Supplementary Material

Functional Impact of the N-terminal Arm of Proline Dehydrogenase from *Thermus thermophilus*

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Table S1. Raw kinetic data of the Proline:DCPIP assay of EE, ΔA, ΔAB, ΔABC and helix αC variants V32D, Y35F and V36D. Each data point provided here is the average of three measurements. This kinetic data was used to determine the kinetic parameters K_m and k_{cat}, which are provided in Table 2.

	EE	**ΔA**	**ΔAB**	**ΔABC**
Proline (mM)	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})
0.5	0.07 ± 0.002	0.08 ± 0.018	0.14 ± 0.005	0.00 ± 0.000
2.5	0.33 ± 0.002	0.29 ± 0.012	0.64 ± 0.008	0.00 ± 0.000
5	0.65 ± 0.015	0.56 ± 0.016	1.16 ± 0.020	0.03 ± 0.002
10	1.36 ± 0.069	1.08 ± 0.000	2.06 ± 0.069	0.06 ± 0.002
20	2.11 ± 0.085	1.90 ± 0.044	3.53 ± 0.060	0.11 ± 0.009
35	3.01 ± 0.129	2.91 ± 0.038	4.90 ± 0.066	0.15 ± 0.004
50	4.06 ± 0.262	3.89 ± 0.008	6.20 ± 0.307	0.20 ± 0.001
75	5.54 ± 0.242	4.94 ± 0.043	7.59 ± 0.027	0.25 ± 0.009
100	6.22 ± 0.357	5.66 ± 0.100	8.38 ± 0.234	0.30 ± 0.014
150	6.89 ± 0.445	7.06 ± 0.274	10.09 ± 0.573	0.37 ± 0.011
200	7.00 ± 0.406	8.08 ± 0.121	10.38 ± 0.219	0.42 ± 0.010

	V32D	**Y35F**	**V36D**
Proline (mM)	k_{obs} (s^{-1})	k_{obs} (s^{-1})	k_{obs} (s^{-1})
0.5	0.01 ± 0.003	0.09 ± 0.009	0.00 ± 0.001
2.5	0.03 ± 0.005	0.35 ± 0.019	0.01 ± 0.002
5	0.05 ± 0.003	0.66 ± 0.017	0.02 ± 0.002
10	0.10 ± 0.010	1.16 ± 0.004	0.02 ± 0.003
20	0.20 ± 0.008	1.88 ± 0.183	0.03 ± 0.003
35	0.33 ± 0.009	2.82 ± 0.087	0.03 ± 0.005
50	0.45 ± 0.009	3.34 ± 0.137	0.04 ± 0.002
75	0.63 ± 0.020	3.95 ± 0.257	0.04 ± 0.003
100	0.75 ± 0.002	5.14 ± 0.489	0.05 ± 0.002
150	1.01 ± 0.042	6.94 ± 0.836	0.06 ± 0.001
200	1.26 ± 0.029	7.93 ± 0.445	0.07 ± 0.001

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TtProDH      MNLDLAYRSFVLGVAGHPQVERLIKHRAKGLVRRYVAGETLEEALKAAEALEREGVHAIL
DrProDH      -MIDQLYRKAVLTVAERPQVEQLARQKMWNLAERFVAGESIESAIQAVQALERDGIAGNL
BjPutA       ---------------------------------QFVLGEDIRAAMKRASGMEQKGYTYSY
GsPutA       ------------------------------------VGETTKEAVKNLEKLRKDGFAAVV
                                                  **  . *::  . :.:.*

TtProDH      DLLGEMVRTEEEARAFQRGLLELVWALAGKP----------WPKYI----------SLKL
DrProDH      DLLGEFIDSPAKCTEFADDVIKLIEAAHAAG----------IKPYV----------SIKL
BjPutA       DMLGEAARTDADAMRYHAAYGRAIEAIAKGCGSEDVR----RNPGISV----------KL
GsPutA       DVLGEATLSEEEAEVYTNTYLELLEALKKEQGSWKGLPGKGGDPGLDWGHAPKVNIAVKP
             *:***   :  ..  :     . : *                   :            *

TtProDH      TQLGLDL-------SEDLALALLREVLREAEPRGVFVRLDMEDSPRVEATLRLYRALR--
DrProDH      SSVGQGKD----ENGEDLGLTNARRIIAKAKEYGGFICLDMEDHTRVDVTLEQFRTLVG-
BjPutA       SALHPRYEEAQKDRVTAELYPRLRDLALMARGRRMGLNIDAEEADRLSISMDLIERLMRD
GsPutA       TALFCLANPQDFEGSVVAILDRMRRIFKKVMELNGFLCIDMESYRHKEIILEVFRRL--K
             : :                    * :   .      : :* *.  : .  :   . *

TtProDH      --EEGFSQVGIVLQSYLYRTEKDLLDLLP------YRPNLRLVKGAYREPKEVA------
DrProDH      --EFGAEHVGTVLQSYLYRSLGDRASLDD------LRPNIRMVKGAYLEPATVA------
BjPutA       PGLAGWDGLGVVVQAFGQRCGAVLEYLHALAEETDRRIMVRLVKGAYWDTEIKLAQVEGL
GsPutA       LEYRDYPHLGIVLQAYLKDNDKDLDDLLAWAKEHKVQISVRLVKGAYWDYETVKAKQN-D
                 .   :* *:*::          *         :  :*:***** :

TtProDH      -----FPDKRLIDAEYLHLGK--LALKEGLYVAFATHDPRIIAELKRYTEAMGIPRSRFE
DrProDH      -----YPDKADVDQNYRRLVF--QHLKAGNYTNVATHDERIIDDVKRFVLAHGIGKDAFE
BjPutA       AGFPVFTSKPATDVSYIANARRLLNMTDRIYPQFATHNAHTVAAILDMAE----DKGSFE
GsPutA       WEVPVWTIKAESDAAYERQARKILENHQICHFACASHNIRTISAVMEMARELNVPEDRYE
                  :  *   *  *              :   *:*: : :  :   .      .. :*

TtProDH      FQFLYGVRPEEQR-RLAREGYTVRAYVPYGR--DWYPYLTRRIAERPENLLLVLRSLVSG
DrProDH      FQMLYGIRRDLQK-QLAAEGYRVRVYLPYGR--DWYAYFSRRIAETPRNAAFVVQGMLKG
BjPutA       FQRLHGMGEALHDIVLKSEGTRCRIYAPVGAHKDLLAYLVRRLLE---------------
GsPutA       FQVLYGMAEPVRKGILKVAG-RIRLYAPYGNMVPGMGYLVRRLLENTANE----------
             ** *:*:    :   *   *   * * * *       *: **: *
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Figure S1. Sequence alignment of TtProDH, DrProDH, and the ProDH domains of BjPutA and GsPutA. The alignment was generated using Clustal Omega [1, 2]. The protein sequences were retrieved from the UniProt Knowlegdebase (UniProtKB). Sequence accession numbers: TtProDH: Q72IB8, DrProDH: Q9RW55, BjPutA: A0A0H4LBV6, GsPutA: Q746X3. For BjPutA and GsPutA, only the sequence comprising the ProDH domain was used (BjPutA: residues 172 – 465 [3], GsPutA: residues 121 – 430 [4]). In green, the conserved Arg-Glu ion pair (Arg288-Glu65 in TtProDH), which is suggested to act as an active site gate, is shown.

References

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