Tubulin Tail Sequences and Post-translational Modifications Regulate Closure of Mitochondrial Voltage-dependent Anion Channel (VDAC)*

Kely L. Sheldon[1], **Philip A. Gurnev, Sergey M. Bezrukov, and Dan L. Sackett**[2]

From the Program in Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

Background: Tubulin C-terminal tail (CTT) peptides bind and block VDAC.
Results: CTT-albumin chimeras show that a single CTT suffices for VDAC blockade and that CTTs differ in potency by more than an order of magnitude.
Conclusion: Small sequence changes or post-translational modifications in CTT result in substantial changes in VDAC blockage.
Significance: Disordered protein tails are not just charged strings, but embody nuanced interaction specificity.

It was previously shown that tubulin dimer interaction with the mitochondrial outer membrane protein voltage-dependent anion channel (VDAC) blocks traffic through the channel and reduces oxidative metabolism and that this requires the unstructured anionic C-terminal tail peptides found on both α- and β-tubulin subunits. It was unclear whether the α- and β-tubulin tails contribute equally to VDAC blockade and what effects might be due to sequence variations in these tail peptides or to tubulin post-translational modifications, which mostly occur on the tails. The nature of the contribution of the tubulin body beyond acting as an anchor for the tails had not been clarified either. Here we present peptide-protein chimeras to address these questions. These constructs allow us to easily combine a tail peptide with different proteins or combine different tail peptides with a particular protein. The results show that a single tail grafted to an inert protein is sufficient to produce channel closure similar to that observed with tubulin. We show that the β-tail is more than an order of magnitude more potent than the α-tail and that the lower α-tail activity is largely due to the presence of a terminal tyrosine. Detyrosination activates the α-tail, and activation is reversed by the removal of the glutamic acid penultimate to the tyrosine. Nitration of tyrosine reverses the tyrosine inhibition of binding and even induces prolonged VDAC closures. Our results demonstrate that small changes in sequence or post-translational modification of the unstructured tails of tubulin result in substantial changes in VDAC closure.

Tubulin is the subunit of microtubules, but it is also a freely diffusing cytoplasmic protein that can find binding partners distinct from those for microtubules. One of these is the mitochondrial outer membrane protein VDAC,[3] which mediates small molecule traffic into and out of the mitochondria (1). It was previously shown that tubulin binding to VDAC blocks channel traffic and reduces oxidative metabolism (2, 3) and that this requires the unstructured anionic tail peptides found on both α- and β-tubulin subunits (2). However, those studies left unclear whether the tails contribute equally to VDAC blockade, as well as the role, if any, of tubulin post-translational modifications, which mostly occur on the tails. Earlier studies showed that anionic polymers could cause VDAC closure, but gave no indication of sequence sensitivity (4, 5).

Here we use single molecule binding studies to show that channel closure is due to the tails themselves, because grafting them to albumin produces closure very similar to that induced by tubulin. We show that the β-tail is ~30-fold more potent than the α-tail and that this difference is largely due to the presence or absence of a terminal tyrosine. Detyrosination activates the α-tail, and activation is reversed by subsequent removal of the next residue. We also show that nitration of tyrosine reverses the tyrosine inhibition of binding and even induces prolonged VDAC closures. Our results demonstrate that small changes in sequence or post-translational modification of the disordered tails of tubulin result in substantial changes in VDAC closure. We expect that this pattern will be found again when comparing the numerous isotypes of α- and β-tubulin, as well as other post-translational modifications. Our study also provides a sensitive system to study the biology of disordered protein sequences.

Experimental Procedures

Materials—Bovine tubulin was obtained from Cytoskeleton (Denver, CO). Lipids were obtained from Avanti Polar Lipids

---

[1] To whom correspondence may be addressed: Emory University School of Medicine, Dept. of Medicine, Cardiology Division, 307 Woodruff Memorial Bldg., 1639 Pierce Dr., Atlanta GA 30322. Tel.: 704-617-1831; E-mail: ksheldo@emory.edu.
[2] To whom correspondence may be addressed: Program in Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, 9000 Rockville Pike, Bethesda MD 20892. Tel.: 301-594-0358; E-mail: sackettd@mail.nih.gov.

[3] The abbreviations used are: VDAC, voltage-dependent anion channel; DMSO, dimethyl sulfoxide; S-4FB, *N*-succinimidyl-4-formylbenzamide; S-HyNic, succinimidyl-6-hydrazino-nicotinamide; CTT, C-terminal tail(s).

