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Supporting information for article:

Na⁺/K⁺ exchange switches the catalytic apparatus of K-dependent plant L-asparaginase

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Table S1 Comparison of amino acid sequences of selected plant-type L-asparaginases (% identity - upper triangle, % similarity - lower triangle)

Created using online version of BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Potassium-dependent enzymes are in **bold**, non-plant homologues are in *italic*. The protein sequences are coded by the following acronyms: PvAspG1 - *Phaseolus vulgaris* K⁺-dependent asparaginase; LIA - *Lupinus luteus* K⁺-independent asparaginase; EcAIII - *Escherichia coli* plant-type asparaginase, *iaaA* gene product; hASNase3 - human asparaginase 3 protein; LjNSE1 - *Lotus japonicus* K⁺-dependent asparaginase, LjNSE2 - *Lotus japonicus* K⁺-independent asparaginase; At3g16150 - *Arabidopsis thaliana* K⁺-dependent asparaginase; At5g08100 - *Arabidopsis thaliana* K⁺-independent asparaginase.

	PvAspG1	LIA	<i>EcAIII</i>	hASNase3	LjNSE1	LjNSE2	At3g16150	At5g08100
PvAspG1	-	60	40	38	91	59	85	59
LIA	71	-	46	41	58	83	58	74
<i>EcAIII</i>	56	61	-	38	41	46	40	45
<i>hASNase3</i>	50	55	53	-	40	41	41	41
LjNSE1	95	70	57	51	-	57	83	58
LjNSE2	73	91	63	56	72	-	57	78
At3g16150	90	72	57	53	89	73	-	57
At5g08100	70	82	61	55	70	83	69	-

Table S2 Corresponding residues in several plant-type L-asparaginase structures

Residues coordinating the metal ions are in **bold**; *italic* font indicates that no such functional element exists in a given protein.

		PvAspG1	LIA	EcAIII		hASNase3	
active site	catalytic nucleophile	Thr196	Thr193	Thr179		Thr168	
	anchor of substrate/product α -carboxyl group	Arg224 Gly249	Arg221 Gly246	Arg207 Gly233		Arg196 Gly222	
	anchor of substrate/product α -amino group	Asp227 Gly247	Asp224 Gly244	Asp210 Gly231		Asp199 Gly220	
	oxyanion hole	Thr246 Gly247	Thr243 Gly244	Thr230 Gly231		Thr219 Gly220	
	GAG motif area	Gly10 Gly11 Gly13	Gly10 Ala12 Val14	Gly11 Gly13	Gly10 Ala12 Ala14	Gly11 Gly13	Gly9 Gly11 Ala12 Gly13
stabilization loop		Leu58 Thr60 Asp61 Pro62 Phe64 Ser66 Arg68	Glu59 Leu63 Asn65 Gly67	Leu59 Asn61 Ile62 His64 Phe65 Asn66 Gly68	Glu60 Glu63 Glu63 Phe66 Asn67 Ala67 Ala68 Ile70	Leu60 Glu62 Pro64 Leu65 Asn67 Ala68 Ala69 Ile70	Glu61 Cys63 Asp57 Asp58 Pro59 Phe61 Asn62 Gly64 Cys65
activation loop		Val111 Met112 Asp113 Lys114 Ser115 Pro116 His117 Ser118	<i>Val112</i> <i>Met113</i> <i>Asp114</i> <i>Lys115</i> <i>Thr116</i> <i>Pro117</i> <i>His118</i>	<i>Val113</i> <i>Met114</i> <i>Glu115</i> <i>Gln116</i> <i>Ser117</i> <i>Pro118</i> <i>His119</i> <i>Val120</i>	<i>Val113</i> <i>Met114</i> <i>Glu115</i> <i>Gln116</i> <i>Ser117</i> <i>Pro118</i> <i>His119</i> <i>Val120</i>	<i>Val108</i> <i>Met109</i> <i>Glu110</i> <i>Lys111</i> <i>Thr112</i> <i>Pro113</i> <i>His114</i> <i>Cys115</i>	
catalytic switch		His117 Arg224 Glu250	<i>His118</i> <i>Arg221</i> <i>Glu247</i>	<i>His119</i> <i>Arg207</i> <i>Glu234</i>	<i>His119</i> <i>Arg207</i> <i>Glu234</i>	<i>His114</i> <i>Arg196</i> <i>Glu223</i>	

