Purification and reconstitution of the antigen transport complex TAP: A prerequisite for determination of peptide stoichiometry, ATP binding and hydrolysis

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Supplemental Information

Supplement Methods

Detergent screen for TAP solubilization-For the detergent screen, the Anatrace soluble detergent kit (Anatrace, Maumee, Ohio, USA) was used, which comprises 114 different detergents as 10% (w/v) stock solution. Detergents with very high CMC were excluded from the screen. TAP containing microsomes (3 mg/ml of protein) in 1x screening buffer (1x PBS, 15% glycerol) were thawed on ice. To standardize the immunoblot signals, 18 µl of microsomes were mixed with 30 µl 3x SDS sample buffer and 42 µl 1x PBS incubated for 20 min at 65°C and stored at -20°C. The rest of the microsomes were divided into 100 µl aliquots. The aliquots were centrifuged at 20,000x g for 6 min at 4°C. The double of the volume of the detergent to add, reaching a ρ-value of two, was removed from the supernatant. The ρ-value is defined as:

$$\rho = \frac{[\mathrm{det}\,ergent] - CMC}{[lipid]}$$

The microsomal lipid concentration was derived from the protein concentration under the assumption of a 1:1 lipid-to-protein ratio (w/w). Subsequently, equal volumes of 10% detergent solution and 2x screening buffer as well as 1 µl of protease inhibitor mix (5 mg/ml AEBSF hydrochloride, 1 mg/ml leupeptin, 0.1 mg/ml aprotinin, 0.5 mg/ml pepstatin A, 15.6 mg/ml benzamidine) were added to end up with a total volume of 100 µl, in which the microsomal pellet was resuspended. After incubation for 30 min at 4°C, the samples were centrifuged at 110.000x g for 30 min at 4°C. 12 µl of the supernatant were mixed with 20 µl of 3x SDS sample buffer and 28 µl 1x PBS, incubated for 20 min at 65°C and stored at -20°C. To follow the long-term stability of TAP, the rest of the supernatant was stored for three days at 4°C. Subsequently, the samples were again centrifuged and an aliquot was treated in the same way for immunoblot analysis as mentioned above. 10 µl of each sample was analyzed by SDS-PAGE (9%), electrotransfered on a PVDF membrane, and immunostained with monoclonal α-TAP1 (148.3) and α-TAP2 (435.3) antibodies. After incubation with horseradish peroxidase coupled anti-mouse antibodies, the membrane was developed by enhanced chemiluminescence and signals were detected and quantified by a Lumi-Imager F1 (Roche, Mannheim, Germany).

Supplemental Figure 1: Peptide binding affinity determined by fluorescence quenching. To analyze peptide binding under equilibrium conditions, quenching of the fluorescence ($\lambda_{ex/em}$ = 470/515 nm) was followed by adding 16 nM of TAP to increasing concentrations of fluorescein-labeled peptide (RRYC$^{(F)}$KSTEL) at 10°C. **A**) Reversibility of peptide binding to TAP. Binding of RRYC$^{(F)}$KSTEL (60 nM) was initiated by adding TAP. After reaching equilibrium, dissociation was started by the addition of 400-fold molar excess of competitor peptide RRYQKSTEL. **B**) Association kinetics at different peptide concentrations. Fluorescence quenching was recorded at different peptide

concentrations and was fitted by a mono-exponential function. **C**) Peptide affinity of TAP. Amplitudes, derived from mono-exponential fits, were plotted against peptide concentration and fitted by a Langmuir (1:1) isotherm. A dissociation constant K_D of 30 ± 5 nM was obtained.

Supplemental Figure 2: ATP hydrolysis kinetics of TAP. The release of inorganic phosphate was determined by the malachite green assay after incubation of 150 nM of purified (open circles) or reconstituted TAP (filled circles) for 10 min at 37°C in the appropriate buffer with or without digitonin, respectively. The peptide specific ATP hydrolysis was fitted by the Michaelis-Menten equation. **A)** ATP concentration dependent kinetics. Phosphate release was determined for increasing concentrations of ATP in the presence or absence of 1 µM high affinity peptide RRYQKSTEL. From the hyperbolic curve fit of peptide specific ATP hydrolysis a $K_{m,ATP}$ of 152 ± 30 µM for purified TAP and 94 ± 20 µM for reconstituted TAP was obtained. **B)** Peptide concentration dependent kinetics. Generation of inorganic phosphate was determined for increasing peptide concentrations in the presence of 3 mM ATP. Fitting the peptide specific ATP hydrolysis resulted in a $K_{m,peptide}$ of 131 ± 62 nM for purified TAP and 62 ± 33 nM for reconstituted TAP.