(Alabaster, AL). Crosslinking reagents were from Solulink (San Diego, CA). Wild-type α-hemolysin was purchased from Merck (Darmstadt, Germany). All peptides were synthesized by Peptide 2.0 (Chantilly, VA), with purity >95% confirmed by HPLC and masses confirmed by mass spectrometry. The default α- and β-peptide sequences were: α (TUBA1A, NP_001257328.1) and β (TUBB, Class I, NP_821133.1).

Protein Purification—VDAC from *Neurospora crassa* mitochondrial outer membranes was isolated and purified as described in Ref. 2 with minor modifications. The wall-less *N. crassa* mutant (FGSC number fz;sg;os-1) was obtained from the Fungal Genetics Stock Center, Kansas City, MO. Cells were lysed in buffered detergent (2.5% Triton X-100, 15% DMSO, 50 mM KCl, 10 mM Tris, 1 mM EDTA, pH 7.0) passed ~10× through a Monoject 200 needle and then ~10× through a Monoject 250 needle. Cell lysate was run over a dry hydroxyapatite:Celite column (2:1), and eluted with ~2 ml of buffered detergent. Four ~500-μl samples were collected and stored at −20 °C.

VDAC from rat liver was isolated using published methods (6) with slight modification. Frozen rat liver fractions (from Dr. Marco Colombini, University of Maryland, College Park, MD) were allowed to thaw, and then centrifuged (10,000 × g) at 4 °C for 10 min. The supernatant was discarded, and membranes were incubated in buffered detergent (see above) for 10 min on ice, vortexing for 20 s every 2 min. Samples were centrifuged (14,000 × g) at 4 °C for 10 min, and the supernatant was run using the same protocol as for *N. crassa* (see above).

Production of Protein-Peptide Constructs—Peptides corresponding to the sequences of tubulin C-terminal tail (CTT) were grafted to BSA (and other proteins) using a "click chemistry"-based protocol using the reaction between hydrazines and carbonyls. The ratio of peptides to protein was kept low (0.5 or less usually) so that most proteins had only one peptide, if they had any (most had none). Peptides were tagged on the N-terminal amine using succinimidyl-6-hydrazino-nicotinamide (HyNic, Solulink). This was added from a DMSO stock to a final concentration of ~7 mM in a solution of the peptide (2 mM) in PBS-borate buffer, pH 8.3– 8.5. Reaction was allowed to proceed for 2 h at room temperature. Tagged peptide was separated from free HyNic using gel filtration on columns of Bio-Gel P2 (Bio-Rad) equilibrated in conjugation buffer (100 mM sodium phosphate, 100 mM NaCl, pH 6.)

BSA (or other proteins) was tagged with *N*-succinimidyl-4-formylbenzamide (S-4FB, Solulink). BSA at 0.15 mM in PBS-borate buffer, pH 8.3– 8.5, was mixed with a sample of DMSO stock of S-4FB to yield a final concentration of 0.33 mM, and the reaction were allowed to proceed for 2 h at room temperature. Unreacted S-4FB was removed by gel filtration using Zeba 7-kDa spin columns (Thermo Fisher Scientific) equilibrated in conjugation buffer.

Conjugation of 4FB-BSA (S-4FB conjugated with BSA) and peptide-S-HyNic was accomplished by combining the tagged protein and peptide at a stoichiometry of 2 peptides to BSA (~0.2 mM peptide-HyNic and 0.1 mM 4FB-BSA). Reaction proceeded overnight at 4 °C. Unconjugated peptide was removed by gel filtration using a Zeba 40-kDa spin column (Thermo Fisher Scientific), equilibrated in PBS.

The concentration of BSA and the concentration of the peptide conjugate were obtained from a UV-visible absorbance spectrum. Absorbance at 350 nm gave the peptide conjugate absorbance from the chromophore produced in the conjugation reaction, and the corrected absorbance at 280 yielded the BSA concentration.

