Supporting Information

for

Critical involvement of the E373-D434 region in the acid sensitivity of a NhaB-type Na^+/H^+ antiporter from *Vibrio alginolyticus*

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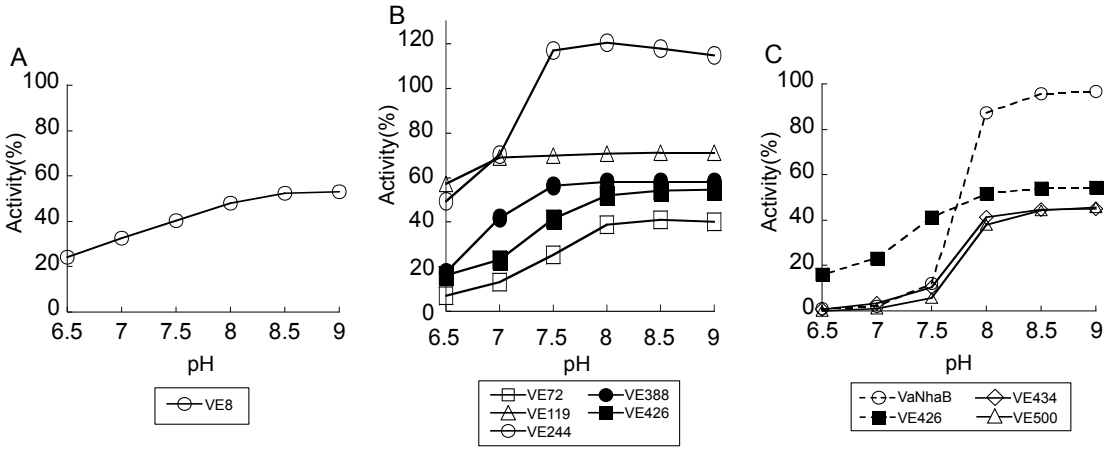

Figure S1. Normalized pH profiles of VE chimeras.
Antiport activities of chimeric proteins at each pH were normalized to the amount of each chimeric protein in membrane vesicles, using the amount of VaNhaB protein as 100% (A, B and C). Amount of each protein was calculated from results of several Western blot analyses using the ImageJ software (Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/).

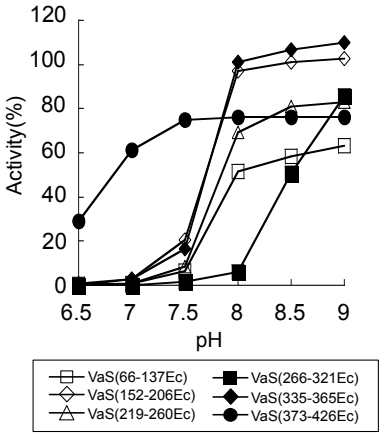

Figure S2. Normalized pH profiles of VaS chimeras.
Normalizations of the activities were performed as in the Legend for Figure S1. Note that results of VaS(1-40Ec) and VaS(479-513Ec) proteins were not included in this figure due to their significantly lower amount in the membrane vesicles.

Table S1. Oligo-DNA primers used in this study.

		Sequence	length(b)
EcNhaB	F	cccgggaggaggggatccatggagatctcctggggccgcgc	41
	R	gtcgactcactcgagaagcgttgctatccagcccatttgc	40
VaNhaB	F	CCCGGGAGGAGGGGATCCATGCCGATATCGCTCGGAAACGC	41
	R	GTCGACTCACTCGAGGTGACCGCCGGAGACTACGGGAGT	39
VE8	F	CCCGGGAGGAGGGGATCCATGCCGATATCGCTCGGAAACGCTctatggcgcaatttttt ggg	62
VE72	F	ATTACAACCTGGTGGTTTACTTgctatcgaagcggtattcatcggcatgacc	52
	R	atgaataccgcttcgatagcAAGTAAACCACCAGGTTGTAATGGGTAGC	49
VE119	F	AGCATTTATTGCTGTTCATTTTTACCcgtttgctgttaagcattcgctccaaaatgctgc	56
	R	ttggagcgaatgcttaacagcaaacgGGTAAAAATGAACAGCAATAAATGCTTC	54
VE244	F	TTGGTTATTTGGTGAATTCCTAATCCGTatgtcgccggtgaccgttccggttctg	55
	R	agaaccggaacggtcaccggcgacatACGGATTAGGAATTCACCAAATAACCAACCCGC	59
VE388	F	ACGCGGTGCTTGCCGTTGAAGATAAAgctcagctgtcgctgttctatattttcaacggtc	60
	R	aaaatatagaacagcgacagctgagcTTTATCTTCAACGGCAAGCACCGCGTCGATAAC	59
VE426	F	AAGTAAAAACAGCACTCATTGAGggcgctatcacgttgaagcaatacgagctg	53
	R	tattgcttcaacgtgatagcgccCTCAATGAGTGCTGTTTTTACTTCATTG	51
VE434	F	TCATTACTCGCGAGCAGTTCGACctgctggcggtcgccattaataccggtacc	53
	R	accggtattaatggcgaccgccagcagGTCGAACTGCTCGCGAGTAATGAGACCCTC	57
VE500	F	TCTAATGGGCATTATGTTCTTCCTAgcccctgtaaccgaatggtttatgcaaatgggctggatagc aacgcttctcgagtgagtcgac	88
	1R	gtcgactcactcgagaagcgttgctatccagcccatttgcataaaccattcggttacaggggcTAGGAAG AACATAATGCCCATTAGA	88
	2R	ataaaccattcggttacaggggcTAGGAAGAACATAATGCCCATTAGACCGAC	57
VaS	F	TCATTGAGGGTgctATTACTttgaagCAGtacgagTTACTGGCAGTTGCTATCAACACTG GTACTAACC	68
	R	ACTGCCAGTAActcgtaCTGcttcaaAGTAATagcACCCTCAATGAGTGCTGTTTTTACT TCATTG	66
EcS	F	aatggaaagtggcCTCatcacgCGCGAGcaaTTCGACctgctggcggtcgccattaataccggtacc	67
	R	ccgccagcagGTCGAAttgCTCGCGcgtgatGAGgccactttccattgccgctttcgcttcgttg	65
VaS(1-40Ec)	F	cccgttaattttcctcatcagcCCATTTGTTGCTGGGTGGTTGCTGGTTG	52
	R	GCAACCACCCAGCAACAAATGGgctgatgaggaaaattaacgggtttacgattaa	55
VaS(66-137Ec)	1F	GGCATTAAAATGCTACCCATTActccccggtggtctgttggctatcgaagc	51
	2F	tgctgtcgctctcttttgcgtgGCCGCTGCATTTTTATCTGCGTTTTTGG	51
	1R	cgatagccaacagaccaccggggagTAATGGGTAGCATTTTAATGCCATTGCAAG	53
	2R	CGCAGATAAAAATGCAGCGGCcacgcaaaaagagagcgacagcagcattttgg	53
VaS(152-206Ec)	1F	TGCGTTTTTGGATGCTTTGACGGTTgtggcggtggtgatcagcgttgcagtcgg	54
	2F	ccgtggctttctgcgtagcctgatgATGCACGCTGGTGTAGGTACTGCGTTAGG	54
	1R	tgcaacgctgatcaccaccgccacAACCGTCAAAGCATCCAAAAACGCAGAT	52
	2R	CGCAGTACCTACACCAGCGTGCATcatcaggctacgcagaaagccacggaactg	54
VaS(219-260Ec)	1F	TAGGTACTGCGTTAGGTGGCGTAatgaccatggtgggcgaaccacagaacct	52
	2F	atttgtggcctgttaacctgcctgCTAGTCGAAAAGCTTAAAGTGTTTGGCTAC	54

