

Supplementary information

A novel purification procedure for active recombinant human DPP4 and the inability of DPP4 to bind SARS_CoV_2

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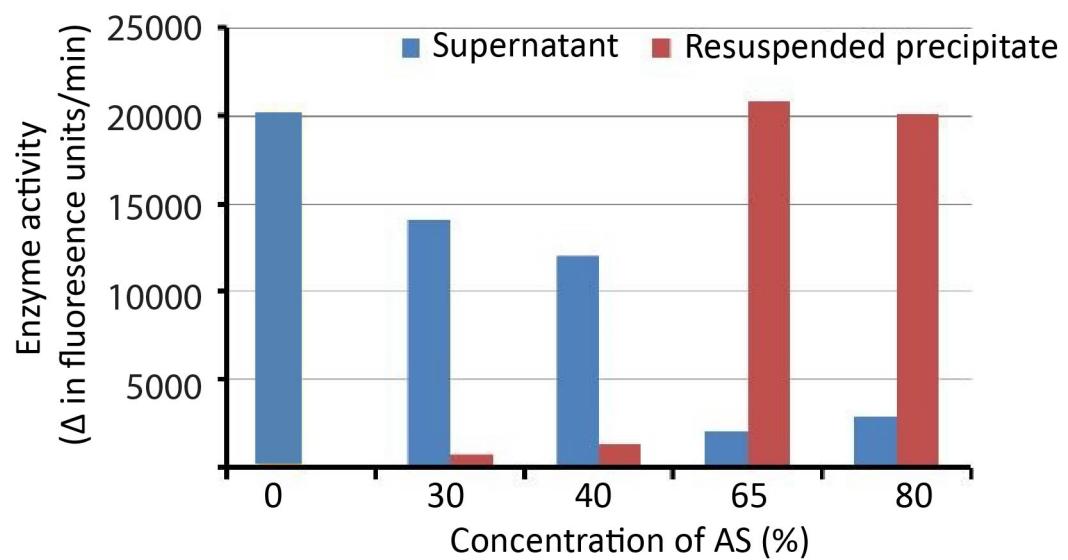
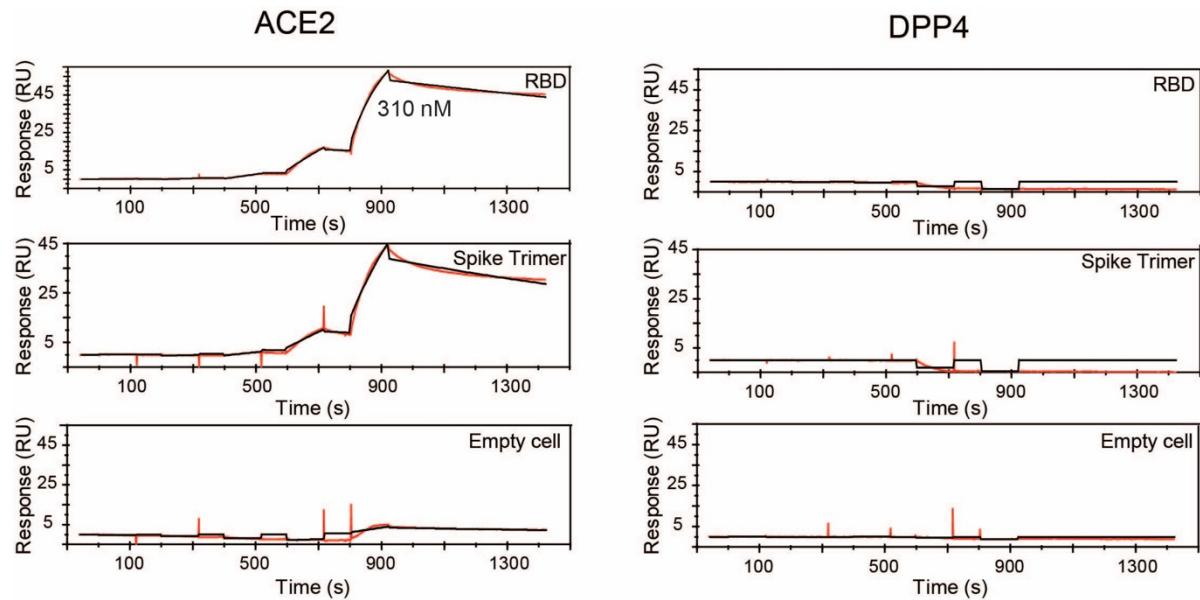