Lipid Bilayers, Channel Reconstitution, and Event Analysis—Lipid bilayer membranes were formed by the lipid monolayer opposition technique from diphytanoyl-phosphatidylcholine, on a circular aperture in a Teflon partition (E. I. DuPont de Nemours) dividing two (cis and trans) compartments of the experimental chamber as described previously (2, 6). VDAC insertion was achieved by adding protein in a 2.5% Triton X-100 solution to the aqueous phase of 1 M KCl buffered with 5 mM Hepes at pH 7.4 in the cis compartment while stirring. α-Hemolysin was added to the cis side solution of the chamber from a diluted water stock solution of ~300 nM containing 8 M urea. Applied transmembrane voltage is defined as positive if potential is higher on the cis side of the chamber. Ion current measurements were performed using Axopatch 200B amplifier (Molecular Devices, Eugene, OR). The output signal was filtered by the amplifier 10-kHz filter and in-line low-pass eight-pole Butterworth filter (Model 9002; Frequency Devices, Ottawa, IL) at 15 kHz and directly saved into the computer memory with a sampling frequency of 50 kHz. Event on-rate and lifetime analysis was performed using ClampFit 10.2 (Molecular Devices) and by ORIGIN 8.2 software (OriginLab, Northampton, MA). BSA constructs were added to the membrane-bathing solutions after channel reconstitution. Statistical analysis of the blockage events was started 15 min after BSA addition to ensure steady state. Lifetimes (τ_{on}) were calculated by fitting a single exponential to logarithmically binned histograms of at least 100 open (unblocked state) events. On-rates were calculated as the inverse of the τ_{on} multiplied by the concentration of chimera added to the bath. Standard deviations are depicted as the average of at least three independent experiments for a given applied potential.

Results

Like many intrinsically disordered protein tails (7), the tubulin CTT are sites of interaction with other proteins and sequence differences between isotypes. The CTT are also a major focus of post-translational modifications that comprise the "tubulin code" (8). In the microtubule, these tails form a charged brush surrounding the microtubule (9–12) and, in this context, the post-translational modifications are recognized to regulate binding of other proteins (13).

The previous study (2) of tubulin-VDAC binding demonstrated that the CTT also have important functions in the dimer, because proteolytic removal of the tails (producing a tubulin globular body without tails) eliminated VDAC blockade. The free tails alone were also unable to cause VDAC closure, indicating that a body is required to anchor the tail, although perhaps this body could be a protein other than tubulin.

To address this, and to examine the tails individually rather than in pairs as in dimeric tubulin, we chose to pursue an approach in which we chemically link a CTT peptide to a non-

beta	ATAEEEEDFGEEAEEEA
beta (-A+Y)	ATAEEEEDFGEEAEEEY
alpha	DSVEGEGEEEGEEY
alpha (-Y)	DSVEGEGEEEGEE
alpha (-E-Y)	DSVEGEGEEEGE

FIGURE 1. **Amino acid sequences of CTT peptides.** Both natural β (TUBB, Class I, NP_821133.1) and α (TUBA1A, NP_001257328.1) CTT sequences are displayed, as well as the peptides containing sequence modifications as described under "Results." *Red* indicates an amino residue with a negative charge.

interacting protein. After trying several other proteins, we chose BSA for this purpose because it is similar in size and charge to the globular body of α- and β-tubulins and does not measurably interact with VDAC. This allowed us to make small libraries of constructs using a click chemistry procedure described under "Experimental Procedures" and to create constructs with non-genetically codable modifications.

The sequences of the CTT peptides are presented in Fig. 1. The sequences correspond to gene-coded human α- and β-CTT (see under "Experimental Procedures" for sequence accession numbers), as well as α-CTT following the removal of the terminal Tyr, the removal of the terminal Tyr and the preceding Glu, and the non-natural sequence of β-CTT terminating in Tyr rather than Ala.

Activity of these BSA-peptide constructs was measured by analyzing the effects of constructs on the ion current through single VDAC channels in the electrophysiological setup described previously (Ref. 2, and also see under "Experimental Procedures"). The constructs were added to the aqueous potassium chloride solutions bathing planar lipid bilayers with reconstituted VDAC molecules. The representative results of the currents through single VDAC channels are shown in Fig. 2, and analysis of the on-rates is given in Fig. 3. As can be seen, the addition of 150 nM BSA-β construct caused intermittent current interruptions similar to those previously found for tubulin-VDAC interactions (2): rapidly reversible (msec) events that reduce channel conductance by ~60%. This single closure level is distinct from the multiple closure levels induced by voltage alone, is characteristic of tubulin binding, and is sufficient to cause channel blockage for molecules the size of ATP and ADP (14).

