Biophysical Characterization of MR1 (K43A)/β2M Complex

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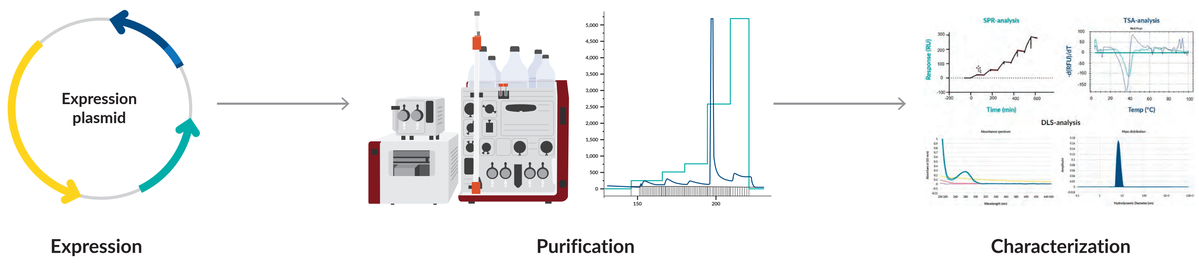
KEY FINDING

A stable, functional, and pure complex of MR1 (K43A)/β2M was isolated. SPR binding analysis confirms binding of complex to vitamin B metabolites.

ABSTRACT

MR1 (MHC-related protein 1) is an antigen-presenting molecule that presents small molecule ligands to mucosal-associated invariant T cells (MAIT cells). MR1 associates with β2-microglobulin (β2M) and can bind with microbial and non-microbial metabolites, which causes the MR1 molecule to translocate from the endoplasmic reticulum to the cell surface. Here we describe the expression and two-step purification of the MR1 (K43A)/β2M stable complex in which size exclusion chromatography (SEC) was able to separate aggregated protein from the stable heterodimer complex.[1] The complex showed minimal aggregation as observed in the thermal shift assay (TSA) and had a melting temperature of 42°C. In addition, the dynamic light scattering (DLS) data showed that 99.7% of the protein species in the complex showed a mean diameter of 7.7 nm. Furthermore, surface plasmon resonance (SPR) analysis confirmed binding of the small molecules diclofenac (sodium salt) (DCF), 6-formylpterin (6-FP) and acetyl-6-formylpterin (Ac-6-FP) with refolded MR1 (K43A)/β2M. Docking studies were performed to obtain predicted poses of the ligand binding to the complex.

EXPERIMENTAL WORKFLOW



RESULTS

Size Exclusion Chromatography and SDS-PAGE

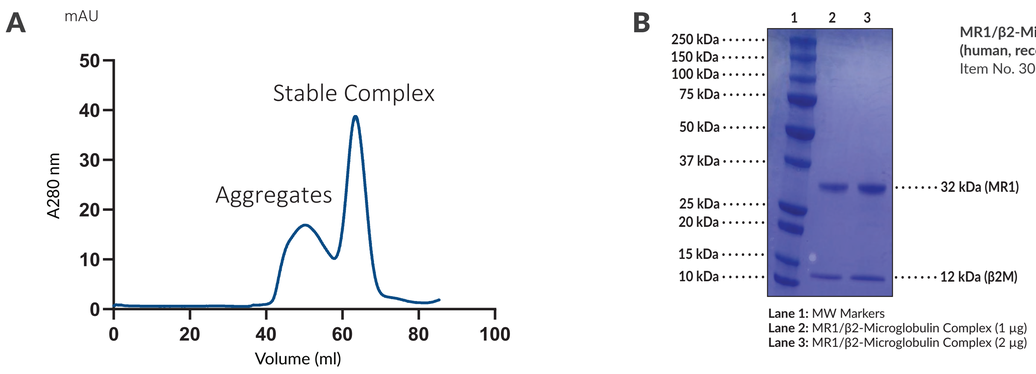


FIGURE 1 – SEC and SDS-PAGE analysis of the refolded MR1 (K43A)/β2M complex.
(A) Chromatogram of stable complex eluting at 62 ml retention volume. **(B)** The MR1 (K43A)/β2M complex separated by 4-20% SDS-PAGE. Bands at 32 kDa and 12 kDa correspond to the predicted molecular weights of MR1 and β2M, respectively.

