Activity Based Protein Profiling of VX Probes in Multiple Rat Tissue Types

Dan Carmany [2], Trevor Glaros, Ph.D. [3], Fu-Lian Hsu, Ph.D. [1], Andrew Walz [1], Fred Berg, Ph.D. [1], Roland Van Den Berg [4], Daan Noort, Ph.D. [4], Jennifer Sekowski, Ph.D. [1]
(1) U.S. Army Edgewood Chemical Biological Center, (2) Battelle Memorial Institute, (3) Excet, Inc., (4) The Netherlands Organization

Overview

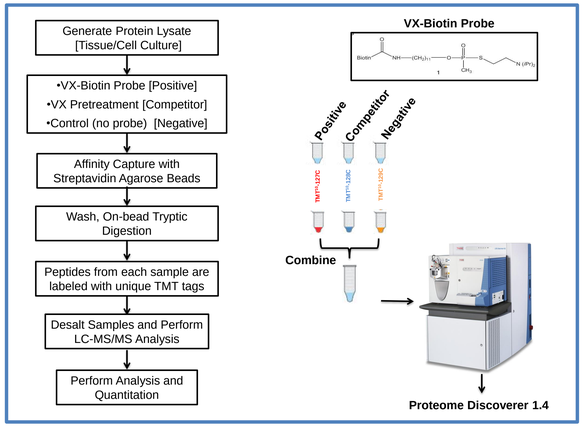
Current countermeasure research uses the traditional reductionist approach of focusing on single mechanisms of action, limiting the solution space for countermeasure development. A systems biology-based approach to this challenge extracts more information from *in vivo* and *in vitro* studies given its advantage of capturing a more integrated picture of the complex molecular response to exposure than traditional single endpoint toxicology assays. The approach employed by the overarching project (CB3810) of which this protein target discovery work is a part, realizes the importance of identifying the myriad of potential vulnerable protein and enzymatic targets for a given chemical.

The activity-based protein profiling (ABPP) assay utilizes a labeled chemical probe to identify and functionally enrich for protein binding from cellular fractions derived from tissues of interest. The tissues of interest are heart , liver, kidney, diaphragm and brain (last two not displayed in this poster). The probe used in this work is a fluorophosphonate probe capable of a one step labeling process. Briefly, a VX "warhead" is attached to a carbon linker chain tipped with a desthiobiotin structure. The VX end of the probe binds covalently to its protein targets. The biotin end binds to strepavidin-agarose beads, which through a centrifugation step, enables pull-down of the protein–probe complexes. Once eluted from the beads and trypsin-digested, the peptides were run through C18-RPLC chromatography (LC) followed by mass spectrometry (MS) on a Thermo Orbitrap Elite Instrument.

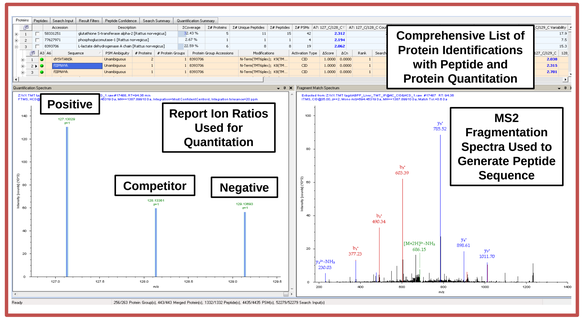
To date, this ABPP assay has identified direct binding enzymes in the heart, kidney and liver (shown in Results tables). Data from the brain and diaphragm was not fully analyzed at the time of poster submission, and is not shown.

Overall, the ABPP work will aid in the understanding of the immediate binding proteins of military chemicals of interest, leading to the efficient discovery of accessible protein biomarkers. This assay also has the benefit of identifying novel pathways of nerve agent toxicity, thus opening opportunities for the development of adjuvant therapeutic strategies.

