# Structural and Kinetic Isotope Effect Studies of Nicotinamidase (Pnc1) from *S. cerevisiae*

Brian C. Smith, Mark A. Anderson, Kelly A. Hoadley, James L. Keck, W. Wallace Cleland, and John M.

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### SUPPORTING INFORMATION

## **Contents:**

- Supporting Discussion. Derivation explaining observed kinetic isotope effects for Pnc1 D51N.
- Table S1. Primers used in Pnc1 mutagenesis.
- Figure S1. Double-reciprocal inhibition plots.
- Figure S2. Multiple-sequence alignment of nicotinamidases.

#### **Supporting Discussion**

*Derivation explaining observed kinetic isotope effects for Pnc1 D51N.* For Pnc1 D51N, both the  $^{15}$ N and  $^{13}$ C KIE are ~0.5% compared to ~1.25% for those of Pnc1 WT (Table 3). We believe this is due to an increase in one of the back reaction off rates. A truncated derivation demonstrating how this is possible is shown below. The entire reaction is described by equation S1.

$$E+A \xrightarrow{k_1} ES \xleftarrow{k_3} ES \xleftarrow{k_5} EX \xleftarrow{k_7} EQ^* \xleftarrow{k_9} EQ \xrightarrow{k_{11}} E+Q \quad (S1)$$

The KIE are on *V/K* which includes all steps up through and including the first irreversible step where  $k_1$  is substrate binding,  $k_2$  is substrate dissociation,  $k_3$  is the formation of the tetrahedral intermediate,  $k_4$  is the breakdown of the tetrahedral intermediate to reform nicotinamide, and  $k_5$  is the isotope sensitive irreversible step (C–N bond cleavage and the loss of NH<sub>3</sub>). *V/K* is expressed by:

$$\frac{V}{KE_{t}} = \frac{\frac{k_{1}k_{3}k_{5}}{k_{2}k_{4}}}{1 + \frac{k_{5}}{k_{4}} + \frac{k_{5}k_{3}}{k_{4}k_{2}}}$$
(S2)

However when the kinetic data are examined we see the  $k_{cat}$  and  $k_{cat}/K_m$  values have decreased compared to the wild-type enzyme. This is due to a decrease in binding of nicotinamide in the D51N mutant which leads to an increase in  $k_2$  and a decrease in  $k_3$ . As such  $k_3/k_2$  would tend to be very small and can be ignored. Then using the <sup>15</sup>N KIE notation the equation becomes:

$${}^{15}\left(\frac{V}{K}\right) = \frac{{}^{15}k_5 + \frac{k_5}{k_4}}{1 + \frac{k_5}{k_4}}$$
(S3)

where  ${}^{15}\left(\frac{V}{K}\right)$  is the measured KIE and  ${}^{15}k_5$  is the intrinsic KIE.  ${}^{15}\left(\frac{V}{K}\right)$  is defined as

 $\frac{{}^{15}k_5 + C_f + {}^{15}K_{eq}}{1 + C_f + C_r}$  where C<sub>f</sub> and C<sub>r</sub> are forward and reverse commitments to catalysis and {}^{15}K\_{eq} is the {}^{15}N

equilibrium isotope effect. In this reaction there is no reverse commitment since the C-N bond cleavage is irreversible and thus  $C_r$  and  ${}^{15}K_{eq}$  can be disregarded and:

$$^{15}\left(\frac{V}{K}\right) = \frac{^{15}k_5 + C_f}{1 + C_f}$$
 (S4)

For this example, an intrinsic isotope effect of 3% will be assumed and then for wild type and D51N we have:

$$1.0122 \pm 0.0002 = \left(\frac{1.0300 + C_{\text{fwt}}}{1 + C_{\text{fwt}}}\right)$$
, and  $C_{\text{fwt}} = 1.50 \pm 0.04$  (S5)

$$1.0045 \pm 0.0006 = \left(\frac{1.0300 + C_{\text{fDSIN}}}{1 + C_{\text{fDSIN}}}\right)$$
, and  $C_{\text{fDSIN}} = 5.50 \pm 0.82$  (S6)

Equation 12 shows that  $C_f = \frac{k_5}{k_4}$  and so the  $C_f$  values are  $\left(\frac{k_5}{k_4}\right)_{WT} \sim 1.5$  and  $\left(\frac{k_5}{k_4}\right)_{Mut} \sim 5.5$ 

These values show that the reaction partitions forward faster in the D51N mutant, resulting in a smaller KIE. The mechanism of this partitioning is unknown but indicates that  $k_5$  (loss of NH<sub>3</sub>) has increased or that breakdown of the tetrahedral intermediate ( $k_4$ ) has decreased.

