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Does VDAC2 have a BH3 Domain For Binding Bax?

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1. Introduction

Mitochondrial outer membrane permeabilization (MOMP) by Bax oligomerization triggers apoptosis. BCL-2 family proteins control apoptosis through their agonist or antagonistic effects on Bax, which is mediated by their conserved BH3 domain. All BH3 domains form an alpha helix containing 5-7 conserved hydrophobic residues and once conserved aspartic acid residue that drive interaction with a canonical hydrophobic groove in Bax and other 'multi-domain' BCl-2 members. BH3 agonists induce Bax oligomerization, whole BH3 antagonists sequester Bax to prevent MOMP. We discovered that voltage dependent anion channels (VDACs) in the MOM contain a putative BH3-like domain. This study aimed to determine if the VDAC2 isoform contains a functional BH3 domain that binds recombinant Bax in a manner similar to the Bim BH3 domain.

2. Methods

2a. 110 nM rBax or vehicle + cF-labeled fluorescent peptide: 60 mins, 4°C in 0.5% octylglucoside

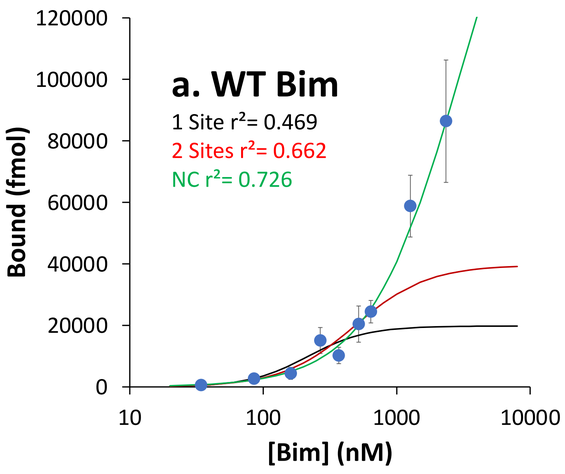
2b. 10 kDa spin filter, 4°C, to separate free (filtered) from bound peptide

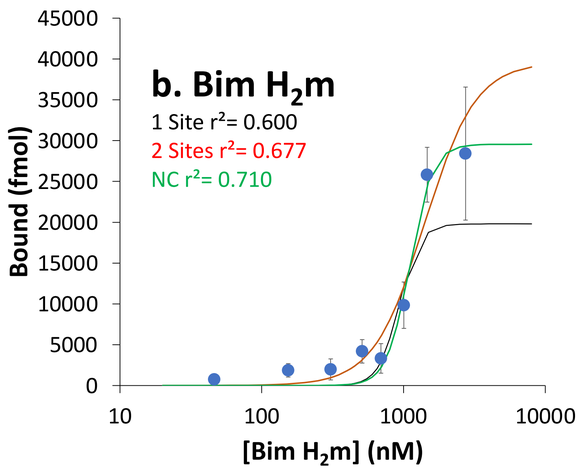
2c. Assess filtrate fluorescence, , λ_{ex} = 494 nm, λ_{em} = 524 nm

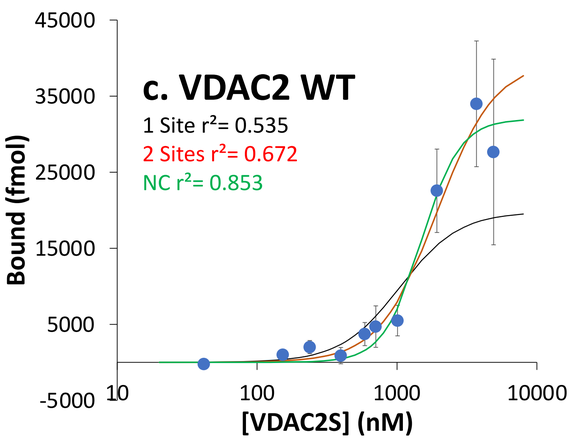
2d. Bim BH3 domain, alignment with putative VDAC1 BH3 domain, and mutations tested