Surprisingly, the addition of BSA-α to the same concentration had virtually no effect on conductance of the channel. This is remarkable because the two peptides are very similar in properties (60–70% Glu or Asp residues in the C-terminal 14 residues) and identical in 6 residues. We confirmed that simple charge was not the critical characteristic, consistent with previous observations with oligonucleotides (4), because VDAC closure was not induced by either of two poly-Glu constructs, BSA-E_{10} (same number of charges as BSA-β) or BSA-E_{15} (more charges, nearly the same length as BSA-β) (data not shown). To test whether the failure of BSA-α to affect the channel conductance was due to its slightly shorter length as compared with the BSA-β construct (Fig. 1), we made a construct using an α-peptide extended by N-terminal addition of the residues ATA so that the construct has the same length and N-terminal sequence as the BSA-β construct. This had no effect on its interaction with VDAC (Fig. 4).
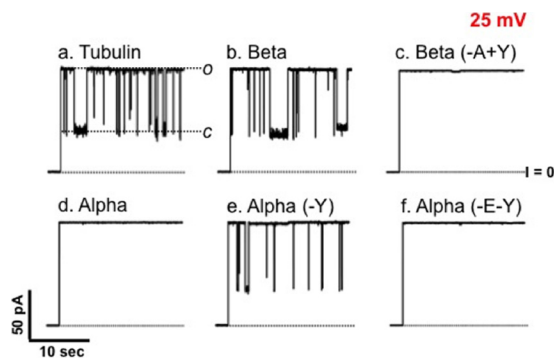

FIGURE 2. **BSA-CTT construct interaction with single VDAC channel is regulated by single terminal CTT amino acid.** *a–f*, current records through single VDAC channels at an applied potential of 25 mV. *a*, 1 nM full-length dimeric tubulin inducing characteristic fluctuations of channel current between the fully open channel (indicated by *o*) and 60% closure (indicated by *c*); *b*, 150 nM BSA-β; *c*, 150 nM BSA-β ($-A+Y$); *d*, 150 nM BSA-α; *e*, 150 nM BSA-α ($-Y$); *f*, 150 nM BSA-α($-EY$).
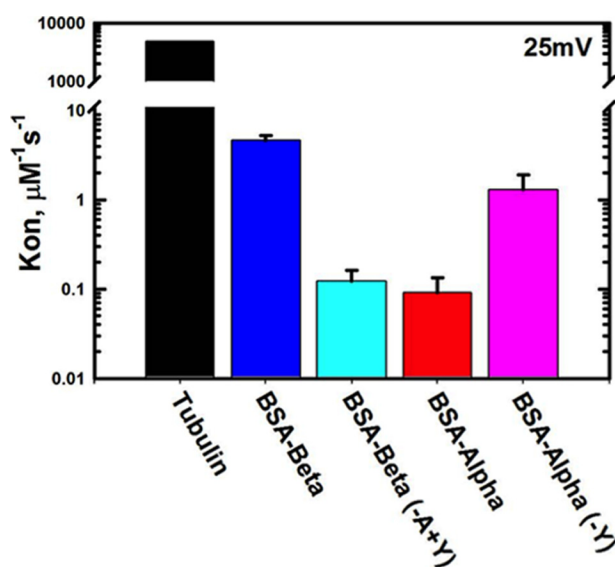

FIGURE 3. **Single amino acid terminal alterations govern peptide interaction with VDAC.** Shown are the on-rate constants (k_{on}) of tubulin binding, and each of the BSA-peptide constructs, with VDAC obtained from the kinetic analysis of the experiments, examples of which are shown in Fig. 2, *a–e*. Values are the average $k_{on} \pm$ S.E. from at least three independent experiments shown for an applied potential of 25 mV.

We also examined the question of channel generality. The *N. crassa* VDAC that we used has advantages for these experiments, because there is only one form (unlike mammalian VDACs, which have several isotypes) and because we wanted continuity with previous publications (2). To address the possibility that the difference in activity between BSA-β and BSA-α might be restricted to this VDAC, we repeated experiments with VDAC isolated from rat liver and found similar differences between the BSA-β and BSA-α constructs. The results presented in Fig. 5a show on-rates of BSA constructs binding as a function of transmembrane voltage for this VDAC. Besides dramatically higher channel affinity found for the β-tail construct, one can see that elevated voltages also increase the frequency of VDAC blockage by the α-tailed BSAs.

