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Chemical Catalysis by the Translocator Protein (18 kDa)

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Supporting Information

ABSTRACT: Translocator proteins (18 kDa) (TSPOs) are conserved integral membrane proteins. In both eukaryotes and prokaryotes, TSPOs interact with porphyrins, precursors of heme, and photosynthetic pigments. Here we demonstrate that bacterial TSPOs catalyze rapid porphyrin degradation in a light- and oxygen-dependent manner. The reaction is inhibited by a synthetic TSPO ligand PK11195 and by mutations of conserved residues, which affect either porphyrin binding or catalytic activity. We hypothesize that TSPOs are ancient enzymes mediating porphyrin catabolism with the consumption of reactive oxygen species.

The translocator protein (18 kDa) (TSPO) is a mitochondrial outer-membrane protein, typically comprising five transmembrane segments (Figure S1A of the Supporting Information).[1,2] TSPO (subtype 1) is an essential protein in mammals, and its knockout is lethal during the early days of gestation.[3] Human TSPO is strongly overexpressed in several cancers, where it correlates with disease progression and poor prognosis,[4] and in activated immune cells, particularly in the brain.[5] In contrast, TSPO levels are diminished in platelets of patients with several psychiatric disorders.[5] A plethora of synthetic ligands targeting TSPO have been developed to image tumors and inflammatory processes,[6] and some ligands have also proven to be therapeutic, particularly because of their anxiolytic effects.[5] TSPO is key to the delivery of cholesterol to the mitochondrial inner membrane, a rate-limiting step of steroidogenesis.[3] However, the cholesterol recognition motif[7,8] is not conserved in bacterial TSPOs (Figure S1B), leaving the question of the ancestral role of TSPOs unanswered. In both eukaryotes and prokaryotes, TSPOs interact with porphyrins[9,10] and bacterial TSPO genes are often part of the bacteriochlorophyll and carotenoid biosynthetic operons, though they are not required for biosynthesis. Hence, it has been suggested that TSPOs transport porphyrins.[2]

To probe the function of TSPOs *in vitro*, we identified a bacterial homologue from *Chlorobium tepidum*, $TSPO_{Ct}$, that retained its structure in detergent (Figure S2A), could be purified to homogeneity, and remained folded upon purification (Figure 1A). Surprisingly, $TSPO_{Ct}$ induced rapid spectral changes in the potential substrate protoporphyrin IX (PPIX). In particular, the Soret absorption band that is due to the conjugated bond system of the porphyrin heterocycle was nearly eliminated (Figure 1B). Chromatographic analysis showed one major product that was more polar than PPIX (Figure 1C) with spectral properties reminiscent of those of biliverdin, a linear tetrapyrrole (Figure S2B). The reaction was also associated with the loss of PPIX fluorescence (Figure S2C), allowing us to examine the activity of unpurified TSPOs from other bacterial species expressed in *Escherichia coli*, which lacks an endogenous TSPO. Two other bacterial TSPOs showed similar activity (Figure S2D).

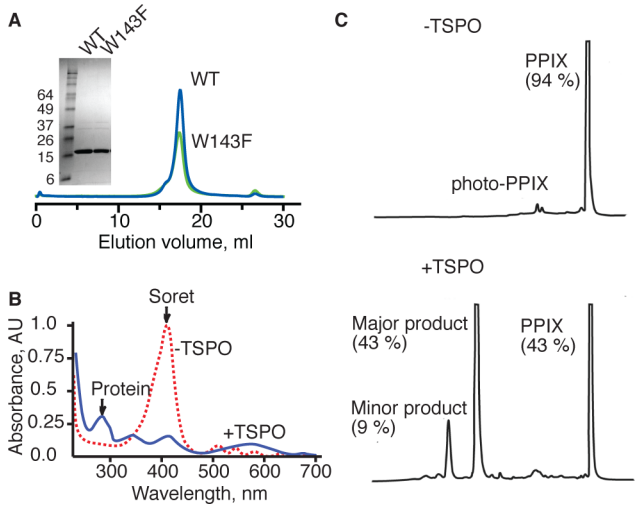

Figure 1. Characterization of $TSPO_{Ct}$. (A) Size exclusion chromatography and sodium dodecyl sulfate−polyacrylamide gel electrophoresis analysis (inset) of the wild type (WT) and W143F mutant. (B) Absorption spectrum of PPIX incubated with and without $TSPO_{Ct}$. (C) HPLC chromatogram of PPIX before and after partial reaction with $TSPO_{Ct}$. The fractions of the total area under the curve are shown in parentheses next to the peaks.

