

Supplementary Information

Table S1. Kinetic constants for PEP³⁻ in WT-RMPK and in T113L-RMPK in the presence of saturating concentrations of the ADP-Mg complex and of the monovalent cation indicated in 100% water. Kinetic data (not shown) were fitted to the Michaelis-Menten equation ($v = V_{max} \cdot S/K_m + S$) (Origin version 7.0). The standard deviations from two experiments are shown. k_{cat}/K_m values are expressed in log form.

M ⁺	WT			T113L		
	K_m (μM)	k_{cat} (s ⁻¹)	Log (k_{cat}/K_m)	K_m (μM)	k_{cat} (s ⁻¹)	Log (k_{cat}/K_m)
K ⁺	47 ± 6.8	963 ± 39	7.31	108 ± 18	725 ± 49	6.83
NH ₄ ⁺	31 ± 3.6	645 ± 26	7.32	24 ± 3	610 ± 19	7.41
Rb ⁺	25 ± 4.2	618 ± 26	7.40	62 ± 6.9	998 ± 34	7.21
Cs ⁺	27 ± 1.7	171 ± 2.6	6.80	34 ± 7.3	193 ± 10	6.76

Table S2. Kinetic constants for the ADP-Mg complex in WT-RMPK and in T113L-RMPK in the presence of saturating PEP³⁻ and the monovalent cation indicated in 100% water. Kinetic data (not shown) were fitted to the Michaelis-Menten equation ($v = V_{max} \cdot S/K_m + S$) (Origin version 7.0). The standard deviations from two experiments are shown. k_{cat}/K_m values are expressed in log form.

M ⁺	WT			T113L		
	K_m (μM)	k_{cat} (s ⁻¹)	Log (k_{cat}/K_m)	K_m (μM)	k_{cat} (s ⁻¹)	Log (k_{cat}/K_m)
K ⁺	379 ± 28	1287 ± 44	6.53	1069 ± 264	831 ± 120	5.89
NH ₄ ⁺	221 ± 30	522 ± 27	6.37	264 ± 25	870 ± 31	6.52
Rb ⁺	374 ± 76	892 ± 84	6.38	716 ± 139	686 ± 52	5.98
Cs ⁺	305 ± 84	164 ± 19	5.73	1009 ± 260	260 ± 30	5.41

Table S3. Kinetic constants for monovalent cations in WT-RMPK and in T113L-RMPK in the presence of saturating PEP³⁻ and ADP-Mg complex in 100% water. The data from Figure 2 were fitted to the Hill equation ($v = V_{max} \cdot S^n/K_{0.5}^n + S^n$) and to the Michaelis-Menten equation ($v = V_{max} \cdot S/K_m + S$) (Origin version 7.0). The standard deviations from two experiments are shown. k_{cat}/K values are expressed in log form.

M ⁺	WT				T113L			
	$K_{0.5}$ (mM)	k_{cat} (s ⁻¹)	n	Log ($k_{cat}/K_{0.5}$)	K (mM) ^a	k_{cat} (s ⁻¹)	n	Log (k_{cat}/K)
K ⁺	11.8 ± 0.9	1068 ± 40	1.4 ± 0.14	4.96	48 ± 10	1036 ± 108	-	4.33
NH ₄ ⁺	4.1 ± 0.4	698 ± 37	1.6 ± 0.25	5.23	7.84 ± 0.8	835 ± 39	1.37 ± 0.16	5.03
Rb ⁺	12 ± 0.8	617 ± 21	1.46 ± 0.12	4.71	32 ± 3.7	1111 ± 57	-	4.54
Cs ⁺	29.7 ± 8.4	240 ± 36	1.36 ± 0.28	3.91	26 ± 4.6	302 ± 32	-	4.06

^a K represents the $K_{0.5}$ and K_m for the data fitted to the Hill and Michaelis-Menten equations, respectively.

Table S4. Kinetic constants for PEP³⁻ in WT-RMPK and in T113L-RMPK in the presence of saturating concentrations of the ADP-Mg complex and of the monovalent cation indicated in 20% DMSO. Kinetic data (not shown) were fitted to the Hill ($v = Vmax \cdot S^n / K_{0.5}^n + S^n$) and Michaelis-Menten ($v = Vmax \cdot S / K_m + S$) equations (Origin version 7.0). The standard deviations from two experiments are shown. k_{cat}/K_{app} values are expressed in log form.

