

**Metallothionein-like peptides involved in sequestration of Zn
in the Zn-accumulating ectomycorrhizal fungus *Russula atropurpurea***

Tereza Leonhardt, Jan Sácký, Pavel Šimek, Jiří Šantrůček and Pavel Kotrba

*Institute of Chemical Technology, Prague, Department of Biochemistry and Microbiology,
Technická 3, 166 28 Prague, Czech Republic*

Author for correspondence: Pavel Kotrba, *E-mail: pavel.kotrba@vscht.cz*,
Tel: (+420) 220445134, Fax: (+420) 220445167

Electronic Supplementary Information:

Table S1 List of the sequences of carbamidomethylated RaZBP fragments identified by using the UHPLC/ESI-Q-TOF-MS/MS analysis

Fig. S1 Electrophoretic analysis of recombinant RaZBP1 produced in *E. coli* BL21(DE3).

The original mass spectrometry data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (<http://www.ebi.ac.uk/pride>; dataset identifier PXD001073; DOI 10.6019/PXD001073).

Table S1 List of the sequences of carbamidomethylated RaZBP fragments identified by using the UHPLC/ESI-Q-TOF-MS/MS analysis as described in Materials and Methods.

Precursor (<i>m/z</i>)	<i>z</i>	Theoretical rel. MW	$\Delta m/z$ (ppm)	Mascot score ^a	Sequence	Position in RaZBP	Carbamidomethyl at position
RaZBP1							
875.9488	5	4374.7127	-1.15	87.13	NGGEGEGKGPCTAQGCQCPAFTGNGSYCSRGGCGHGYNQHSG	12-53	11, 16, 18, 28, 33
853.1420	5	4260.6698	0.88	93.94	GGEGEGKGPCTAQGCQCPAFTGNGSYCSRGGCGHGYNQHSG	13-53	10, 15, 17, 27, 32
830.3319	5	4146.6268	-0.91	44.95	EGEGKGPCTAQGCQCPAFTGNGSYCSRGGCGHGYNQHSG	15-53	8, 13, 15, 25, 30
804.5217	5	4017.5843	-3.08	50.67	GEGKGPCTAQGCQCPAFTGNGSYCSRGGCGHGYNQHSG	16-53	7, 12, 14, 24, 29
958.8863	4	3831.5202	-1.05	72.25	GKGPCTAQGCQCPAFTGNGSYCSRGGCGHGYNQHSG	18-53	5, 10, 12, 22, 27
744.7908	4	2975.1341	0.02	49.71	CQCPAFTGNGSYCSRGGCGHGYNQHSG	27-53	1, 3, 13, 18
RaZBP2							
864.5485	5	4317.7025	0.84	85.89	GGGEGGRGACTVSGCQCPGFTGNYANCARQGCGHGYNQHSD	13-53	10, 15, 17, 27, 32
841.7404	5	4203.6595	1.41	61.05	GGEGRGACTVSGCQCPGFTGNYANCARQGCGHGYNQHSD	15-53	8, 13, 15, 25, 30
752.7100	5	3758.5151	-0.33	48.28	GRGACTVSGCQCPGFTGNYANCARQGCGHGYNQHS	18-51	5, 10, 12, 22, 27
991.1526	4	3960.5740	1.81	113.00	GRGACTVSGCQCPGFTGNYANCARQGCGHGYNQHSD	18-53	5, 10, 12, 22, 27
667.7761	4	2667.0728	0.98	67.75	PGFTGNYANCARQGCGHGYNQHSD	30-53	10, 15
838.6725	3	2512.9985	-1.20	61.93	FTGNYANCARQGCGHGYNQHSD	32-53	8, 13

^a Cut-off score for the 5% confidence threshold was calculated by Mascot as 43.

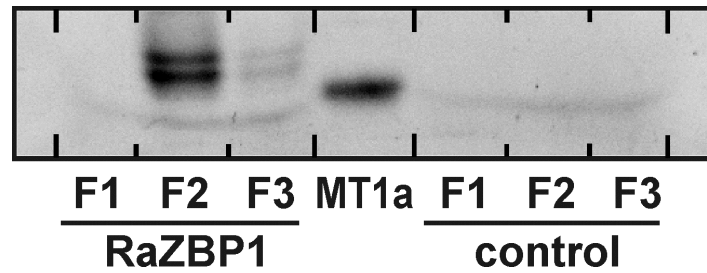


Fig. S1 Electrophoretic analysis of recombinant RaZBP1 produced in *E. coli* BL21(DE3). The 4-h expression of RaZBP1 under the control of T7 promoter of pET-29b (Novagen) was initiated with IPTG, and the harvested cells were disintegrated by sonication in the presence of 1 mM DTT. A partially purified peptide was obtained from the cell-free extract in which proteins were heat-precipitated and metals removed after acidification with TFA (pH 1.05) by desalting on a PD-10 column (GE Healthcare) packed with Sephadex G-25. The aliquots of eluted 0.5 ml F1, 3 ml F2 and 1 ml F3 fractions and the 6.1-kDa rabbit MT1a were labeled with SBD-F. The same treatment was used to obtain the control preparation from the extracts of *E. coli* cells that were not transformed with RaZBP1.