Supplemental Table S1: Detergent screen for TAP solubilization.

* percentage of TAP solubilized in comparison to TAP solubilized by SDS

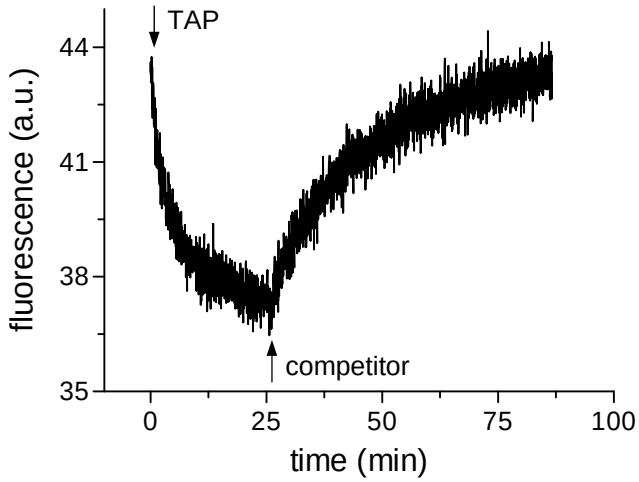
Detergent	Mw (g/mol)	CMC (mM)	[detergent] at ρ = 2 (mM)	TAP1 at day 0 (%)*	TAP1 at day 3 (%)*	TAP1 day 0 / day 3	TAP2 at day 0 (%)*	TAP2 at day 3 (%)*]	TAP2 day 0 / day 3
ANAMEG®-7	335.40	19.50	28.10	4.88	0.01	0.00	7.82	0.01	0.00
ANAPOE®-20	1228.00	0.06	8.66	0.24	0.02	0.08	0.34	0.10	0.29
ANAPOE®-35	1198.00	0.09	8.69	11.94	6.42	0.54	13.26	8.90	0.67
ANAPOE®-58	1122.00	0.00	8.60	52.04	51.13	0.98	55.40	54.67	0.99
ANAPOE®-80	1310.00	0.01	8.61	0.00	0.00	0.00	0.21	0.00	0.00
ANAPOE®-C10E6	423.00	0.90	9.50	0.00	0.00	0.00	2.00	0.49	0.25
ANAPOE®-C10E9	555.00	1.30	9.90	10.63	11.33	1.07	19.76	19.41	0.98
ANAPOE®-C12E8	539.00	0.01-0.09	8.69	157.26	199.15	1.27	48.66	40.80	0.84
ANAPOE®-C12E9	583.00	0.05	8.65	31.46	22.68	0.72	47.59	34.38	0.72
ANAPOE®-C12E10	627.00	0.20	8.80	27.88	17.16	0.62	45.18	35.58	0.79
ANAPOE®-C13E8	553.00	0.10	8.70	32.74	18.06	0.55	24.80	27.03	1.09
ANAPOE®-X-100	647.00	0.23	8.83	39.39	39.13	0.99	45.38	39.00	0.86
ANAPOE®-X-114	536.00	0.20	8.80	72.90	62.07	0.85	36.46	28.73	0.79
ANAPOE®-X-405	1967.00	0.81	9.41	0.00	0.00	0.00	0.00	0.00	0.00
ANZERGENT® 3-10	307.60	39.00	47.60	75.22	11.06	0.15	36.74	6.42	0.17
ANZERGENT® 3-12	335.50	2.80	11.40	24.75	73.16	2.96	43.09	42.27	0.98
ANZERGENT® 3-14	363.60	0.20	8.80	53.88	44.39	0.82	76.53	66.52	0.87
C-DODECAFOS™	349.17	22.00	30.60	27.01	2.24	0.08	3.31	0.30	0.09
C-HEGA®-9	363.50	108.00	116.60	11.58	1.75	0.15	28.50	2.12	0.07
C-HEGA®-10	377.50	35.00	43.60	9.97	0.09	0.01	34.42	0.60	0.02
C-HEGA®-11	391.50	11.50	20.10	29.41	0.02	0.00	40.19	1.49	0.04
CYCLOFOS™-3	306.91	43.00	51.60	0.00	0.00	0.00	0.00	0.00	0.00