Thermal Shift Assay

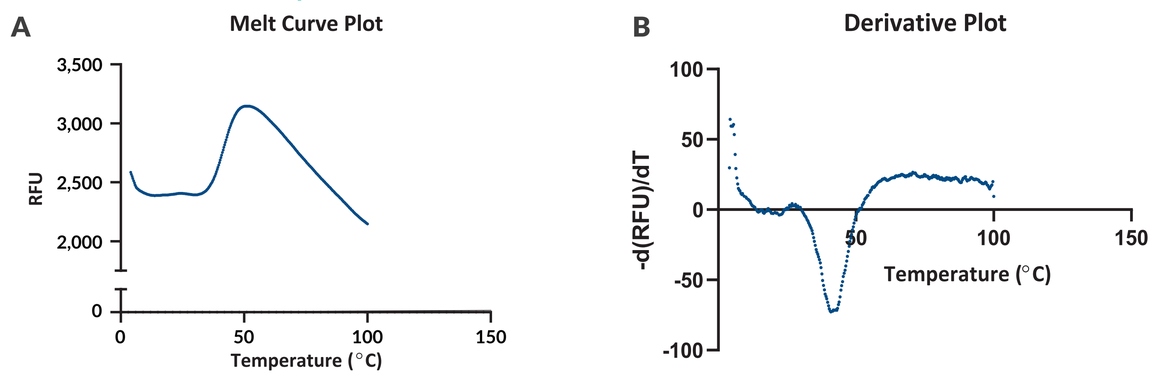


FIGURE 2 – TSA analysis of the refolded MR1 (K43A)/β2M complex.
50 µl of the MR1 (K43A)/β2M complex (~3 µg) was mixed with 0.25 µl SYPRO® Orange Protein Gel Stain (Sigma), and the mixture was heated in Bio-Rad CFX96™ Real-Time PCR system with an increment of 0.3°C/cycle. The thermal melt curve is well-defined and shows minimal aggregation. The melting temperature (T_m, °C) of the MR1 (K43A)/β2M complex is 42°C. **(A)** Relative fluorescent unit (RFU) *versus* temperature (°C). **(B)** Derivative plot.

Dynamic Light Scattering

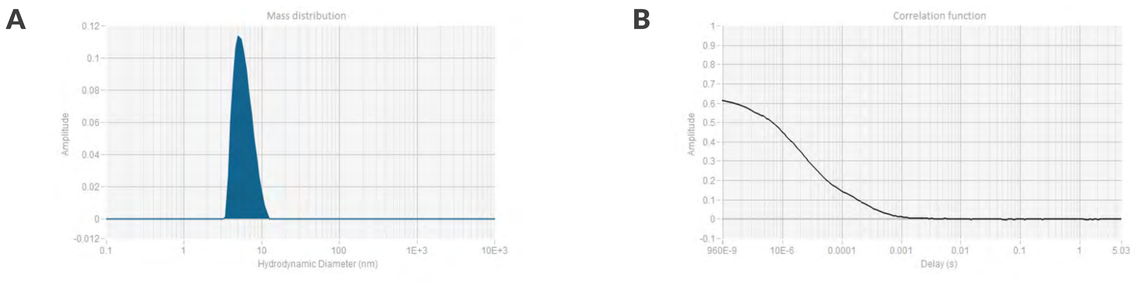


FIGURE 3 – DLS analysis of the refolded MR1 (K43A)/β2M complex.
99.7% of protein species had a hydrodynamic diameter of 7 nm. The predicted molecular weight of the complex was 40.9 kDa. The polydispersity index (PDI) is 0.28. **(A)** Mass distribution plot shows a sharp, single peak with minimal shoulder, indicating less aggregation and a uniform particle sizing. **(B)** Correlation function represents the signal-to-noise ratio, and the decay indicates that the protein complex in solution is minimally aggregated.

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RESULTS CONT.

SPR Binding Analysis

TABLE 1 – Variables obtained from SPR analysis using Biacore™ Insight Software.

	Diclofenac (sodium salt) (DCF)	6-Formylpterin (6-FP)	Acetyl-6-formylpterin (Ac-6-FP)	6,7-dimethyl-8-Ribityl-lumazine (DMRL)
Complex concentration	MR1/β2M 0.01 mg/ml	MR1/β2M 0.02 mg/ml	MR1/β2M 0.015 mg/ml	MR1/β2M 0.01 mg/ml
1:1 Binding k_a (M^{-1} s^{-1})	1.56e+03	8.55e+03	2.09e+04	NA
k_d (s^{-1})	4.48e-02	1.2e-02	1.28e-02	NA
K_D (M)	2.88e-05	1.41e-06	6.16e-07	NA

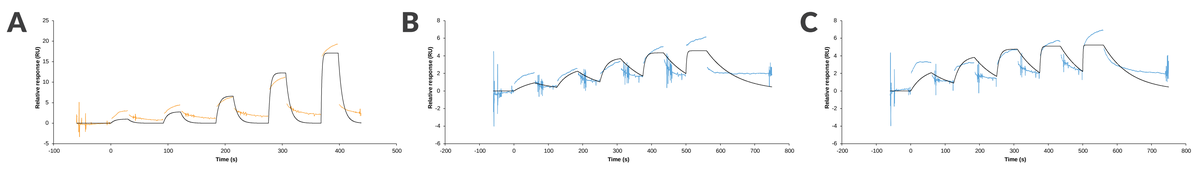


FIGURE 4 – SPR measurements of small molecules binding to MR1 (K43A)/β2M complex.
Sensorgrams of varying concentrations (up to 50 µM) of **(A)** DCF, **(B)** 6-FP, and **(C)** Ac-6-FP were flowed over a CM5 Sensor Chip (Cytiva) with MR1 (K43A)/β2M immobilized to the surface through an antibody capture method using αMR1 mAb clone 26.5 (BioLegend). DCF was used as a positive control. NA indicates data not available.