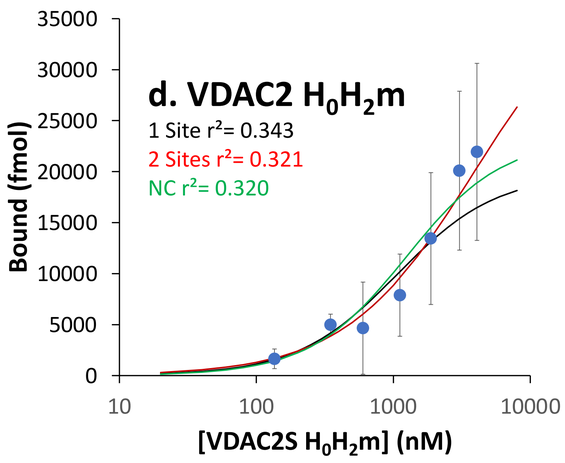
	H_0		H_1	H_2	H_3	H_4		H_5	
Bim	D	L	R	P E I R I A Q E	L	R	R	I G D E	F N E T Y T R R –
Bim H_2m	D	L	R	P E I R I A Q E	A	R	R	I G D E	F N E T Y T R R - -
Vdac2S	-	-	S	I P P P Y A D	L	G	K	A A R D	I F N K G F G F G L V
Vdac2S H_0H_2m	-	-	S	I P G P Y A D	A	G	K	A A R D	I F N K G F G F G L V
Vdac2S H_1H_3m	-	-	S	I P P P A A D	L	G	K	A G R D	I F N K G F G F G L V
Vdac2S H_2H_4m	-	-	S	I P P P Y A D	A	G	K	A A R D	I G N K G F G F G L V
Vdac2S H_3H_5m	-	-	S	I P P P Y A D	L	G	K	A G R D	I F N K G A G F G L V
Vdac2S Dm	-	-	S	I P P P Y A D	L	G	K	A A R R	I F N K G Y G F G L V