Experimental Workflow



Quantitative TMT Analysis using Proteome Discoverer

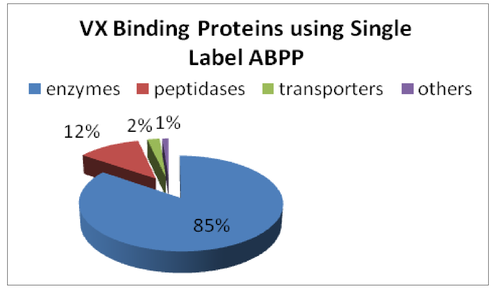


Results

Heart Protein ID	Coverage	# Unique Peptides	# Peptides	# PSMs	Pos/Neg	Pos/Comp	MW [kDa]
liver carboxylesterase 1	43.17	3	20	427	100.000	2.318	60.2
platelet-activating factor acetylhydrolase IB subunit beta	27.95	5	5	26	21.051	2.365	25.6
carboxylesterase 3 precursor	50.27	9	21	217	20.529	10.780	62.1
cholinesterase	27.30	13	13	68	12.826	6.354	67.7
arylformamidase	14.38	4	4	17	12.649	5.727	34.3
neutral cholesterol ester hydrolase 1	38.24	14	14	74	11.775	1.669	45.8
prolyl endopeptidase	63.10	34	34	314	11.613	4.886	80.7
aldo-keto reductase family 1, member C-like 1	51.23	13	14	65	9.123	4.843	37.2
carboxylesterase-like	39.65	6	17	122	8.450	4.184	62.1
phosphatidylcholine-sterol acyltransferase precursor	21.82	6	6	31	7.370	1.691	49.8
glutathione S-transferase Mu 2	47.71	8	10	47	6.567	4.077	25.7
hormone-sensitive lipase	25.09	18	18	74	6.027	2.606	116.7
monoglyceride lipase	66.67	15	15	146	5.898	3.605	33.5
group XV phospholipase A2	10.65	4	4	17	5.577	2.153	47.4
acylamino-acid-releasing enzyme	17.08	9	9	35	5.365	3.019	81.3
retinoid-inducible serine carboxypeptidase precursor	8.85	3	3	14	4.808	1.794	51.1
platelet-activating factor acetylhydrolase IB subunit gamma	24.14	5	5	21	4.223	1.822	25.8
ADP/ATP translocase 1	37.58	5	12	71	4.128	2.265	33.0
enoyl-CoA hydratase, mitochondrial precursor	33.10	9	9	28	3.982	2.372	31.5
phosphate carrier protein, mitochondrial precursor	14.61	5	5	12	3.813	1.783	39.4
acyl-protein thioesterase 2	40.26	6	6	39	3.724	2.173	24.8
isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial precursor	28.69	9	9	36	2.786	1.939	39.6
succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	6.02	3	3	7	2.530	1.754	50.3
tubulin alpha-4A chain	39.06	3	13	76	2.469	1.854	49.9
carnitine O-palmitoyltransferase 1, muscle isoform	5.70	3	3	9	2.288	2.173	88.2
medium-chain specific acyl-CoA dehydrogenase, mitochondrial precursor	17.34	6	6	31	2.284	1.612	46.5
acyl-CoA synthetase family member 2, mitochondrial precursor	23.25	11	11	39	2.178	1.827	67.8
carboxylesterase 2-like	8.38	3	4	15	2.159	1.548	62.8

Kidney Protein ID	Coverage	# Unique Peptides	# Peptides	# PSMs	Pos/Neg	Pos/Comp	MW [kDa]
glutathione S-transferase alpha-1	37.84	2	8	111	50.478	12.677	25.6
retinoid-inducible serine carboxypeptidase precursor	36.50	15	15	204	16.453	7.952	51.1
carboxylesterase 3 precursor	49.56	10	20	187	15.430	7.587	62.1
monoglyceride lipase	67.33	15	15	277	13.545	7.973	33.5
arylformamidase	54.25	11	11	182	12.966	8.849	34.3
acyl-protein thioesterase 1	56.52	9	9	75	12.475	8.942	24.7
lysosomal protective protein	20.18	8	8	61	11.074	2.018	51.2
carboxylesterase-like	27.96	2	11	105	11.055	6.314	62.1
carboxylesterase 2G	35.89	12	18	189	10.839	5.288	62.9
platelet-activating factor acetylhydrolase IB subunit beta	8.30	2	2	6	10.300	8.486	25.6
carboxylesterase 2C precursor	41.53	5	18	145	10.265	3.624	62.1
carboxylesterase 2 isoform 1 precursor	34.23	3	15	141	9.994	5.910	62.4
glutathione S-transferase alpha-2	37.84	2	8	84	9.861	6.904	25.6
monoacylglycerol lipase ABHD6	10.39	4	4	16	9.130	4.973	38.3
arylacetamide deacetylase	7.54	3	3	17	8.400	2.773	45.7
glutathione S-transferase P	36.19	4	5	31	7.792	4.286	23.4
liver carboxylesterase 3 precursor	52.05	17	21	271	7.509	1.771	61.6
prolyl endopeptidase	50.85	28	28	198	6.953	2.394	80.7
hormone-sensitive lipase	13.01	11	11	38	6.677	4.684	116.7
dipeptidyl peptidase 4	24.77	18	18	99	6.639	1.945	87.9
S-formylglutathione hydrolase	12.77	3	3	5	6.258	2.909	31.3
glutathione S-transferase alpha-4	27.93	2	6	22	6.121	3.736	25.5
acylamino-acid-releasing enzyme	25.00	14	14	51	5.759	3.978	81.3
sialate o-acetylesterase	21.22	8	8	55	5.700	2.449	60.4
neutral cholesterol ester hydrolase 1	30.15	9	9	40	5.241	3.473	45.8
solute carrier family 22 member 6	11.98	8	8	30	5.110	4.183	60.7
SEC23-interacting protein	2.90	3	3	4	4.958	3.326	110.9
acyl-protein thioesterase 2	25.97	4	4	17	4.852	3.296	24.8
phosphatidylcholine-sterol acyltransferase precursor	7.50	3	3	21	4.642	1.798	49.8
acyl-coenzyme A thioesterase 2, mitochondrial	27.15	4	10	46	4.493	4.288	49.6
carboxylesterase 2-like	17.29	2	10	89	4.368	2.855	62.8
enoyl-CoA hydratase, mitochondrial precursor	10.00	2	2	9	4.179	4.355	31.5
fatty acid synthase	13.37	28	28	88	4.127	2.152	272.5
sn1-specific diacylglycerol lipase beta	4.04	3	3	11	4.106	1.792	73.7
carboxylesterase-like	35.12	5	16	119	3.946	3.094	62.2
glutathione S-transferase Mu 2	36.70	3	7	44	3.783	3.595	25.7
platelet-activating factor acetylhydrolase 2, cytoplasmic	41.79	16	16	68	3.429	3.171	43.5
platelet-activating factor acetylhydrolase IB subunit gamma	10.34	3	3	9	2.883	4.435	25.8
PREDICTED: hypothetical protein	5.95	5	6	20	2.641	2.102	148.5