CYCLOFOS™-5	334.95	4.50	13.10	61.05	38.72	0.63	80.60	52.15	0.65
CYCLOFOS™-6	349.17	2.68	11.28	61.05	67.89	1.11	91.13	82.26	0.90
CYCLOFOS™-7	363.30	0.62	9.22	79.47	69.47	0.87	95.97	85.48	0.89
CYGLU®-3	308.40	28.00	36.60	0.00	0.00	0.00	0.00	0.00	0.00
CYMAL®-2	452.50	120-104	112.60	26.45	8.93	0.34	38.48	12.90	0.34
CYMAL®-3	466.50	34.5-29	43.10	38.11	4.59	0.12	50.39	7.19	0.14
CYMAL®-4	480.50	7.6-7.3	16.20	28.09	5.59	0.20	43.33	11.69	0.27
CYMAL®-5	494.50	2; 2.4-5	13.60	39.94	16.81	0.42	46.75	25.69	0.55
CYMAL®-6	508.50	0.56	9.16	51.30	28.66	0.56	51.07	32.54	0.64
CYMAL®-7	522.50	0.19	8.79	42.41	37.32	0.88	35.37	62.70	1.77
CYPFOS™-3	293.30	180.00	188.60	0.00	0.00	0.00	0.00	0.00	0.00
2,6-Dimethyl-4-heptyl-b-D-maltopyranoside	472.21	27.50	36.10	0.00	0.00	0.00	0.00	0.00	0.00
2-Propyl-1-pentyl maltopyranoside	455.50	42.50	51.10	0.00	0.00	0.00	0.00	0.00	0.00
FOS-CHOLINE®-8	295.40	114.00	122.60	8.68	6.96	0.80	9.36	10.07	1.08
FOS-CHOLINE®-9	309.40	39.50	48.10	69.62	70.68	1.02	61.90	64.29	1.04
FOS-CHOLINE®-10	323.40	11.00	19.60	63.62	63.31	1.00	57.06	42.25	0.74
FOS-CHOLINE®-11	337.40	1.85	10.45	92.80	84.80	0.91	120.28	104.72	0.87
FOS-CHOLINE®-12	351.50	1.50	10.10	123.20	105.60	0.86	90.09	85.38	0.95
FOS-CHOLINE®-13	365.50	0.75	9.35	92.80	92.00	0.99	121.70	73.58	0.60
FOS-CHOLINE®-14	379.50	0.12	8.72	116.80	106.40	0.91	76.42	120.28	1.57
FOS-CHOLINE®-15	393.50	0.07	8.67	83.73	75.90	0.91	95.35	69.77	0.73
FOS-CHOLINE®-16	407.50	0.01	8.61	92.77	65.06	0.70	82.56	72.67	0.88
FOS-CHOLINE®-ISO-9	309.00	32.00	40.60	0.00	0.00	0.00	0.00	0.00	0.00
FOS-CHOLINE®-ISO-11	337.40	26.60	35.20	0.00	0.00	0.00	0.00	0.00	0.00
FOS-CHOLINE®-ISO-11-6U	337.16	25.80	34.40	0.00	0.00	0.00	0.00	0.00	0.00
FOS-CHOLINE®-UNSAT-11-10	335.40	6.20	14.80	60.45	50.23	0.83	42.76	33.26	0.78
FOS-MEA®-8	267.01	22.00	30.60	0.00	0.00	0.00	0.00	0.00	0.00