The $TSPO_{Ct}$-mediated reaction rate was proportional to protein concentration and dependent on light and oxygen (Figure 2A,B and Figure S3A−E). These results suggest that the process was oxidative in nature and mediated by reactive oxygen species generated when energy was transferred from photoexcited PPIX. Notably, singlet oxygen, the most abundant reactive oxygen species, may not be involved because addition of azide or conducting the experiments in deuterium oxide[11] had no effect (Figure S3F). Importantly, spontaneous PPIX photo-oxidation is inefficient in solution and occurs on the vinyl groups in R2 positions (Figure S4A).[12] In contrast, the $TSPO_{Ct}$-mediated reaction was comparatively rapid (Supplementary Discussion and Figure S5), resulted in drastic spectral changes, and occurred in porphyrins lacking vinyl groups (Figure S4A−C). Interestingly, a metalloporphyrin Zn(II)-

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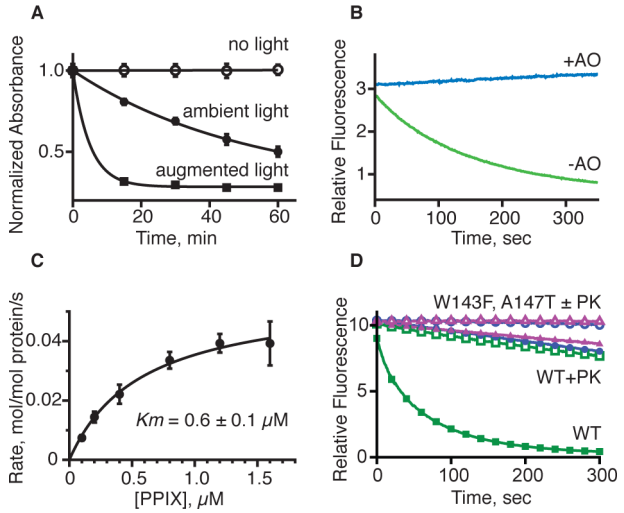

Figure 2. Reaction requirements. (A) PPIX (10 μM) absorbance at 410 nm in the presence of 5 μM $TSPO_{Ct}$ under variable light. (B) PPIX (10 μM) fluorescence in the presence of 1 μM $TSPO_{Ct}$ with and without the ascorbate oxidase oxygen scavenging system (AO). (C) Concentration dependence of the initial rate of PPIX oxidation. K_m was determined by fitting to the Michaelis–Menten equation. (D) PPIX (10 μM) fluorescence recorded in the presence of 1 μM WT $TSPO_{Ct}$ (green) and the W143F (blue) or A147T (purple) mutant in the absence and presence of 10 μM PK11195 (PK).

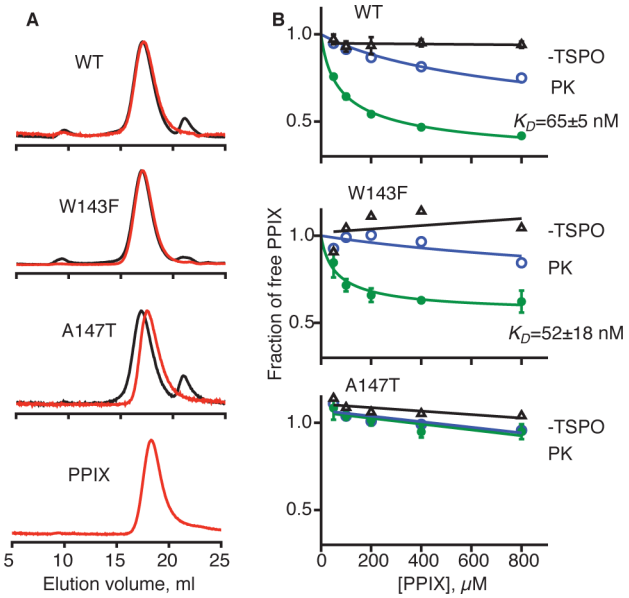

Figure 3. PPIX binding. (A) Size exclusion chromatography of $TSPO_{Ct}$ variants co-injected with PPIX. The protein and PPIX were followed by absorbance at 280 nm (black) and 410 nm (red), respectively. Elution of PPIX alone in detergent micelles is also shown. (B) Equilibrium binding of PPIX in the absence and presence of 10 μM PK11195. Controls in the absence of protein are also shown. The data for WT and the W143F mutant were fit to the quadratic binding equation (eq S2) with the dissociation constants (K_D) shown next to the graphs.

PPIX, but not Sn(II)PPIX or heme, also served as a $TSPO_{Ct}$ substrate (Figure S4D). Finally, each $TSPO_{Ct}$ molecule mediated multiple rounds of PPIX oxidation (Supplementary Discussion) with a K_m of ~0.6 μM in a 1 mM *n*-dodecyl β-D-maltopyranoside (DDM) solution (Figure 2C). Collectively, our data suggest that $TSPO_{Ct}$ provides a catalytic mechanism and is a bona fide enzyme.