Figure S1: Precipitation of soluble DPP4 from culture supernatant at various saturation levels of ammonium sulfate. Solid ammonium sulfate (AS) at 0%, 35%, 40%, 65% and 80% (w/v) was added to Sf9 cell culture supernatant. DPP4 enzyme activity was measured in the supernatant and re-suspended precipitate.

Figure S2. Surface plasmon resonance assay characterising binding between DPP4 and SARS-CoV-2 RBD and spike with a biotin CAPture chip. Experimental data are shown in red. Calculated fits using a 1:1 binding model are shown in black. Gradient concentrations of (A) ACE2 at ACE2 at 0.50 nM, 2.5 nM, 12 nM, 62 nM and 310 nM and (B) DPP4 at 1.6 nM, 8.0 nM, 40 nM, 200 nM and 1000 nM were flowed over the chip surface bound with RBD and spike glycoprotein.



ACE2	K_d	K_{on}	K_{off}
RBD	1.4×10^{-8}	2.8×10^4	3.8×10^{-4}
Spike	4.0×10^{-8}	1.5×10^4	6.0×10^{-4}

Supplementary Material A: Methods of assay and storage for purified recombinant soluble human DPP4 protein.

Dipeptidyl peptidase IV, EC#: 3.4.14.5, is also known as DPIV, DPP-IV, DPP4, adenosine deaminase binding protein (ADAbp), thymocyte activation molecule (THAM).

This is an active soluble form of human DPP-IV, residues 29-766. Six histidines were added at the C-terminus.

Molecular Weight: approximately 90,000 daltons [50]

Source:

The cDNA, Genbank ID M80536, was from human lymphocytes [51]. The expression construct was made by PCR from human liver RNA and expressed in a baculovirus / Sf9 insect cell system following the methods described by[33, 50].

Unit Definition:

One unit will produce 1.0 μ mole of p-nitroaniline from Gly-Pro p-nitroanilide per min in reaction buffer: 100 mM Tris-HCl at pH 7.6. All reagents were prepared in this reaction buffer.

The extinction co-efficient of pNA at 405 nm was $6.993 \text{ mM}^{-1} \text{ cm}^{-1}$. This was calculated from a pNA titration.

Assay in 96-well microplate [43, 49]

295 μ l H-Gly-Pro p-nitroanilide (Bachem: L-1880, or Sigma-Aldrich: G0513, at 1 mM) were added to 5 μ l DPP-IV sample (enzyme) in each well and the reaction was incubated at 37 degrees Celsius. The OD was monitored continuously at 405 nm from time zero until 10 minutes or until the OD exceeded 1.0. Enzyme activity was converted to the change in absorbance units per minute. OD (405 nm) was measured using a FLUOstar Omega microplate reader (BMG LABTECH). Enzyme activity (Units/mL) was calculated using an extinction coefficient of

6.993 in the formula:

[total vol of 300 μ l x change in OD / minutes] / [6.993 x 2.5 μ l sample volume].

Authentic pNA (Sigma cat# 185310) was used to calculate the extinction coefficient at 405 nm from a standard curve. pNA standard solutions (300 μ l) were prepared in reaction buffer.

Assay in cuvette:

0.42 ml GlyPro p-nitroanilide (Bachem: L-1880 or Sigma: G0513)(1 mM) was added to 0.005 ml of DPP-IV sample (enzyme) being tested in a 0.5 ml cuvette. The reaction was incubated at ambient temperature and the OD monitored continuously at 405 nm from time zero until 10 minutes or until the OD exceeds 1. The change in absorbance units per minute was calculated. OD (405 nm) was measured using a spectrophotometer. Enzyme activity (Units/ml) was calculated using the calculated extinction coefficient (E) in the formula:

[total vol of 0.425 ml x change in OD / minutes] / [E x 0.005 ml sample volume].