FOS-MEA®-10	295.01	5.25	13.85	49.34	11.48	0.23	35.23	0.00	0.00
FOSFEN™-9	385.21	1.35	9.95	27.84	0.00	0.00	54.31	0.00	0.00
FOSFENETH™-4	343.18	13.80	22.40	29.02	0.00	0.00	60.34	0.00	0.00
HEGA®-8	351.50	109.00	117.60	0.00	0.00	0.00	0.00	0.00	0.00
HEGA®-9	365.50	39.00	47.60	0.00	0.00	0.00	0.00	0.00	0.00
HEGA®-10	379.50	7.00	15.60	38.62	13.46	0.35	57.22	7.02	0.12
MEGA-8	321.40	79.00	87.60	0.00	0.00	0.00	0.00	0.00	0.00
NOPOL-FOS™	331.40	42.50	51.10	6.85	0.00	0.00	18.37	3.23	0.18
Decyltrimethylammoniumchloride	236.00	2.95	11.55	25.04	0.00	0.00	47.29	0.00	0.00
Dodecyltrimethyl-ammoniumchloride	263.89	0.05	8.65	15.97	12.98	0.81	5.47	1.99	0.36
Hexadecyltrimethylammonium chloride	320.00	0.00	8.60	143.13	167.22	1.17	22.77	24.90	1.09
Octadecyltrimethylammonium chloride	348.10	0.03	8.63	0.00	0.00	0.00	0.00	0.00	0.00
Tetradecyltrimethylammonium chloride	279.90	0.03	8.63	98.99	36.48	0.37	29.03	21.09	0.73
n-Heptyl-β-D-glucopyranoside	278.40	70.00	78.60	0.00	0.00	0.00	0.00	0.00	0.00
n-Octyl-β-D-glucopyranoside	292.40	18-20; 23.4	31.60	0.00	0.00	0.00	0.00	0.00	0.00
n-Nonyl-β-D-glucopyranoside	306.40	3.5-6.5	15.10	0.00	0.00	0.00	0.00	0.00	0.00
n-Octyl-β-D-maltopyranoside	454.40	19.50	28.10	25.72	5.05	0.20	19.68	4.65	0.24
n-Nonyl-β-D-maltopyranoside	468.50	6.00	14.60	68.19	7.10	0.10	45.58	12.51	0.27
n-Decyl-β-D-maltopyranoside	482.60	1.80	10.40	41.60	16.33	0.39	31.24	15.38	0.49
n-Undecyl-α-D-maltopyranoside	496.60	0.58	9.18	61.43	16.46	0.27	27.90	11.92	0.43
n-Undecyl-β-D-maltopyranoside	496.60	0.59	9.19	95.38	59.34	0.62	35.17	33.20	0.94
n-Dodecyl-α-D-maltopyranoside	510.60	0.15	8.75	30.90	8.38	0.27	28.01	9.81	0.35
n-Dodecyl-β-D-maltopyranoside	510.60	0.12-0.17	8.77	38.28	28.68	0.75	26.85	24.54	0.91

n-Tridecyl-β-D-maltopyranoside	524.60	0.024-0.033	8.63	50.22	24.68	0.49	33.10	25.00	0.76
n-Tetradecyl-β-D-maltopyranoside	538.60	0.01	8.61	57.85	13.99	0.24	44.68	9.89	0.22
n-Heptyl-β-D-thioglucopyranoside	294.40	29.00	37.60	0.00	0.00	0.00	0.00	0.00	0.00
n-Octyl-β-D-thiomaltopyranoside	470.60	8.50	17.10	25.00	0.00	0.00	23.00	0.00	0.00
n-Nonyl-β-D-thiomaltopyranoside	484.60	3.20	11.80	24.00	0.00	0.00	x	x	x
n-Decyl-β-D-thiomaltopyranoside	498.60	0.90	9.50	22.00	7.70	0.35	53.60	16.12	0.30
n-Undecyl-β-D-thiomaltopyranoside	512.70	0.21	8.81	55.36	27.10	0.49	67.18	44.79	0.67
n-Dodecyl-β-D-thiomaltopyranoside	526.60	0.05	8.65	38.56	26.98	0.70	64.72	33.74	0.52
n-Decyl-N,N-dimethylglycine	243.40	19.00	27.60	75.54	23.22	0.31	59.51	29.87	0.50
n-Dodecyl-N,N-dimethylglycine	271.40	1.50	10.10	85.84	63.09	0.73	63.19	49.69	0.79
Sodium decanoyl sarcosine	265.40	52.00	60.60	133.82	115.17	0.86	67.76	31.28	0.46
Sodium dodecanoyl sarcosine	293.40	14.40	23.00	111.88	89.07	0.80	31.25	24.83	0.79
Octaethylene glycol monododecyl ether (C12E8)	538.77	0.09	8.69	75.05	70.66	0.94	26.40	21.63	0.82
Pentaethylene glycol monodecyl ether (C10E5)	378.55	0.81	9.41	26.25	0.00	0.00	17.01	0.00	0.00
Tetraethylene glycol monooctyl ether (C8E4)	306.45	8.00	16.60	0.00	0.00	0.00	0.00	0.00	0.00
Sucrose monododecanoate	524.61	0.30	8.90	30.14	11.81	0.39	28.50	17.64	0.62
Dimethyldecylphosphine oxide	218.30	4.66	13.26	8.86	0.00	0.00	0.00	0.00	0.00
n-Tetradecyl-N,N-dimethylamine-N-oxide (TDAO)	257.46	0.24-0.29	8.89	57.98	36.32	0.63	48.90	22.80	0.47
n-Dodecyl-N,N-dimethylamine-N-oxide (DDAO)	229.41	0.14; 1-2	10.60	53.72	79.79	1.49	49.13	36.55	0.74