The $TSPO_{Ct}$-mediated reaction was inhibited by a ligand of the mammalian TSPOs, isoquinoline carboxamide PK11195,[13] and by mutations of residues conserved between $TSPO_{Ct}$ and the mammalian TSPOs, W143F and A147T [human TSPO numbering (Figure S1 and Figure 2D)]. During size exclusion chromatography, PPIX co-eluted with WT $TSPO_{Ct}$ and with the W143F mutant but trailed behind the A147T mutant, suggesting that the latter has a reduced affinity for PPIX (Figure 3A). Also in equilibrium binding experiments, in which we measured the fraction of free PPIX after removal of the His-tagged $TSPO_{Ct}$ with immobilized metal affinity beads, the A147T mutant failed to bind PPIX tightly. In contrast, both WT protein and W143F mutant bound PPIX with affinities of 50–70 nM in 1 mM DDM. PK11195 inhibited this binding. On average, the apparent number of PPIX binding sites was 0.5 ± 0.1 (n = 10), suggesting that either not all protein was active or one PPIX molecule binds per $TSPO_{Ct}$ dimer. Consistently, a low-resolution electron microscopy structure of a *Rhodobacter sphaeroides* TSPO showed dimeric assembly.[14]

The A147T mutation mimics a known human single-nucleotide polymorphism associated with reduced steroid hormone levels,[15] a lower affinity for a class of synthetic ligands,[16,17] and increased instances of psychiatric disorders.[18,19] Our results suggest that the A147T mutation disrupts the PPIX binding site, which may be shared with these synthetic ligands.

The W143F mutation has no effect on protein stability (Figure 1A) or PPIX binding but abrogates PPIX degradation. Remarkably, W143 is conserved in >99% of all TSPO sequences, suggesting that W143 is essential for function in all TSPOs and hinting that the catalytic activity that we observe in bacterial TSPOs is a conserved property. Previous experiments suggest that TSPOs from diverse organisms participate in porphyrin metabolism, possibly with the involvement of reactive oxygen species. For example, rapid degradation of excess PPIX in mammalian cells depends on both TSPO and reactive oxygen species, although it is unclear whether TSPO acts on PPIX directly.[20] In *R. sphaeroides*, TSPO regulates expression of bacteriochlorophyll biosynthetic genes by controlling levels of unidentified tetrapyrroles in response to the simultaneous presence of light and oxygen.[10] Remarkably, mammalian TSPO mimics this role when expressed in a *tspo* knockout bacterial strain.[21]

In summary, our data suggest that TSPOs catalyze degradation of PPIX with the consumption of reactive oxygen species, which may originate from photoexcitation of PPIX itself, as in our *in vitro* reactions, or from cellular processes, including malfunctioning electron transfer chains and enzymatic reactions. This role of TSPO is consistent with its overexpression in bacteria and plants under stress.[22−25] Decreasing cellular levels of porphyrins could protect cells of diverse evolutionary origin from oxidative damage by decreasing the levels of pro-oxidant heme and, perhaps, by generating signaling molecules in a manner similar to that of heme oxygenase-1.[26] We further hypothesize that cholesterol transport by TSPOs may have evolved as an additional functionality in higher eukaryotes. Such "moonlighting" is not rare in biology[27] and has been observed in other membrane proteins.[28]

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■ ASSOCIATED CONTENT

Supporting Information

Methods, Supplementary Discussion, Figures S1−S5, and references. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

Accession numbers of the genes used: AAM72885, AAB89774, AAF24291, AAC00363, and AF65585.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Snyder, S. H., Verma, A., and Trifiletti, R. R. (1987) *FASEB J. 1*, 282−288.

(2) Papadopoulos, V., Baraldi, M., Guilarte, T. R., Knudsen, T. B., Lacapere, J. J., Lindemann, P., Norenberg, M. D., Nutt, D., Weizman, A., Zhang, M. R., and Gavish, M. (2006) *Trends Pharmacol. Sci. 27*, 402−409.

(3) Papadopoulos, V., Amri, H., Boujrad, N., Cascio, C., Culty, M., Garnier, M., Hardwick, M., Li, H., Vidic, B., Brown, A. S., Reversa, J. L., Bernassau, J. M., and Drieu, K. (1997) *Steroids 62*, 21−28.

(4) Mukherjee, S., and Das, S. K. (2012) *Curr. Mol. Med. 12*, 443−457.

(5) Rupprecht, R., Papadopoulos, V., Rammes, G., Baghai, T. C., Fan, J., Akula, N., Groyer, G., Adams, D., and Schumacher, M. (2010) *Nat. Rev. Drug Discovery 9*, 971−988.