In addition, we tested BSA constructs with a completely different β-barrel channel-forming protein, α-hemolysin (15), known for its ability to interact with an array of biopolymers,
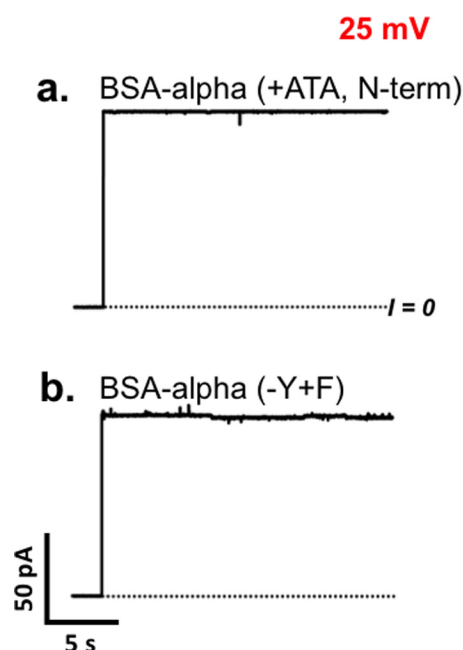
25 mV

FIGURE 4. **Neither the addition of N-terminal ATA nor terminal substitution (Tyr to Phe) to the α-peptide activates interaction with VDAC.** *a* and *b*, current record through single VDAC channel at an applied potential of 25 mV in the presence of 150 nM BSA-α (+ATA, N-terminal (*N-term*)) (*a*) or 150 nM BSA-α (−Y+F) (*b*).

including unstructured proteins (16, 17). We found that the BSA-β and BSA-α constructs interact with α-hemolysin in a similar voltage-dependent manner; however, the voltage required to induce the channel-BSA interaction is significantly larger (Fig. 5*b*). Most importantly, the results obtained with α-hemolysin demonstrate that BSA-β and BSA-α constructs do not exhibit any measurable specificity in blocking this channel (Fig. 5*b*, *bottom panel*). The corresponding on-rates virtually coincide in the whole range of the studied voltages. In other words, α-hemolysin does not seemingly discriminate between the two constructs, thus supporting our conjecture regarding the sequence specificity in interaction of tubulin tails with mitochondrial VDACs.

We then looked at differences in the peptides to understand the difference in activity between BSA-β and BSA-α. We found that removing the terminal Tyr from BSA-α (BSA-α(−Y)) restored activity to this construct, whereas replacing the terminal Ala with Tyr on BSA-β (BSA-β(−A+Y)) quenched activity (Figs. 2 and 3). Surprisingly, removing from BSA-α the penultimate Glu as well as the terminal Tyr (BSA-α(−EY)) resulted in lost activity. Replacing the terminal Tyr with nitrotyrosine yielded a construct that was not only able to cause closure, but actually caused quite extended closures (Fig. 6).

Discussion

These results show that VDAC blocking by tubulin dimer is by the unstructured tail, and not by the tubulin body, because grafting β-CTT to BSA (and to other proteins; data not shown) induced the same blocking events as tubulin. These results also show that grafting of the tails to *a* body is required because we previously showed that the free tail peptides alone do not cause closure (2), but, importantly, that grafting need not be to the

tubulin body itself. The difference in affinity between constructs with BSA and tubulin (on-rates, Fig. 3) is likely due to a lipid-dependent affinity of tubulin body for the membrane (18) as has been often reported (reviewed in Ref. 19). The significantly reduced activity of BSA-α as compared with BSA-β in blocking VDAC was surprising, hinting that small differences in peptide sequence or chemical structure may be significant, and suggesting that the activity we reported with tubulin dimer was due almost solely to β-tubulin CTT-VDAC interaction.

To search for differences between the peptides that explain the more than an order of magnitude difference in activity, we started with a construct that corresponds to the product of a major tubulin post-translational modification: the removal of the terminal Tyr residue of the α-CTT. This evolutionarily ancient post-translational modification of tubulin is usually related to microtubule stability and binding of other proteins to the microtubule (13), although also implicated in rapid shifts during cellular signaling (20, 21).

Surprisingly, removing the terminal Tyr from the α-CTT peptide to form the construct BSA-α(−Y) activated the construct-VDAC binding to a potency close to that of the BSA-β construct (Figs. 2 and 3). Furthermore, replacement of the terminal Ala with a Tyr on β-CTT peptide (BSA-β(−A+Y)) reduced construct-VDAC binding activity by an amount similar to the difference between BSA-α and BSA-α(−Y) (Figs. 2 and 3). These results show that the presence or absence of a tyrosine residue at the end of the tail peptide is the controlling element in determining whether these tail peptides are potent VDAC blockers or not, apparently overriding other differences between the peptides preceding the C terminus. Replacing the terminal Tyr with a terminal Phe (BSA-α(−Y+F)) produced a construct as inactive as the α-CTT construct, BSA-α (Fig. 4), suggesting that a bulky aromatic group at the C terminus is the inactivating moiety.