Liver Protein ID	Coverage	# Unique Peptides	# Peptides	# PSMs	Pos/Neg	Pos/Comp	MW [kDa]
carboxylesterase 3 precursor	52.21	11	23	349	15.987	12.347	62.1
arylformamidase	60.13	12	12	154	12.746	10.657	34.3
acyl-protein thioesterase 1	13.91	3	3	18	12.051	3.570	24.7
carboxylesterase 5	30.70	7	13	112	8.314	4.865	62.4
carboxylesterase 2C precursor	35.47	5	16	138	8.423	4.329	62.1
arylacetamide deacetylase	31.91	11	11	92	10.148	4.779	45.7
carboxylesterase-like	30.44	2	13	206	9.963	7.887	62.1
liver carboxylesterase 3 precursor	55.61	19	23	305	8.304	2.310	61.6
carboxylesterase 2G	25.18	8	13	102	9.378	7.907	62.9
glutathione S-transferase Mu 1	41.74	4	10	45	6.512	3.432	25.9
carboxylesterase 2A	40.68	7	17	263	7.227	6.500	61.8
retinoid-inducible serine carboxypeptidase precursor	24.34	10	10	64	6.939	6.961	51.1
sialate o-acetylesterase	4.61	2	2	39	6.148	4.818	60.4
omega-amidase NIT2	18.84	5	5	12	5.144	3.338	30.7
lysosomal protective protein	5.26	2	2	9	5.763	2.481	51.2
acyl-coenzyme A amino acid N-acyltransferase 2	38.28	6	15	98	4.970	2.450	46.0
neutral cholesterol ester hydrolase 1	26.23	9	9	32	5.838	4.011	45.8
monoglyceride lipase	60.07	13	13	113	4.201	3.809	33.5
group XV phospholipase A2	16.46	8	8	34	4.450	2.451	47.4
carboxylesterase 2 isoform 1 precursor	37.46	4	16	142	4.906	4.760	62.4
carboxylesterase-like	32.80	4	15	97	3.895	3.747	62.2



Discussion

Characterization of the direct and indirect actions of chemical threat agents is critical for the development of effective detection methods, diagnostic tests, and therapeutics. To aid in this characterization and to better understand the toxicity of chemical warfare agents, an ABPP assay was developed using VX as the subject molecule. The assay is able to isolate and quantify targets of VX from tissue lysates. Using tandem mass tag (TMT) labeling, pull down proteins are quantitatively analyzed without the gel electrophoresis step that is typically used in ABPP. The protein targets are confirmed by the addition of VX as a competitor molecule. To date, our results demonstrate that VX targets a wide range of expected and novel proteins in rat heart, liver, and kidney tissues. This information, along with data from brain and lung diaphragm muscle (data not shown) will be analyzed further to better understand the direct binding targets and elucidate secondary toxicity mechanisms of VX. This understanding will add in the development of novel diagnostic and therapeutic strategies against VX, and potentially other nerve agents.

Conclusions

- Expected and novel proteins bound VX
- Common and tissue-specific proteins bound VX
- Ongoing comprehensive analysis of heart, kidney, liver, diaphragm muscle, brain, and blood (some data not shown)
- Data suggests potential novel diagnostic and adjuvant therapeutic targets

Acknowledgements: The authors thank the Defense Threat Reduction Agency/Joint Science and Technology Office for their assistance and funding of this work under Project CB2198. The views expressed in this presentation are those of the authors and do not necessarily reflect official policy or the position of the Department of Defense or the U.S. Government.

Approved for Public Release