A



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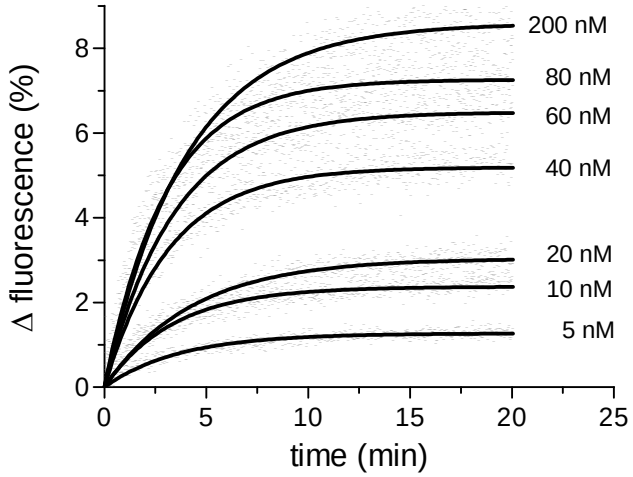

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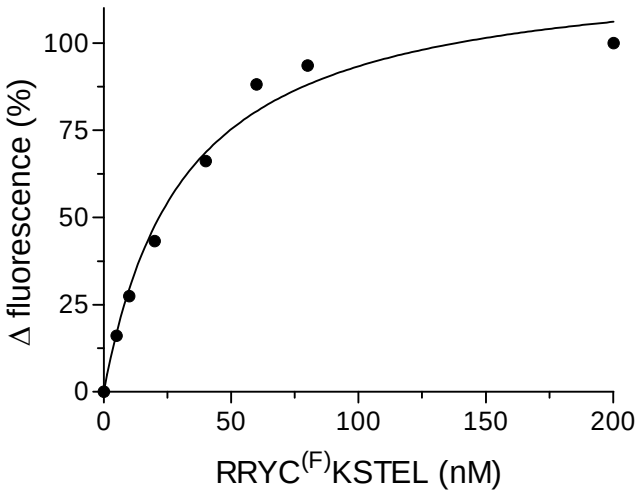

Supplement Figure 1 Herget et al.

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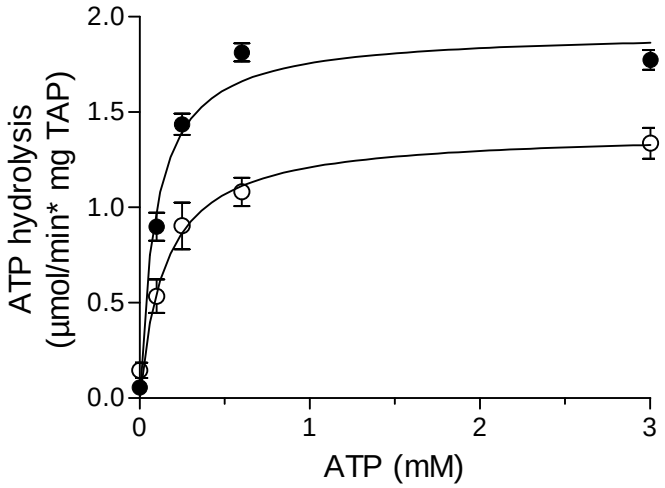

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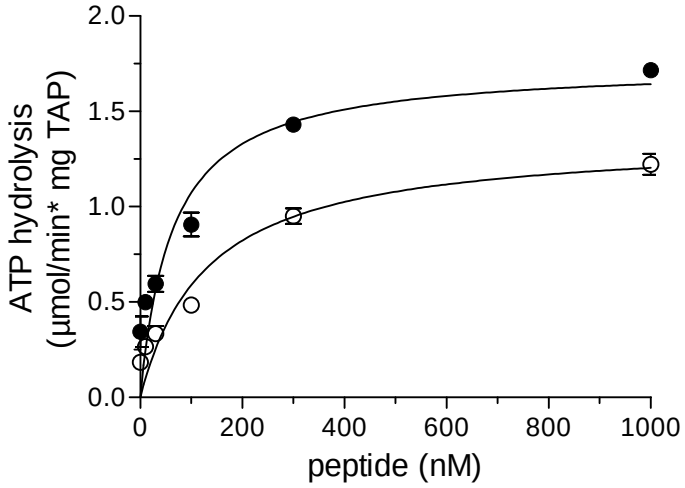

Supplement Figure 2 Herget et al.