BIOCHEMICAL CHARACTERIZATION OF HUMAN GBP5

William Blass[1], **Taiwo Scholes Adewole**[1], **Qian Yin**[1,2]

[1]Department of Biological Science. [2]Institute of Molecular Biophysics

BACKGROUND

Guanylate-binding proteins (GBPs) are interferon-inducible large GTPases involved in the innate immune response. These proteins hydrolyze GTP and participate in cellular defense against intracellular pathogens. Although members of the GBP family share structural similarities, their enzymatic activity and biological roles differ. Understanding these differences can help clarify how individual GBPs contribute to immune signaling pathways.

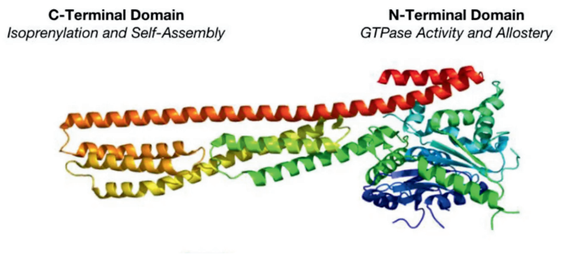

FIGURE 1. STRUCTURE OF GUANYLATE-BINDING PROTEINS. GBPS ARE LARGE INTERFERON-INDUCIBLE GTPASES COMPOSED OF AN N-TERMINAL LARGE GTPASE (LG) DOMAIN RESPONSIBLE FOR NUCLEOTIDE BINDING AND HYDROLYSIS, FOLLOWED BY A-HELICAL MIDDLE (MD) AND GTPASE EFFECTOR (GED) DOMAINS THAT REGULATE OLIGOMERIZATION AND FUNCTION. ADAPTED FROM HONKALA ET AL., 2020.

OBJECTIVE

The goal of this study was to:

- Express recombinant GBP5 in E. coli
- Purify GBP5 using multiple chromatography techniques
- Measure GTP hydrolysis using a malachite green phosphate assay
- Compare enzymatic activity between GBP1, GBP3, and GBP5

RECOMBINANT EXPRESSION OF GBP5

- Human GBP5 was cloned into a pET28α expression vector containing an N-terminal His-SUMO affinity tag to enable purification.
- The construct was expressed in E. coli and induced for recombinant protein production.
- Cells were lysed and the expressed protein was purified using sequential chromatographic methods.

FIGURE 2.PURIFICATION WORKFLOW FOR RECOMBINANT GBP5

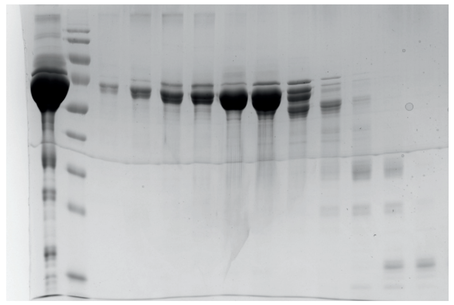

FIGURE 3. SDS-PAGE ANALYSIS OF GBP5FL PURIFICATION BY SIZE-EXCLUSION CHROMATOGRAPHY FOLLOWING Q ION EXCHANGE. LANE 1: LOAD AFTER Q COLUMN PURIFICATION. LANE 2: PAGERULER PROTEIN LADDER. LANES F1–F8: SEQUENTIAL SEC FRACTIONS. PEAK FRACTIONS (F3–F6) SHOW GBP5FL AT ~67 KDA, CONSISTENT WITH THE EXPECTED MOLECULAR WEIGHT.

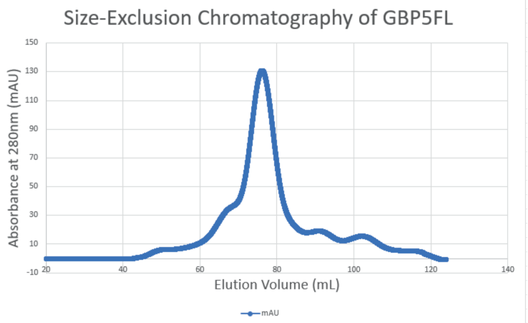

FIGURE 4. SIZE-EXCLUSION CHROMATOGRAPHY PROFILE OF GBP5FL FOLLOWING Q ION EXCHANGE PURIFICATION. PROTEIN ELUTION WAS MONITORED BY ABSORBANCE AT 280 NM. THE MAJOR PEAK AT ~75 ML CORRESPONDS TO GBP5FL, WITH PEAK FRACTIONS (F3–F6) USED FOR DOWNSTREAM ANALYSIS.

GTPase Activity Assay (Malachite Green)

GTP hydrolysis activity was measured using a malachite green phosphate detection assay. Purified GBP proteins (0.5 µM) were incubated with 200 µM GTP at 37 °C. Reactions were quenched with EDTA and malachite green reagent was added to detect released inorganic phosphate. Absorbance was measured at 620 nm and phosphate concentration was determined using a phosphate standard curve. Control reactions containing GTP alone or protein without GTP were included to account for spontaneous hydrolysis and background signal.

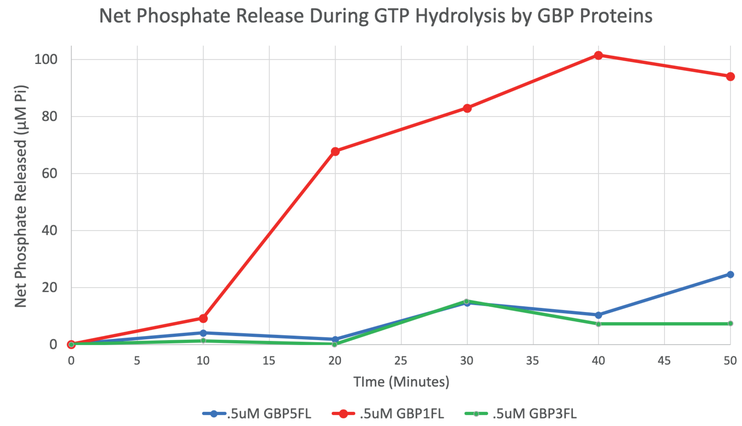

FIGURE 5. GTP HYDROLYSIS ACTIVITY OF GBP PROTEINS MEASURED USING A MALACHITE GREEN ASSAY. NET PHOSPHATE RELEASE WAS CALCULATED BY SUBTRACTING SPONTANEOUS GTP HYDROLYSIS MEASURED IN CONTROL REACTIONS. DATA REPRESENT THE MEAN OF REPLICATE EXPERIMENTS.

Key Findings/Results

Purified GBP5 exhibited measurable GTPase activity, confirming that the recombinant protein retained catalytic function following purification.

- GBP5 displayed relatively low GTP hydrolysis compared to GBP1, but showed slightly greater activity than GBP3 under the same assay conditions.
- These comparisons indicate that GBP family members possess different intrinsic catalytic efficiencies despite sharing a conserved GTP-binding domain.
- Previous studies suggest that GBP1 displays higher intrinsic GTPase activity due to enhanced catalytic efficiency and GTP-dependent oligomerization. GBP5 has also been reported to associate with intracellular membranes through prenylation, suggesting that membrane interactions may contribute to its catalytic activation. The reduced activity observed in vitro may therefore reflect the absence of membrane-associated regulatory factors.

References: Honkala et al., Cells (2020) • Britzen-Laurent et al., PLOS ONE (2010) • Kim et al., Nat Rev Immunol (2011) • Praefcke & Herrmann, Nat Rev Mol Cell Biol (2004) • Shenoy et al., Science (2012)