Pnc1 mutant	Primers
D8A	5′-CGAGATGAAGACTTTAATTGTTGTTGCTATGCAAAATGATTTTATTTCACC-3′
	5′-GGTGAAATAAAATCATTTTGCATAGCAACAACAATTAAAGTCTTCATCTCG-3′
D8N	5′-CGAGATGAAGACTTTAATTGTTGTTAATATGCAAAATGATTTTATTTCACC-3′
	5′-GGTGAAATAAAATCATTTTGCATATTAACAACAATTAAAGTCTTCATCTCG-3′
D8E	5′-CGAGATGAAGACTTTAATTGTTGTTGAGATGCAAAATGATTTTATTTCACC-3′
	5′-GGTGAAATAAAATCATTTTGCATCTCAACAACAATTAAAGTCTTCATCTCG-3′
D51A	5'-GTGGTCACCAGAGCTTGGCACCCTTCC-3'
	5'-GGAAGGGTGCCAAGCTCTGGTGACCAC-3'
D51N	5'-GTGGTCACCAGAAATTGGCACCCTTCC-3'
	5'-GGAAGGGTGCCAATTTCTGGTGACCAC-3'
H53A	5'-GGTCACCAGAGATTGGGCCCCTTCCAGAC-3'
	5'-GTCTGGAAGGGGCCCAATCTCTGGTGACC-3'
H94A	5'-TGTGGCCCGTAGCCTGTGTGAAAAACACC-3'
	5'-GGTGTTTTTCACACAGGCTACGGGCCACA-3'
K122A	5'-GATTGTCGACGCGGGTTTCTTGACTGACC-3'
	5'-GGTCAGTCAAGAAACCCGCGTCGACAATC-3'
K122R	5'-GATTGTCGACAGGGGTTTCTTGACTGACC-3'
	5'-GGTCAGTCAAGAAACCCCTGTCGACAATC-3'
C167A	5'-AGCTTTGGAGTATGCTGTCAAAGCCACCG-3'
	5'-CGGTGGCTTTGACAGCATACTCCAAAGCT-3'

Table S1.         Primers used in Pnc1 mutagenesis	••
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**Figure S1.** Double-reciprocal inhibition plots. Initial rates were determined as described under Experimental Procedures. (left) Nicotinic acid exhibits competitive inhibition toward nicotinamide during the Pnc1-catalyzed reaction. The following nicotinic acid concentrations were used: 0 ( $\bullet$ ), 200 ( $\bullet$ ), 400 ( $\bullet$ ), 800 ( $\bullet$ ), and 1200 µM ( $\bullet$ ). (right) Nicotinaldehyde exhibits competitive inhibition toward nicotinamide during the Pnc1-catalyzed reaction. The following nicotinaldehyde concentrations were used: 0 ( $\bullet$ ), 1 ( $\bullet$ ), 2 ( $\bullet$ ), 4 ( $\bullet$ ), and 6 µM ( $\bullet$ ). Data were fit to competitive inhibition models using KinetAsyst as described in Experimental Procedures. All reactions were performed at pH 7.5.