In the absence of a terminal Tyr, other small changes in sequence can determine activity. One such change is the removal of the Glu residue preceding the Tyr, producing α-CTT(−EY), the known variant "Δ2-tubulin" (22). Although the C-terminating residue is Glu in both BSA-α(−Y) and BSA-α(−EY), the tail with two residues deleted is as inactive as the full-length tail (Fig. 2).

We then tested the effect of incorporation of nitrotyrosine (NO$_2$-Tyr) at the terminus of full-length α-CTT. The removal and replacement of Tyr from the end of the α-CTT is a normal post-translational modification cycle, but this can be altered when NO$_2$-Tyr is introduced due to constitutive synthesis, oxidative stress, or other means (see review in Ref. 23). It has been suggested that the incorporation of NO$_2$-Tyr into α-tubulin may be harmful *in vivo* and that the observed accumulation of Δ2-tubulin in patient tumors provided resistance to chemotherapy agents by rendering this tubulin incapable of retyrosination-driven incorporation of NO$_2$-Tyr produced in response to chemotherapy (24, 25). However, the nature of the putative harmful effect(s) of NO$_2$-Tyr incorporation was not revealed (26). We tested a BSA-α-NO$_2$ construct and found that it differed remarkably from BSA-α (Fig. 6); it is able to cause channel closure to the same 60% closed state as BSA-β or intact tubulin, and more remarkably, it causes closures that are very long (more than 10 min), essentially irreversible on the time scale of the experiment.

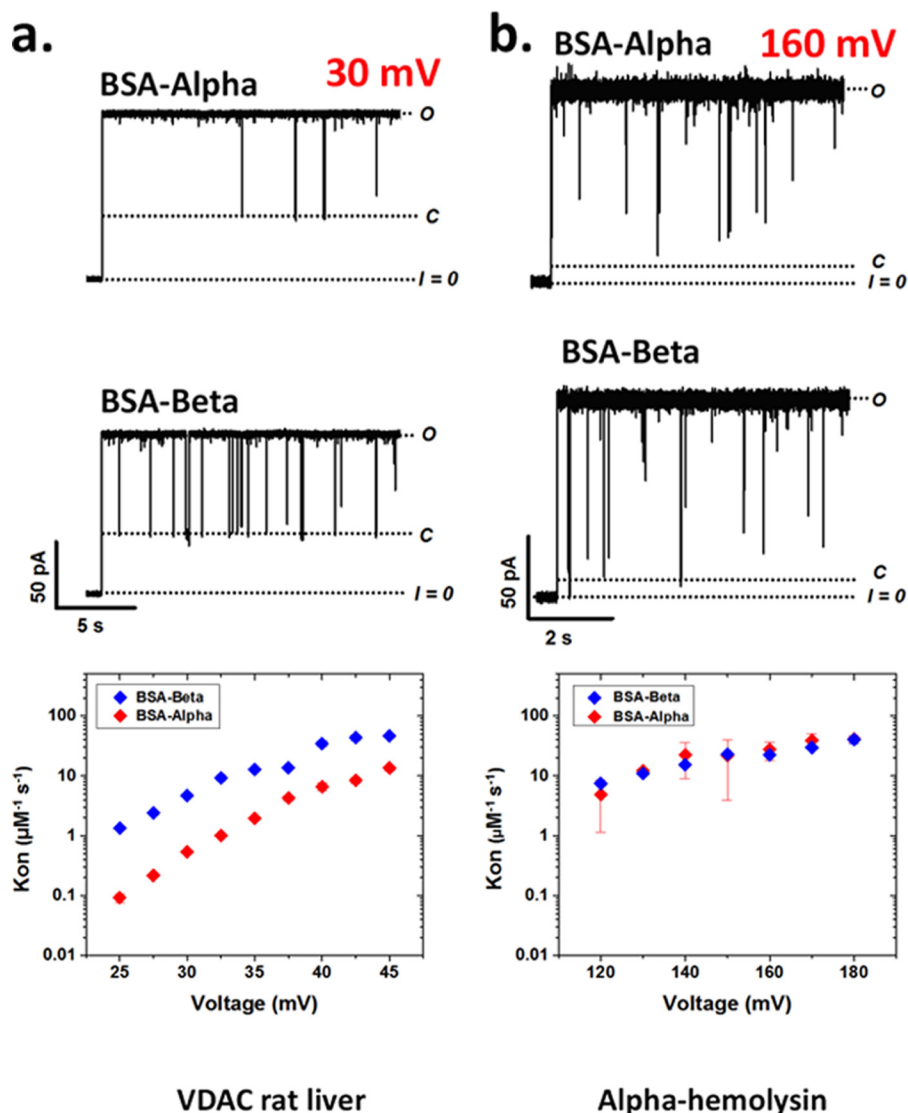**FIGURE 5. VDAC of different origin shows similar specificity to the tail sequence, whereas α-hemolysin is sequence-insensitive.** *a* and *b*, records of the current through VDAC isolated from rat liver (*a*, *two top panels*) and through α-hemolysin (*b*, *two top panels*) in the presence of 100 nM of the BSA-α and BSA-β constructs. *Bottom panels* in *a* and *b* show the dependences of the on-rates on the applied voltage.
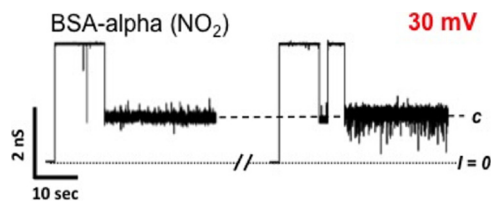