M ⁺	WT			T113L			
	K_{app} (μM) ^a	k_{cat} (s ⁻¹)	n	Log (k_{cat}/K_{app})	$K_{m(app)}$ (μM)	k_{cat} (s ⁻¹)	Log ($k_{cat}/K_{m(app)}$)
K ⁺	24 ± 1.1	858 ± 17	1.62 ± 0.12	7.56	44 ± 7.8	714 ± 46	7.22
NH ₄ ⁺	11.2 ± 1.5	439 ± 23	1.61 ± 0.34	7.59	28 ± 3.8	776 ± 30	7.44
Rb ⁺	13.8 ± 0.9	548 ± 12	1.87 ± 0.24	7.6	30 ± 3.4	616 ± 17	7.31
Cs ⁺	2.9 ± 0.6	162 ± 4	-	7.75	5.2 ± 0.98	204 ± 5	7.59

^a K_{app} represents the $K_{0.5(app)}$ and $K_{m(app)}$ for the data fitted to the Hill and Michaelis-Menten equations, respectively.

Table S5. Kinetic constants for the ADP-Mg complex in WT-RMPK and in T113L-RMPK in the presence of saturating PEP³⁻ and the monovalent cation indicated in 20% DMSO. Kinetic data (not shown) were fitted to the Michaelis-Menten equation ($v = Vmax \cdot S / K_{m(app)} + S$) (Origin version 7.0). The standard deviations from two experiments are shown. $k_{cat}/K_{m(app)}$ values are expressed in log form.

M ⁺	WT			T113L		
	$K_{m(app)}$ (μM)	k_{cat} (s ⁻¹)	Log ($k_{cat}/K_{m(app)}$)	$K_{m(app)}$ (μM)	k_{cat} (s ⁻¹)	Log ($k_{cat}/K_{m(app)}$)
K ⁺	99.7 ± 13.2	993 ± 39	7.00	252 ± 30	1131 ± 60	6.65
NH ₄ ⁺	71 ± 15	472 ± 23	6.82	124 ± 19	579 ± 26	6.67
Rb ⁺	83 ± 11	674 ± 28	6.91	156 ± 29	621 ± 39	6.6
Cs ⁺	44 ± 14	174 ± 10	6.6	73 ± 13	232 ± 10	6.5

Table S6. Kinetic constants for monovalent cations in WT-RMPK and in T113L-RMPK in the presence of saturating PEP³⁻ and ADP-Mg complex in 20% DMSO. The data from Figure 3 were fitted to the Michaelis-Menten ($v = Vmax \cdot S / K_m + S$) and to the Hill ($v = Vmax \cdot S^n / K_{0.5}^n + S^n$) equations (Origin version 7.0). The standard deviations from two experiments are shown. k_{cat}/K_{app} values are expressed in log form.

M ⁺	WT			T113L				
	K_{app} (mM) ^a	k_{cat} (s ⁻¹)	n	Log (k_{cat}/K_{app})	$K_{0.5(app)}$ (mM)	k_{cat} (s ⁻¹)	n	Log ($k_{cat}/K_{0.5(app)}$)
K ⁺	1.5 ± 0.18	889 ± 36	-	5.76	1.8 ± 0.4	587 ± 78	1.15 ± 0.10	5.50
NH ₄ ⁺	0.6 ± 0.09	432 ± 33	1.3 ± 0.19	5.84	0.92 ± 0.1	425 ± 33	1.81 ± 0.27	5.66
Rb ⁺	1.4 ± 0.23	640 ± 50	1.18 ± 0.16	5.67	1.8 ± 0.22	548 ± 35	1.3 ± 0.1	5.49
Cs ⁺	3.8 ± 0.6	198 ± 16	-	4.72	7.3 ± 2	216 ± 38	1.32 ± 0.19	4.47

^a K_{app} represents the $K_{0.5(app)}$ and $K_{m(app)}$ for the data fitted to the Hill and Michaelis-Menten equations, respectively.

Figure S1. Stick model of the water-protein and water-water interactions in the vicinity of O γ of T113 of pyruvate kinase from rabbit muscle. The dashed lines show hydrogen bond distances. Water molecules (red spheres) are illustrated. This figure was constructed from the coordinates of PDB 2G50 [10] using the program, PYMOL v0.99.