	1R	ctgtggttcgcccaccatggtcatTACGCCACCTAACGCAGTACCTACACC	52
	2R	AAACACTTTAAGCTTTTCGACTAGcaggcaggttaacaggccacaaatcag	51
VaS(266-321Ec)	1F	ACTTGTGCTCTAGTCGAAAAGCTTcgttggtttggctacggtgaaacgctgcc	53
	2F	tgcgctggcgttgcatctggcggaaGTAGGCTTAATTGGTCTTTCAGTGATCATC	55
	1R	gcgtttcaccgtagccaaaccaacgAAGCTTTTCGACTAGAGCACAAGTGATGAG	55
	2R	TCACTGAAAGACCAATTAAGCCTACttccgccagatgcaacgccagcgcagtcacc	56
VaS(335-365Ec)	1F	TCTTTCAGTGATCATCTTAGCGACGtcattgaccggtgtcaccgatgagcatgc	54
	2F	actgttgacggtgtttttctcggtaGTTGCGGTAATCATCGACCAAGAGTTG	52
	1R	tcatcggtgacaccggtcaatgaCGTCGCTAAGATGATCACTGAAAGACC	50
	2R	TCTTGGTCGATGATTACCGCAACtaccgagaaaaacaccgtcaacagtgcgg	52
VaS(373-434Ec)	1F	TATCGTTGCGGTAATCATCGACCAAcaactgttttcgccaattattcagtttgtg	55
	2F	cgctatcacgttgaagcaatacgagTTACTGGCAGTTGCTATCAACACTGGTAC	54
	1R	tgaataattggcgaaaacagttgTTGGTCGATGATTACCGCAACGATAGAG	51
	2R	AGTGTTGATAGCAACTGCCAGTAActcgtattgcttcaacgtgatagcgccac	53
VaS(479-513Ec)	1F	TCGTCTGTCTTACGGTAGAATGGTTtggatggccctgccttacaccctcgtcctg	55
	2F	gcaaatgggctggatagcaacgcttAGTGGTGATCTGACTCCCGTAGTCTCCGGC	55
	1R	agggtgtaaggcagggccatccaAACCATTCTACCGTAAGACAGACGAATTAATGG	56
	2R	AGACTACGGGAGTCAGATCACCACTaagcgttgctatccagcccatttgcataaacc	57
VE8(371-431Va)	1F	tcgcggtgattatcgaccaaGAGTTGTTTAAACCAGTTATCGACGCGGTGC	51
	2F	ACTCGCGAGCAGTTCGACTTActggcggtcgccattaataccggtaccaatc	52
	1R	TAACTGGTTTAAACAACTCttggtcgataatcaccgcgactaccgag	47
	2R	accggtattaatggcgaccgccagTAAGTCGAACTGCTCGCGAGTAATGAGACCCTC	57
2HA	F*	GATCCGTCGACCTCGAGTACCCTTACGATGTTCCAGATTATGCTaacggcTACCCATAT GATGTACCAGATTATGCTtgaCTGCA	85
	R*	GtcaAGCATAATCTGGTACATCATATGGGTAgccgttAGCATAATCTGGAACATCGTAA GGGTACTCGAGGTCGACG	77

Restriction enzyme recognition sites are underlined, and sequences derived from VaNhaB and EcNhaB are shown as upper case and lower case, respectively.
* Oligo nuculeotide linkers used for construction of pHG165-2HA.