Authentic pNA (Sigma cat# 185310) was used to deduce extinction coefficient at 405 nm from standard curve. pNA standard solutions (0.425 ml) were prepared in the same reaction buffer defined above.

Storage temperature: -20 degrees Celsius [minus 40 to zero is acceptable, with glycerol]. Zero to +10 degrees Celsius is acceptable when the pure DPP-IV is stored without glycerol.

Shipping Temperature: zero to -20 degrees Celsius is preferred (minus 40 to plus 15 is acceptable, when the DPP-IV is with glycerol).

Suggested shelf-life: >1 year when stored appropriately. We have observed activity retained for more than 2 years.

Thermal stability/activity: unstable above 40 degrees Celsius.

Components of solution : 10 mM Tris-HCl pH 7.6, 200 mM NaCl, 1 mM EDTA, 10 % glycerol. May also contain 1 mM DTT.

Optimum pH: 7.4 (6.8 – 7.8)

Protein Concentration assay: Bradford microassay.

Some **DPP-IV Inhibitors[15]** (Kirby et al 2010): Sitagliptin (Merck – MK0431; Januvia), vildagliptin (Novartis – LAF237; Galvus), saxagliptin (Bristol-Meyers Squibb – BMS477118; Onglyza), alogliptin (Takeda – Syr322), linagliptin (Boehringer Ingelheim – BI1356; Tradjenta), teneligliptin (Daiichi Sankyo), trelagliptin (Takeda - SYR472), dutogliptin (Phenomix - PHX1149), gemigliptin (LG Life Sciences).

References

15. Kirby, M.S., Yu, D.M.T., O'Connor, S.P., & Gorrell, M.D. (2010). Inhibitor selectivity in the clinical application of dipeptidyl peptidase-4 inhibition. *Clinical Science*, 118(1), 31-41. doi:10.1042/CS20090047
33. Park, J., Knott, H.M., Nadvi, N.A., Collyer, C.A., Wang, X.M., Church, W.B., & Gorrell, M.D. (2008). Reversible inactivation of human dipeptidyl peptidases 8 and 9 by oxidation. *The Open Enzyme Inhibition Journal*, 1, 52-61. doi: 10.2174/1874940200801010052
43. Sinnathurai, P., Lau, W., Vieira de Ribeiro, A.J., Bachovchin, W.W., Englert, H., Howe, G., . . . & Gorrell, M.D. (2018). Circulating fibroblast activation protein and dipeptidyl peptidase 4 in rheumatoid arthritis and systemic sclerosis. *International Journal of Rheumatic Diseases*, 21(11), 1915-1923. doi:10.1111/1756-185X.13031
49. Keane, F.M., Yao, T.-W., Seelk, S., Gall, M.G., Chowdhury, S., Poplawski, S.E., . . . & Gorrell, M.D. (2014). Quantitation of fibroblast activation protein (FAP)-specific protease activity in mouse, baboon and human fluids and organs. *FEBS Open Bio*, 4, 43-54. doi:org/10.1016/j.fob.2013.12.001
50. Ajami, K., Pitman, M.R., Wilson, C.H., Park, J., Menz, R.I., Starr, A.E., . . . & Gorrell, M.D. (2008). Stromal cell-derived factors 1 alpha and 1 beta, inflammatory protein-10 and interferon-inducible T cell chemo-attractant are novel substrates of dipeptidyl peptidase 8. *FEBS Letters* 582(5), 819-825. doi:10.1016/j.febslet.2008.02.005
51. Tanaka, T., Duke-Cohan, J.S., Kameoka, J., Yaron, A., Lee, I., Schlossman, S.F., & Morimoto, C. (1994). Enhancement of antigen-induced T-cell proliferation by soluble CD26/dipeptidyl peptidase IV. *Proceedings of the National Academy of Sciences United States of America*, 91(8), 3082-6.