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Structural basis for antibiotic action of the B1 antivitamin 2'-methoxy-thiamine

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Supplementary Tables

Supplementary Table 1. Kinetic and thermodynamic constants of *E. coli* transketolase reconstituted with either ThDP or MThDP.

	Steady-state kinetic constants [a]			*Cofactor activation* [b]	*Cofactor binding* [c]	
	k_{cat} [s^{-1}]	K_m^{app} [mM]	k_{cat}/K_m^{app}	k_{obs} [s^{-1}]	K_D cofactor [µM]	K_m^{app} [µM]
	X5P+R5P	X5P	[$s^{-1}mM^{-1}$]	H/D-Exchange	Fluorescence	Activity
*Ec*TK$_{ThDP}$	51.3 ± 0.4	0.34 ± 0.01	150.9	313 ± 41	0.23 ± 0.01	2.0 ± 0.2
*Ec*TK$_{MThDP}$	1.9 ± 0.1	0.36 ± 0.06	5.3	2.9 ± 1.1	0.09 ± 0.02 [d]	21.9 ± 2.2
	(27-fold↘)	(no change)	(28-fold ↘)	(108-fold↘)	(2.5-fold↘)	(11-fold ↗)
					13.39 ± 1.99 [d]	
					(58-fold ↗)	

[a] macroscopic steady-state kinetic constants k_{cat}, K_m^{app} (X5P) and k_{cat}/K_m^{app} were determined using an enzymatic activity assay (conversion of donor X5P and acceptor R5P to products S7P and G3P)

[b] k_{obs} of H/D-exchange at C2 of enzyme-bound cofactor as a measure of cofactor activation was derived from rapid quench-flow/^{1}H NMR spectroscopy

[c] equilibrium binding constants for ThDP and MThDP were estimated by both fluorescence quenching experiments as well as by steady-state enzymatic activity assay (see above) using different cofactor concentrations in the assay mixture

[d] two different binding regimes were observed in the fluorescence quenching experiments indicating a high-affinity binding (20% amplitude) and a major medium-affinity (80% amplitude) binding site or species

Supplementary Table 2. Data collection and refinement statistics.

	*Ec*TK MThDP resting state [#]	*Ec*TK MThDP Michaelis complex with X5P [#]
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	89.6 101.9 133.0	89.8, 102.1, 133.1
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	50 – 0.92 (0.95-0.92)*	50 – 0.95 (1.03 – 0.95)*
R_{merge}	9.5 (61.4)	8.1 (88.6)
$I / \sigma I$	9.73 (2.22)	12.46 (2.42)
Completeness (%)	93.8 (61.6)	98.9 (96.6)
Redundancy	4.2 (2.9)	6.1 (5.0)
Refinement		
Resolution (Å)	47.29 – 0.92	47.66 – 0.95
No. reflections	780599	751409
R_{work} / R_{free}	9.48 / 10.64	9.86 / 11.02
No. atoms		
Protein	6290/6201	6187/6202
Ligands (MThDP/X5P)	39/39	53/53
Water	1638	1558
Ligands (other)	180	146
B-factors (Å^2)		
Protein	9.1/8.8	9.1/9.0
Ligands (MThDP/X5P)	8.9	9.2
Water	23.0	23.3
Ligands (other)	14.1	14.9
R.m.s. deviations		
Bond lengths (Å)	0.008	0.008
Bond angles (°)	1.44	1.21

[#] Single crystal.

*Values in parentheses are for highest-resolution shell.

Supplementary Table 3. Enzymatic activities and cofactor affinities of *E. coli* and human ThDP enzymes with genuine ThDP and antivitamin-derived MThDP (*E. coli* and human TK: this study; all other enzymes: reference 11). Note that strong inhibition with relative activities <10% (highlighted in red) is exclusively observed for *E. coli* enzymes, while human enzymes retain high enzymatic activities with MThDP.