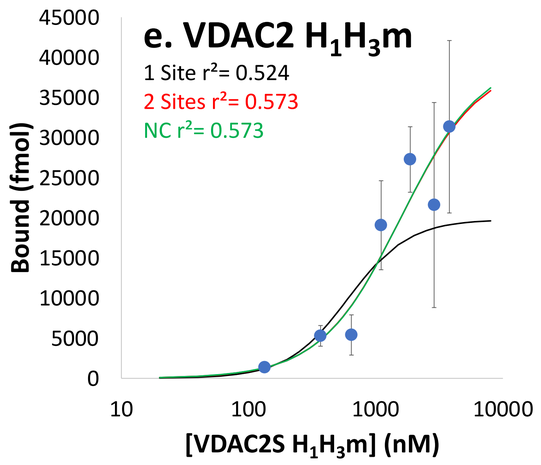
3. Equilibrium Binding With Regression

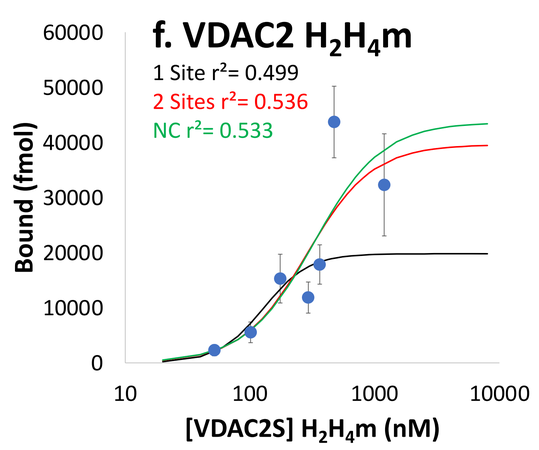


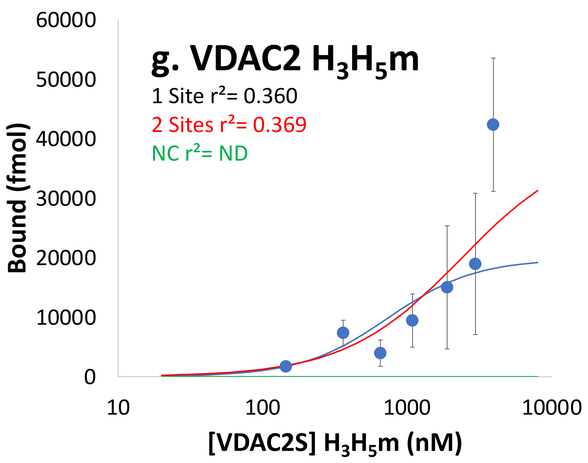


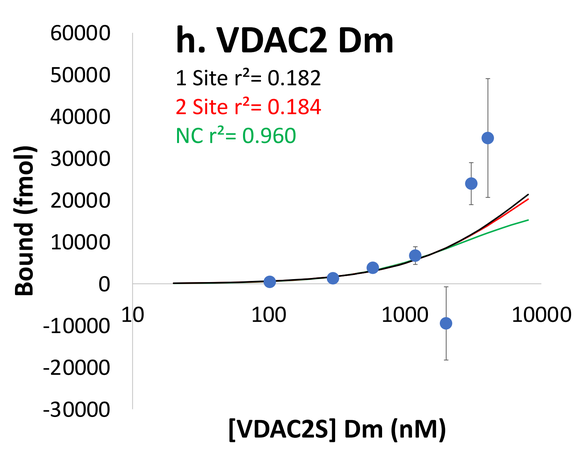












Peptide sequences tested are shown in 2d. Data are mean ± SEM of 5-8 experiments, with each concentration run in triplicate. Equilibrium binding curves were fit to the data assuming one binding site per monomer (black, 19800 fmol), two sites per monomer (red, 39600 fmol), or no constraint (green).

4. Modeled Results

	K_d (nM)			B_{max} (fmol)			Hill Number		
	1 Site	2 Sites	No Constraint	1 Site	2 Sites	No Constraint	1 Site	2 Sites	No Constraint
VDAC2S	-	-	-	-	-	-	-	-	-
WT	1,048	1,938	1,496	19,800	39,600	32,007	2.06	2.10	3.17
VDAC2S [H_0H_2] Mutant	1,061	3,824	1,242	19,800	39,600	23,279	1.19	0.93	1.23
VDAC2S [H_1H_3] Mutant	602	1,552	1,584	19,800	39,600	40,148	1.82	1.38	1.37
VDAC2S [H_2H_4] Mutant	128	287	326	19,800	39,600	43,643	2.41	1.66	1.59
VDAC2S [H_3H_5] Mutant	739	2,353	ND	19,800	39,600	ND	1.44	1.08	ND
VDAC2S [D] Mutant	2,649	7,641	10,090	19,800	39,600	47,895	1.09	0.96	0.94
Bim	-	-	-	-	-	-	-	-	-
WT	216	484	3,021	19,800	39,600	204,808	1.96	1.60	1.26
Bim [H_2] Mutant	928	1,408	1,101	19,800	39,600	29,561	6.00	2.42	5.46

5. Conclusions

5a. Bax binds the putative BH3 domain of VDAC2 with low micromolar affinity

5b. Bax may have a second non-canonical receptor site for VDAC2

5c. However, further experiments are necessary to assess potential nonspecific binding

5d. The conserved aspartic acid residue between H_3 & H_4 is important for Bax binding VDAC2

5e. The double mutants H_1H_3, H_2H_4, and H_3H_5 do not adversely affect Bax affinity for VDAC2

5f. VDAC2 residues H_1 to H_5 may not be as important for binding as in known BH3 domains