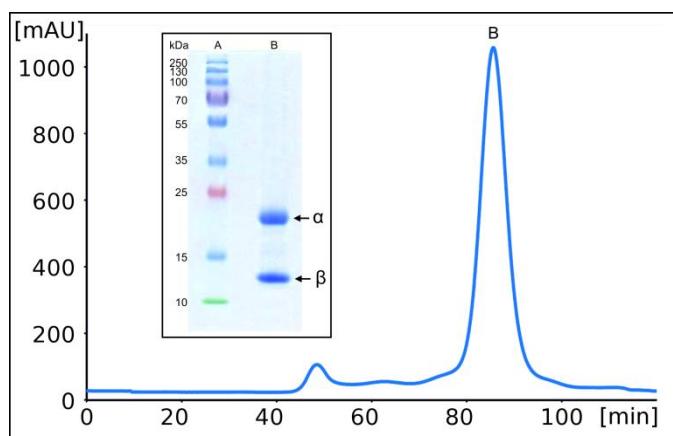


Figure S1 Elution profile from gel filtration chromatography of PvAspG1 using a GE Hiload 16/60 Superdex 200 column, with subsequent analysis of the main peak B using 14% SDS-PAGE (inset; lane A, Page Ruler Plus, Thermoscientific; lane B, 10 µg protein sample from peak B). The main peak B corresponds to a molecular mass of ~70 kDa (calibrated using retention times of proteins with known molecular mass), demonstrating that the purified protein is a dimer of two units, each corresponding to the full sequence of PvAspG1. Absorbance was measured at 280 nm in arbitrary units (mAU). The SDS-PAGE electrophoregram shows that the purified protein consists exclusively of subunits α (21.0 kDa) and β (13.6 kDa).

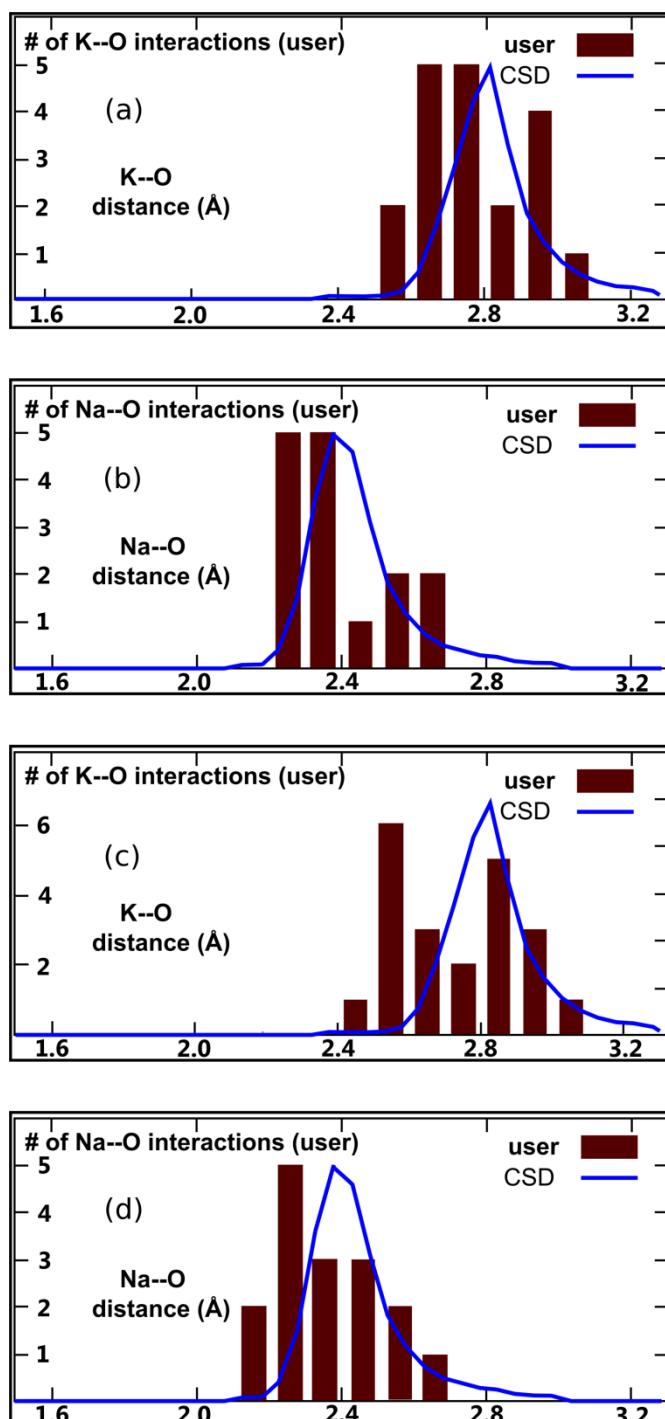


Figure S2 Histograms of metal-ligand distances in PvAspG1-K (a), PvAspG1-Na (b), and PvAspG1-K/Na (c, d). The graphs were prepared using the CSGID CheckMyMetal (CMM) server (Zheng *et al.*, 2014).

Reference

Zheng, H., Chordia, M. D., Cooper, D. R., Chruszcz, M., Muller, P., Sheldrick, G. M. & Minor, W. (2014). *Nature Protocols* **9**, 156-170.