	Relative activity with MThDP [#]	Binding constant K_D (ThDP) in µM	Binding constant K_D (MThDP) in µM
***E. coli* enzymes**			
TK	3-4% [a]	0.23 ± 0.01	0.09 ± 0.02
			13.39 ± 1.99
PDHc E1	6-11% [a]	7.0 ± 0.9	2.7 ± 0.6
OGDHc E1	74-80% [b]	tight binding [c]	n.d.[d]
DXPS	9-14% [a]	3.5 ± 0.1	6.2 ± 0.2
Human enzymes			
TK	97% [b]	tight binding [c]	n.d.[d]
PDHc E1	50-75% [a]	7.2 ± 0.5	16.2 ± 4.5
OGDHc E1	89% [b]	tight binding [c]	n.d.[d]

Abbreviations: TK, transketolase; PDHc, pyruvate dehydrogenase complex; OGDHc, oxoglutarate dehydrogenase complex; DXPS, 1-deoxy-D-xylulose 5-phosphate synthase

[#] enzymatic activity after reconstitution with [a] or in presence of MThDP [b] relative to activity with genuine cofactor ThDP

[a] reconstituted with MThDP

[b] in presence of MThDP

[c] no true equilibrium binding constant can be estimated due to quasi-irreversible binding

[d] not determined

Supplementary Table 4. Predicted and experimental binding affinity differences (ΔΔG, in kcal/mol) between ThDP and MThDP.

	***Ec*TK**	***Hs*TK**	***Ec*PDH**	***Hs*PDH**
Predicted ΔΔG	+3.10 ± 0.10	+0.14 ± 0.15	−0.08 ± 0.10	−0.53 ± 0.29
Experimental ΔΔG	+2.40 [a]	n.a.[b]	−0.82 [a]	+0.48 [a]

[a] A positive ΔΔG value indicates that MThDP has lower affinity than ThDP for the specified enzyme, while a negative ΔΔG value indicates that MThDP has higher affinity. For predicted ΔΔG values, the mean and its standard error are reported.

[b] The affinity of *Hs*TK for MThDP is unknown.

Supplementary Table 5. Results of the binding free energy calculations used for validation.

Force Field	Protein	Transformation	$\Delta\Delta G_{exp}$	$\Delta\Delta G_{calc}$	$\Delta\Delta G_{calc}$ (restrained)
Amber	*Ec*TK	ThDP → MThDP	+2.40	+0.36 ± 0.21	+3.10 ± 0.10
Amber	*Ec*TK	ThDP → TP1[a]	−0.05	+0.36 ± 0.13	+0.87 ± 0.12
Amber	*Ec*TK	ThDP → TP2[a]	−1.08	−0.69 ± 0.14	−0.17 ± 0.14
Amber	*Ec*TK	WT → E411A[a]	−0.30	+0.09 ± 0.27	+0.23 ± 0.14
Amber	*Ec*PDH	ThDP → MThDP	−0.82	−0.18 ± 0.07	−0.08 ± 0.10
Charmm	*Ec*TK	ThDP → MThDP	+2.40	−0.18 ± 0.15	+0.93 ± 0.05
Charmm	*Ec*TK	ThDP → TP1	−0.05	+0.58 ± 0.12	+0.59 ± 0.06
Charmm	*Ec*TK	ThDP → TP2	−1.08	+0.40 ± 0.11	+0.99 ± 0.10
Charmm	*Ec*TK	WT → E411A	−0.30	+0.66 ± 0.32	+0.13 ± 0.09
Charmm	*Ec*PDH	ThDP → MThDP	−0.82	−0.36 ± 0.08	−0.57 ± 0.04

ThDP: *thiamine diphosphate*; MThDP: 2'-*methoxythiamin diphosphate*; TP1: *4'-desamino ThDP*; TP2: *N3'-pyridyl ThDP*; WT: *wild-type.*

Scatter plots of these results are shown in Extended Data Fig. 6a-d. The Amber99sb*-ILDN/GAFF(v2.1) force field is referred to as "Amber", and the Charmm36/CGenFF(v3.0.1) force files is referred to as "Charmm". All ΔΔG values are in kcal/mol. For the calculated ΔΔG values, the mean and standard error are reported.

[a] data taken from: Asztalos, P., *PhD thesis*, 2008, Martin-Luther University Halle-Wittenberg, Germany