FIGURE 6. Nitro-Tyrosine activates α-peptide interaction with VDAC inducing semi-permanent blockages. Shown is a conductance record through single VDAC channel at an applied potential of 30 mV in the presence of 1 μM BSA-α-NO2. Interaction of peptide with VDAC induces characteristic current blockage (*c*), but in a non-reversible manner. *Dotted line* indicates zero current. The break in the *x* axis indicates a forced reopening of the channel by brief application of 0 mV, followed by a reapplication of 30 mV.

We conclude from these studies that the interaction between the tubulin dimer and VDAC is determined by the disordered tail peptides and that small changes in sequence or post-translational modification of these tails result in substantial changes in VDAC closure. We expect this pattern to be repeated when the numerous isotypes of α- and β-tubulin are compared.

Because different tissues express differing tubulin isotypes and differing post-translational modifications, this suggests that VDAC closure, and therefore mitochondrial regulation, may change in different tissues. These results also demonstrate that disordered protein tails, even if highly charged, are not just charged strings, but embody nuanced specificity for interaction.

References

1. Colombini, M. (2004) VDAC: the channel at the interface between mitochondria and the cytosol. *Mol. Cell. Biochem.* **256–257,** 107–115

2. Rostovtseva, T. K., Sheldon, K. L., Hassanzadeh, E., Monge, C., Saks, V., Bezrukov, S. M., and Sackett, D. L. (2008) Tubulin binding blocks mitochondrial voltage-dependent anion channel and regulates respiration. *Proc. Natl. Acad. Sci. U.S.A.* **105,** 18746–18751

3. Maldonado, E. N., Sheldon, K. L., DeHart, D. N., Patnaik, J., Manevich, Y., Townsend, D. M., Bezrukov, S. M., Rostovtseva, T. K., and Lemasters, J. J. (2013) Voltage-dependent anion channels modulate mitochondrial metabolism in cancer cells: regulation by free tubulin and erastin. *J. Biol. Chem.* **288,** 11920–11929

4. Tan, W., Loke, Y. H., Stein, C. A., Miller, P., and Colombini, M. (2007) Phosphorothioate oligonucleotides block the VDAC channel. *Biophys. J.* **93,** 1184–1191.

5. Szabò, I., Bàthori, G., Tombola, F., Coppola, A., Schmehl, I., Brini, M., Ghazi, A., De Pinto, V., and Zoratti, M. (1998) Double-stranded DNA can be translocated across a planar membrane containing purified mitochondrial porin. *FASEB J.* **12,** 495–502

6. Sheldon, K. L., Maldonado, E. N., Lemasters, J. J., Rostovtseva, T. K., and Bezrukov, S. M. (2011) Phosphorylation of voltage-dependent anion channel by serine/threonine kinases govern its interaction with tubulin. *PLoS ONE* 6, e25539

7. Uversky, V. N. (2013) The most important thing is the tail: multitudinous functionalities of intrinsically disordered protein termini. *FEBS Lett.* **587,** 1891–1901

8. Verhey, K. J., and Gaertig, J. (2007) The tubulin code. *Cell Cycle* **6,** 2152–2160

9. Sackett, D. L., Bhattacharyya, B., and Wolff, J. (1985) Tubulin subunit carboxyl termini determine polymerization efficiency. *J. Biol. Chem.* **260,** 43–45