In Silico Docking Against MR1 (K43A)/β2M

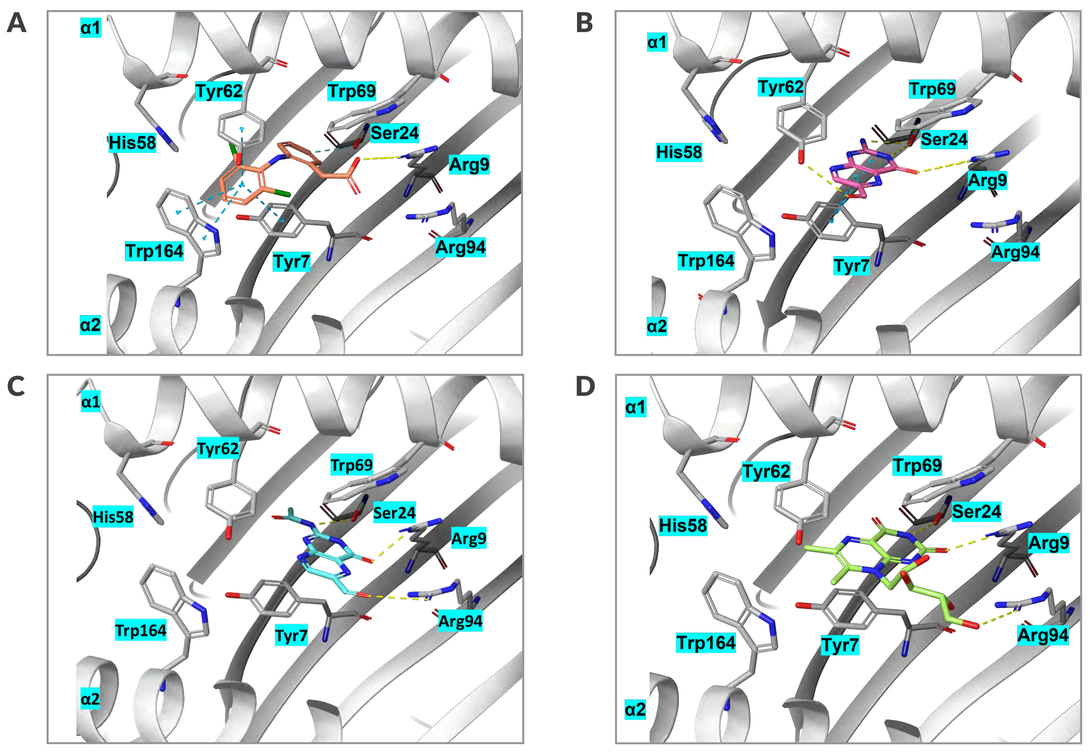
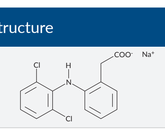
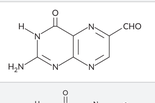
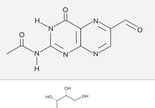
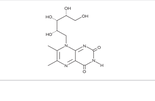


FIGURE 5 – *In silico* docking study of compounds against MR1 (K43A)/β2M complex.
Predicted poses of four different compounds docked against MR1 (K43A)/β2M complex (PDB ID 5D5M). *In silico* docking studies were performed using the Glide docking tool in Schrödinger Maestro 13 software.[2] The MR1 (K43A)/β2M complex is shown in grey, and compounds are shown in colored stick models. Key residues on the complex are shown as grey sticks. Compounds are predicted to bind in the antigen binding cleft, which is both polar and hydrophobic, with four aromatic residues. **(A)** DCF, **(B)** 6-FP, **(C)** Ac-6-FP, and **(D)** DMRL.

TABLE 2 – Docking results of different compounds against the MR1 (K43A)/β2M complex (PDB ID 5D5M).
Best pose was obtained for top hits using their Emodel values. Glide gscore is a prediction of ligand affinity, Emodel score is a measure of pose strength and validity, and MM/GBSA dG Bind is a prediction of ligand binding free energy.

	Structure	Glide gscore (kcal/mol)	Glide Emodel	MM/GBSA dG Bind
Diclofenac (sodium salt) (DCF)		-8.737	-69.813	-39.79
6-Formylpterin (6-FP)		-7.209	-54.236	-40.30
Acetyl-6-formylpterin (Ac-6-FP)		-8.049	-69.399	-50.48
6,7-dimethyl-8-Ribityllumazine (DMRL)		-7.869	-69.595	-59.42

CONCLUSIONS

- MR1 (K43A)/β2M complex was expressed in a bacterial system and purified.
- MR1 (K43A)/β2M was purified as a stable complex with minimal aggregation and had a melting temperature of 42°C.
- SPR binding analysis showed the interaction of DCF (positive control) and various vitamin B metabolites to MR1 (K43A)/β2M.
- Docking studies showed the predicted binding poses of the small molecules that showed binding in SPR.

References

1. Kjer-Nielsen, L., Patel, O., Corbett, A.J. *et al.* MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* **491(7426)**, 717–723 (2012).
2. Friesner, R.A., Murphy, R.B., Repasky, M.P., *et al.* Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J. Med. Chem.* **49(21)**, 6177–6196 (2006).