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M. tuberculosis PncA	19	LΑ	V T	G	GΑ	Α-				L.	A F	₹A	1.5	5 D	ΥL	. A	ΕA	AC	)Y-	·н	Н١	/ V /	ΑT	ĸ	F	H I	DP	G	DΗ	FS	G	ΓР				62
S. pneumoniae PncA	21	LT	ΑG	ΞA	ΡA	Q -			- A	A I	S C	D A	1.5	5 K	VТ	R	LΑ	FE	RQ	G D	ΥI	F	FΤ	1	A I	I E	ΕN	D	CF	ΗP	Ε·					64
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C. elegans Pnc1a	109	LΚ	10	ΞD	G D	AG	δQ	ΕP	S 5	5 A	ΙT	Р	LN	ΙE	LL	Q	LS	SV	VD-		L١	۷ v	ΥT	КΙ	)WI	ΗP	ΗN	н	IS	FL	. s (	QΑ	ΗN	SE	۶R۱	/ 163
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S cerevisiae Pnc1	120	vn		S F	ιт	DR	F	v v	S A	A F	нг	211	w N	JF	нк	т			Y		K F	чн.	гр	F \	/ Y	iv	G V		IF	Y C	v	< A	ТА	1 9		A 176
C elegans Prc1h	198	I M		ŝv	DP	YI	D	s v	S A	A F	ΝГ	) N	NO	R	S K	т	FI	FI	) I		RF	= N	ם ו	Δ \	, v	ΙA	GI		v D	ic	v	R F	тс	I F		/ 254
C elegans Prc2	190	IK	KC	5 A	סע	YV	, D.		S A	A F	SI	2 N	c c	5 1	ĸc	ŝ	FI	FA			ĸ		IN	Δ \	/ 1 0	30	GI		ע י	ic	v	йн	ті	кг		\$ 246
C elegans Prc1a	218	I M		5 V	DP	v i	D	s v	S A	A F	ΝГ	2 N	NO	R	S K	т	FI	FI	21		RF	= N	חו	Δ	, v		GI		י ח י	ic	v	R F		1 1		1 274
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M. tuberculosis PncA	148	RN	١G L	L A	ΤR	νL	. v	DL	ΤA	٩G	VS	5 -	- /	۱D	ТΤ	v	A A	LI	ΕĒ	ИR	Т	۱S ۱	VΕ	L١	/ C	s s										186
S. pneumoniae PncA	146	ΝL	GY	í D	ΙE	ιv	/ к	ΡA	V A	٩S	IV	V -	- F	ΡE	Νŀ	١Q	FΑ	LO	GΗ	FΚ	NT	L	G A	к	. V I	ΣE	ΝL	N	ΕL	SE						191
A. baumannii PncA	169	ΚQ	GF	FK	ΤL	V I	E	D A	C١	(G	1 0	<b>)</b> -	LN	١G	SΙ	Ε.	Q A	wo	2 T I	NQ	QC	QG	v v	R	Q	sт	DL	. L I	ΝE	C -						214
S. cerevisiae Pnc1	177	ΕL	GY	Υ K	ΤТ	٧L	. L	DΥ	ΤF	۲P	1 9	S D	DF	ΡE	V I	Ν	кν	КΙ	ΕE	LK	Ał	1 N	I N	٧V	DI	< -										216
C. elegans Pnc1b	255	ΚQ	N F	FL	ΑA	vı	P	ΕC	s A	٩G	LT	Г-	- 1	К	GΙ	Е	ΕS	ΕN	ΛA	FΚ	кс	QG	V A	м	S	< D	ΕA	R	GΙ	ΤE	G	GΕ	LΡ	RI	w١	V 309
C. elegans Pnc2	247	КН	i G F	FL	т с	ΞV	/ к	S G	S١	(G	LS	5 -	- 9	ΣL	ΚN	۱D	ΕA	N	(M	FQ	ΚF	R G I	V A	Т	D	ΣE	M A	Q	LI	SR	R	ΕA	FΡ	11	w	I 301
C. elegans Pnc1a	275	ΚQ	N F	FL	ΑA	vı	P	ЕC	S A	٩G	LT	Г-	- 1	к	GΙ	Е	ΕS	ΕN	ΛA	FΚ	кс	QG	V A	м	S	< D	ΕA	R	GΙ	ТЕ	G	GΕ	LΡ	RI	w	V 329
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**Figure S2.** Multiple-sequence alignment of the nicotinamidases for which structures have been solved from *Pyrococcus horikoshii* (PDB entries 1ILW and 1IM5), *Mycobacterium tuberculosis* (PDB entry 3PL1), *Streptococcus pneumoniae* (PDB entries 3O90, 3O91, 3O92, 3O93, and 3O94), *Acinetobacter baumannii* (PDB entries 2WT9 and 2WTA), and *Saccharomyces cerevisiae* (PDB entry 2H0R and this work). Eukaryotic nicotinamidases from *Caenorhabditis elegans* and *Drosophila melanogaster* are also shown for comparison. The alignment was generated using ClustalW (*59*).