10. Sackett, D. L., and Wolff, J. (1986) Proteolysis of tubulin and the substructure of the tubulin dimer. *J. Biol. Chem.* **261,** 9070–9076

11. Aiken, J., Sept, D., Costanzo, M., Boone, C., Cooper, J. A., and Moore, J. K. (2014) Genome-wide analysis reveals novel and discrete functions for tubulin carboxy-terminal tails. *Curr. Biol.* **24,** 1295–1303

12. Freedman, H., Luchko, T., Luduena, R. F., and Tuszynski, J. A. (2011) Molecular dynamics modeling of tubulin C-terminal tail interactions with the microtubule surface. *Proteins* **79,** 2968–2982

13. Sirajuddin, M., Rice, L. M., and Vale, R. D. (2014) Regulation of microtubule motors by tubulin isotypes and post-translational modifications. *Nat Cell Biol.* **16,** 335–344

14. Gurnev, P. A., Rostovtseva, T. K., and Bezrukov, S. M. (2011) Tubulin-blocked state of VDAC studied by polymer and ATP partitioning. *FEBS Lett.* **585,** 2363–2366

15. Song, L., Hobaugh, M. R., Shustak, C., Cheley, S., Bayley, H., and Gouaux, J. E. (1996) Structure of staphylococcal α-hemolysin, a heptameric transmembrane pore. *Science* **274,** 1859–1866

16. Movileanu, L., Schmittschmitt, J. P., Scholtz, J. M., and Bayley, H. (2005) Interactions of peptides with a protein pore. *Biophys. J.* **89,** 1030–1045

17. Gurnev, P. A., Yap, T. L., Pfefferkorn, C. M., Rostovtseva, T. K., Berezhkovskii, A. M., Lee, J. C, Parsegian, V. A., and Bezrukov, S. M. (2014) α-Synuclein lipid-dependent membrane binding and translocation through the α-hemolysin channel. *Biophys. J.* **106,** 556–565

18. Rostovtseva, T. K., Gurnev, P. A., Meng-Yang Chen, M.-Y., and Bezrukov, S. M. (2012) Membrane lipid composition regulates tubulin interaction with mitochondrial voltage-dependent anion channel. *J. Biol. Chem.* **287,** 29589–29598

19. Wolff J. (2009) Plasma membrane tubulin. *Biochim. Biophys. Acta* **1788,** 1415–1433

20. Rothwell, S. W., Nath, J., and Wright, D. G. (1993) Rapid and reversible tubulin tyrosination in human neutrophils stimulated by the chemotactic peptide, fMet-Leu-Phe. *J. Cell. Physiol.* **154,** 582–592

21. Robinson, J. M., and Vandré, D. D. (1995) Stimulus-dependent alterations in macrophage microtubules: increased tubulin polymerization and detyrosination. *J. Cell Sci.* **108,** 645–655

22. Paturle-Lafanechère, L., Manier, M., Trigault, N., Pirollet, F., Mazarguil, H., and Job, D. (1994) Accumulation of delta 2-tubulin, a major tubulin variant that cannot be tyrosinated in neuronal tissues and in stable microtubule assemblies. *J. Cell Sci.* **107,** 1529–1543

23. Murray, J., Oquendo, C. E., Willis, J. H., Marusich, M. F., and Capaldi, R. A. (2008) Monitoring oxidative and nitrative modification of cellular proteins: a paradigm for identifying key disease related markers of oxidative stress. *Adv. Drug. Deliv. Rev.* **60,** 1497–1503

24. Eiserich, J. P., Estévez, A. G., Bamberg, T. V., Ye, Y. Z., Chumley, P. H., Beckman, J. S., and Freeman, B. A. (1999) Microtubule dysfunction by posttranslational nitrotyrosination of α-tubulin: a nitric oxide-dependent mechanism of cellular injury. *Proc. Natl. Acad. Sci. U.S.A.* **96,** 6365–6370

25. Lafanechère, L., Courtay-Cahen, C., Kawakami, T., Jacrot, M., Rüdiger, M., Wehland, J., Job, D., and Margolis, R. L. (1998) Suppression of tubulin tyrosine ligase during tumor growth. *J. Cell Sci.* **111,** 171–181

26. Bisig, C. G., Purro, S. A., Contín, M. A., Barra, H. S., and Arce, C. A. (2002) Incorporation of 3-nitrotyrosine into the C-terminus of α-tubulin is reversible and not detrimental to dividing cells. *Eur. J. Biochem.* **269,** 5037–5045