



Article

Posttranslational Acylations of the Rat Brain Transketolase Discriminate the Enzyme Responses to Inhibitors of ThDP-Dependent Enzymes or Thiamine Transport

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Supplementary data

Table S1. The peptides used for the relative quantifications by MS. The acylated (P1, P3, P5) and reference (P2, P4, P6–9) peptides of TKT, the peptides of reference proteins actin (ActB, P10–P12) and tubulin (Tubb3, P13–P15), and the peptides of sirtuin 5 (Sirt5, P16–P18) are shown. Dependent on the number of the lysine, arginine and histidine residues in a peptide, several charged precursor variants (2+, 3+ or 4+) with monoisotopic precursor mass and the C13-isotopomeric variants ([M+1] and [M+2]) are detected to increase the quantification accuracy. ox – Met oxidation (+16), cam – Cys carbamidomethylation (+57), n.a. – not analyzed.

N	Peptide	Specification	C13-isotopomeric variants of the precursors		
			monoisotopic	[M+1]	[M+2]
P1	SK(mal)DDQVTVIGAGVTLHEALAAAEM(ox)LKK	Malonyl-K499	966.5023+++	966.8366+++	967.1708+++
			725.1286++++	n.a.	n.a.
P2	SKDDQVTVIGAGVTLHEALAAAEM(ox)LKK	K499 de-malonylated	703.6285++++	703.8792++++	704.1298++++
			563.1042; +5	563.3048; +5	563.5053; +5
P3	K(ac)ISSDLDGHPVPK	Acetyl-K102	717.8830++	718.3845++	718.8858++
			478.9244+++	479.2587+++	479.5930+++
P4	ISSDLDGHPVPK	K102 deacetylated	632.8302++	633.3317++	633.8330++
			422.2226+++	422.5569+++	422.8911+++
P5	M(ox)EGYHK(ac)PDQQLQALKDTANR	Acetyl-K6	843.7553+++	844.0896+++	844.4237+++
P6	QAFTDVTATGSLGQGLGAAC(cam)GM(ox)AYTGK	TKT	1274.5940++	1275.0954++	1275.5963++
P7	LGQSDPAPLQHGVVDVYQK	TKT	1012.0158++	1012.5172++	1013.0186++
			675.0130+++	675.3473+++	675.6815+++
P8	TSRPENAIHYSNNEDFQVGQAK	TKT	1241.1039++	1241.6053++	1242.1067++
			827.7383+++	828.0726+++	828.4069+++
P9	SVPM(ox)STVFYPSDGVATEK	TKT	965.9588++	966.4603++	966.9613++
P10	VAPEEHPVLLTEAPLNPK	ActB	977.5358++	978.0373++	978.5387++
			652.0263+++	652.3606+++	652.6949+++
P11	DLYANTVLSGGTTM(ox)YPIGIADR	ActB	1116.0361++	1116.5375++	1117.0386++
P12	QEYDESGPSIVHR	ActB	758.8550++	759.3564++	759.8577++
			506.2391+++	506.5734+++	506.9076+++
P13	EVDEQM(ox)LAIQSK	Tubb3	703.8452++	704.3467++	704.8474++
P14	LATPTYGDLNHLVSATM(ox)SGVTTSR	Tubb3	1311.1656++	1311.6670++	1312.1682++
			874.4462+++	874.7805+++	875.1146+++
P15	YLTVAIVFR	Tubb3	535.3057++	535.8071++	536.3085++
P16	GVPVAEFNM(ox)ETTPATNR	Sirt5	925.4411++	925.9425++	926.4435++
P17	NKEPNPGHLAIAQC(cam)EAR	Sirt5	952.9734++	953.4748++	953.9758++
			635.6514+++	635.9856+++	636.3196+++
P18	VVVITQNIDELHR	Sirt5	768.4306++	768.9321++	769.4334++