(6) Scarf, A. M., and Kassiou, M. (2011) *J. Nucl. Med. 52*, 677−680.

(7) Li, H., Yao, Z., Degenhardt, B., Teper, G., and Papadopoulos, V. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 1267−1272.

(8) Jamin, N., Neumann, J. M., Ostuni, M. A., Vu, T. K., Yao, Z. X., Murail, S., Robert, J. C., Giatzakis, C., Papadopoulos, V., and Lacapere, J. J. (2005) *Mol. Endocrinol. 19*, 588−594.

(9) Verma, A., Nye, J. S., and Snyder, S. H. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 2256−2260.

(10) Yeliseev, A. A., and Kaplan, S. (1999) *J. Biol. Chem. 274*, 21234−21243.

(11) Dalton, J., McAuliffe, C. A., and Slater, D. H. (1972) *Nature 235*, 388.

(12) Cox, G. S., and Whitten, D. G. (1982) *J. Am. Chem. Soc. 104*, 516−521.

(13) Pike, V. W., Halldin, C., Crouzel, C., Barre, L., Nutt, D. J., Osman, S., Shah, F., Turton, D. R., and Waters, S. L. (1993) *Nucl. Med. Biol. 20*, 503−525.

(14) Korkhov, V. M., Sachse, C., Short, J. M., and Tate, C. G. (2010) *Structure 18*, 677−687.

(15) Costa, B., Pini, S., Gabelloni, P., Da Pozzo, E., Abelli, M., Lari, L., Preve, M., Lucacchini, A., Cassano, G. B., and Martini, C. (2009) *Endocrinology 150*, 5438−5445.

(16) Owen, D. R., Yeo, A. J., Gunn, R. N., Song, K., Wadsworth, G., Lewis, A., Rhodes, C., Pulford, D. J., Bennacef, I., Parker, C. A., StJean, P. L., Cardon, L. R., Mooser, V. E., Matthews, P. M., Rabiner, E. A., and Rubio, J. P. (2011) *J. Cereb. Blood Flow Metab. 32*, 1−5.

(17) Kreisl, W. C., Jenko, K. J., Hines, C. S., Hyoung Lyoo, C., Corona, W., Morse, C. L., Zoghbi, S. S., Hyde, T., Kleinman, J. E., Pike, V. W., McMahon, F. J., and Innis, R. B. (2012) *J. Cereb. Blood Flow Metab. 33*, 53−58.

(18) Nakamura, K., Yamada, K., Iwayama, Y., Toyota, T., Furukawa, A., Takimoto, T., Terayama, H., Iwahashi, K., Takei, N., Minabe, Y., Sekine, Y., Suzuki, K., Iwata, Y., Pillai, A., Nakamoto, Y., Ikeda, K., Yoshii, M., Fukunishi, I., Yoshikawa, T., and Mori, N. (2006) *Am. J. Med. Genet., Part B 141B*, 222−226.

(19) Costa, B., Pini, S., Martini, C., Abelli, M., Gabelloni, P., Landi, S., Muti, M., Gesi, C., Lari, L., Cardini, A., Galderisi, S., Mucci, A., Lucacchini, A., and Cassano, G. B. (2009) *Psychiatr. Genet. (London, U.K.) 19*, 110−111.

(20) Zeno, S., Veenman, L., Katz, Y., Bode, J., Gavish, M., and Zaaroor, M. (2012) *Curr. Mol. Med. 12*, 494−501.

(21) Yeliseev, A. A., Krueger, K. E., and Kaplan, S. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 5101−5106.

(22) Davey, M. E., and de Bruijn, F. J. (2000) *Appl. Environ. Microbiol. 66*, 5353−5359.

(23) Anthony, J. R., Warczak, K. L., and Donohue, T. J. (2005) *Proc. Natl. Acad. Sci. U.S.A. 102*, 6502−6507.

(24) Frank, W., Baar, K. M., Qudeimat, E., Woriedh, M., Alawady, A., Ratnadewi, D., Gremillon, L., Grimm, B., and Reski, R. (2007) *Plant J. 51*, 1004−1018.

(25) Guillaumot, D., Guillon, S., Deplanque, T., Vanhee, C., Gumy, C., Masquelier, D., Morsomme, P., and Batoko, H. (2009) *Plant J. 60*, 242−256.

(26) Gozzelino, R., Jeney, V., and Soares, M. P. (2010) *Annu. Rev. Pharmacol. Toxicol. 50*, 323−354.

(27) Jeffery, C. J. (2003) *Trends Genet. 19*, 415−417.

(28) Menon, I., Huber, T., Sanyal, S., Banerjee, S., Barre, P., Canis, S., Warren, J. D., Hwa, J., Sakmar, T. P., and Menon, A. K. (2011) *Curr. Biol. 21*, 149−153.