	
61 W 38.9	62 E 38.9	63 N 38.9	64 D 38.9	65 E 38.9	66 C 38.9	67 A 38.9	68 Q 38.9	69 K 38.9	70 K 50.1	71 I 29.9	72 I 29.9	73 A 29.9	74 E 29.9	75 K 29.9	76 T 29.9	77 K 29.9	78 I 29.9	79 P 29.9	80 A 29.9	81 V 29.9	82 F 29.9	83 K 29.9	84 I 29.9	85 D 29.9	86 A 29.9	87 L 29.9	88 N 29.9	89 E 29.9	90 N 29.9	
91 K 8.9	92 V 8.9	93 L 8.9	94 V 8.9	95 L 8.9	96 D 8.9	97 T 8.9	98 D 8.9	99 Y 8.9	100 K 50.1	101 K 29.9	102 Y 29.9	103 L 29.9	104 L 29.9	105 F 29.9	106 C 29.9	107 M 29.9	108 E 29.9	109 N 29.9	110 S 29.9	111 A 29.9	112 E 29.9	113 P 29.9	114 E 29.9	115 Q 29.9	116 S 29.9	117 L 29.9	118 V 29.9	119 C 29.9	120 Q 29.9	
121 C 29.9	122 L 29.9	123 V 29.9	124 R 29.9	125 T 29.9	126 P 29.9	127 E 29.9	128 V 29.9	129 D 29.9	130 D 29.9	131 E 29.9	132 A 29.9	133 L 29.9	134 E 29.9	135 K 29.9	136 F 29.9	137 D 29.9	138 K 27.4	139 A 27.4	140 L 27.4	141 K 27.4	142 A 27.4	143 L 27.4	144 P 27.4	145 M 27.4	146 H 27.4	147 I 27.4	148 R 27.4	149 L 27.4	150 S 27.4	
151 F 29.9	152 N 29.9	153 P 29.9	154 T 29.9	155 Q 29.9	156 L 29.9	157 E 29.9	158 E 29.9	159 Q 29.9	160 C 29.9	161 H 29.9	162 I 29.9																			

Glycated residues are shown rimmed. Alternatively, the percental increase is represented by the green intensity.

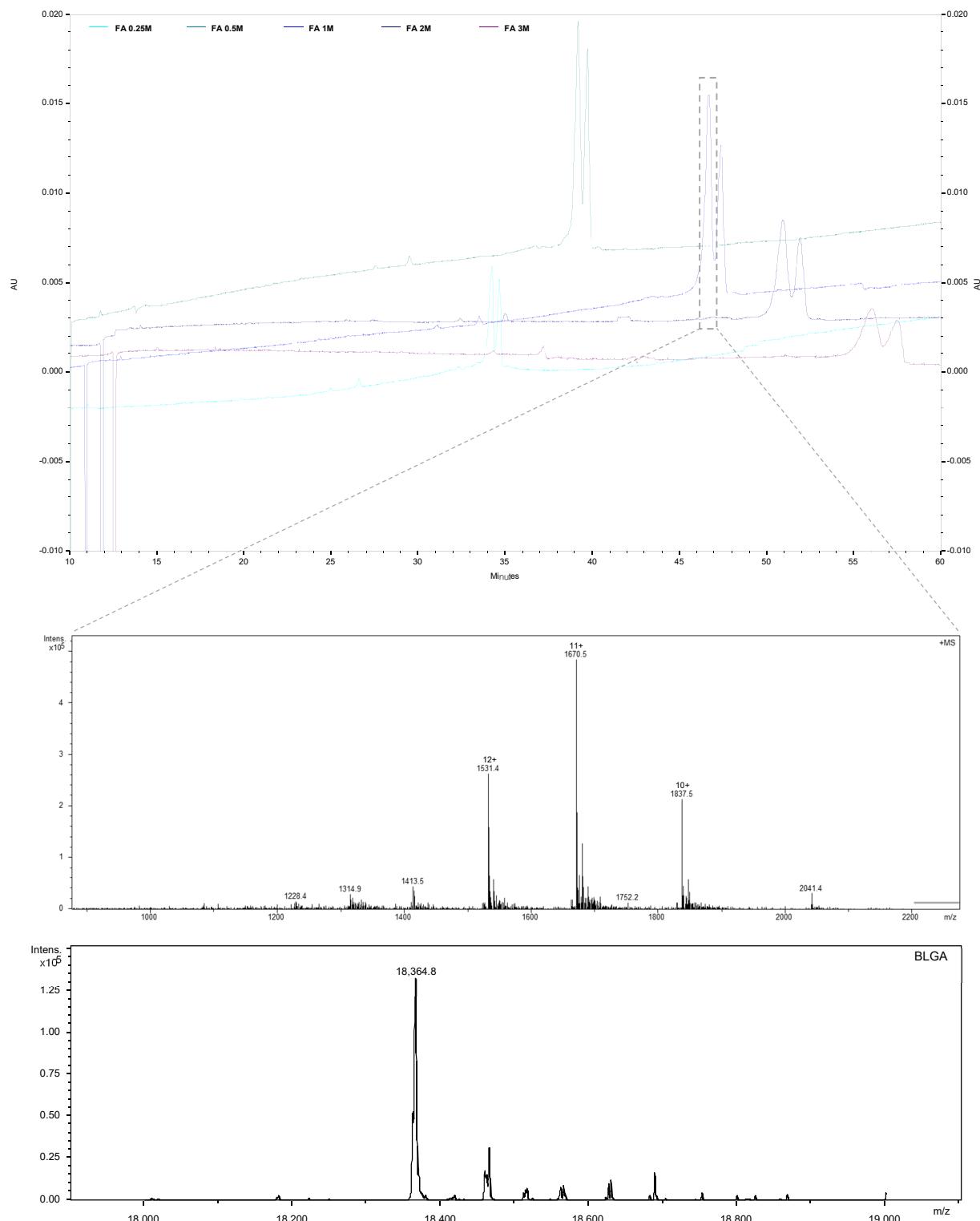


Figure S1 - CE-MS analysis of BLG. (Upper) Electropherogram. (Middle) Profile spectrum. (Bottom) Deconvoluted spectrum of BLGA. Using a triple layer coating and a capillary of 90 cm with a BGE of FA 1M best separation of BLGA and BLGB was observed (BLGA was the first peak to elute, secondly BLGB).