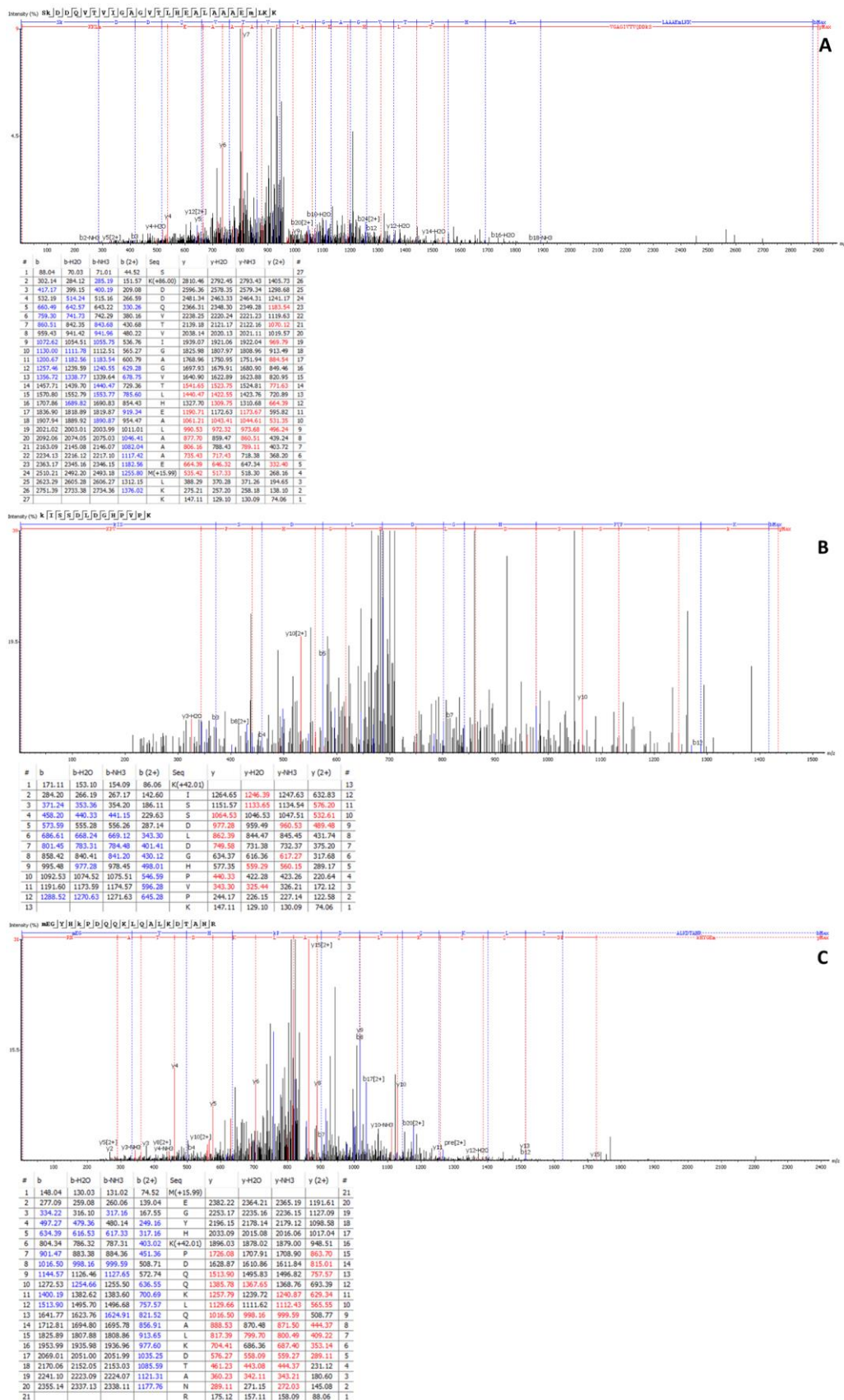


Figure S1. Representative MS/MS spectra of the TKT peptides containing acylated residues. A – The peptide with malonylated K499 residue. B – The peptide with acetylated K102 residue. C – The peptide with acetylated K6 residue. The images of the spectra are created by PEAKS Studio Xpro. The fragment masses of the peptides indicated in the

tables below the MS/MS spectra, unambiguously determine the site of the modification, even if the second site is present in the sequence.

Table S2. The p -values for statistical significance of the differences between the control and experimental groups of rats shown in Figure 1. The significances estimated by the Student and Mann-Whitney tests are compared.

Test	Oxythiamine (OT) vs. Control		Metformin + Amprolium (M+A) vs. Control	
	Student	Mann-Whitney	Student	Mann-Whitney
Mal-K499	0.0002	0.0006	0.202	0.181
Sirt5	0.003	0.009	0.108	0.251
Ac-K102	0.278	0.198	0.598	0.557
Ac-K6	0.483	0.357	0.146	0.121