# Interferon-γ Induced Human Guanylate Binding Protein-2: A Path Less Discovered

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## Abstract

Interferon–gamma–inducible large GTPases, Guanylate Binding Proteins (GBPs) have recently emerged as a central player in host defense against viral, bacterial, and protozoan infections. Most of the studies encompass biochemical and biological functions of the human GBP-1 (hGBP-1). hGBP-2, a close homolog of hGBP-1, in recent times has gained attention due to its association with several carcinomas. The present mini-review provides a detailed understanding of the molecular mechanism of GTPase activity of this protein and also illustrates how it differs from hGBP-1 with respect to GMP formation. We also exchange our views on probable reasons for lower GMP formation in hGBP-2, which may have implications in its biological functions.

**Keywords:** Large GTPases; Human guanylate; Binding protein; Allosteric regulation; Conformational change; Dimerization

## Introduction

Guanylate-binding proteins (GBPs) are interferon-gamma-inducible large GTPases, found in all eukaryotes, from protists to vertebrates [1]. In humans, seven GBP genes and one pseudogene are identified and they cluster on chromosome 1q22.2 [2]. Unlike small GTPases, these proteins have low guanine nucleotides binding affinity and do not require GTPase-activating protein (GAP) to stimulate their enzymatic activity. Instead, GBPs trigger their activity in a cooperative manner by undergoing nucleotide-dependent self-assembly, a property they share with fellow members of the dynamin superfamily [3]. GBP's emerging role as a defence protein against a variety of pathogens, inflammatory diseases, and cancer has gained importance in recent times. In this short commentary, we describe structural, biochemical, and biophysical properties of GBPs, and later we integrate this knowledge to understand their biological functions.

## Literature Review

### Structure of GBPs

They are multi-domain large GTPases with molecular masses of about 65-73 kDa. In year 2000, the crystal structure of full-length human guanylate binding-protein-1 (hGBP-1) was solved in the absence (PDB 1DG3) [4] and presence of a non-hydrolysable GTP analogue, guanosine-5'-[(β, γ)-imido] triphosphate, GppNHp (PDB 1F5N) [5]. The basic architecture consists of an N-terminal GTP-binding domain and a C-terminal purely helical domain. These two domains are linked by an intermediate region, consisting of a α-helix and a two-stranded β-sheet. The GTP-binding domain is structurally homologous to small GTPases with conserved catalytic regions (Switch-I and Switch-II). However, due to certain insertions and deletions of residues, this domain is large in size. Based on the crystal structure, two unique regions are identified and termed as a phosphate cap and a guanine cap, which shield the γ-phosphate and guanine base binding site from the solvent, respectively. The C-terminal helical domain is further subdivided into three regions: Region-I (α7-α11), Region-II (α12), and Region-III (α13). Overall, α12 and α13 helices fold back and position next to the GTP-binding domain (α4) and interact electrostatically, which regulates GTPase activity [6]. hGBP-2 is a close homolog of hGBP-1 sharing nearly 78% sequence identity, thereby suggesting a similar domain architecture [7].

### Biochemical properties of GBPs

Similar to dynamin, GBPs share the properties of low binding affinity for guanine nucleotides (micromolar range), substrate-induced self-assembly, and high intrinsic rate of GTP hydrolysis [3–5]. Besides these properties, GBPs have a unique ability to hydrolyse GTP to both GDP and GMP through successive cleavages of the two phosphates [8,9]. However, the ratio of GMP to GDP formation varies among different isoforms [7-10]. Among the GBP family, the biochemical properties and biological functions of hGBP-1 have been studied to a great extent followed by murine GBP-2 [11], murine GBP-5 [12], hGBP-5 [10,13], and hGBP-2 [14-16], while little is known about others. We will be discussing hGBP-2 extensively using hGBP-1 as a model protein. hGBP-1, in contrast to other small GTPases, does not require GAP for its stimulated GTPase activity [5]. Instead, stimulation of GTPase activity happens due to conformational

changes in the GTP-binding domain triggered by substrate-induced protein assembly formation. Briefly, hGBP-1 is a monomer in solution and forms a dimer upon GTP binding. This dimer undergoes the first phosphate cleavage initiated by conformational changes in the GTP-binding domain led by its electrostatic interactions with α 12 helix of the helical domain. Once the ⊡-phosphate is cleaved, either the dimer dissociates yielding GDP and Pi, or remains in the dimeric form to undergo a second round of conformational change to form a tetramer [17]. The critical step for tetramer formation is the separation of α 12 helix from GTP-binding domain and their self-association, while the protein is still bound to GDP.Pi [18]. In the transition-state assembly (4 GDP.Pi. hGBP-1), the protein undergoes second phosphate cleavage resulting in GMP as the end product. Each step of assembly-induced phosphate cleavage is intensely associated with conformational changes in the GTP-binding domain mediated by the helical domain in a stepwise manner [17]. It is important to note that external GDP cannot act as a substrate for direct GMP formation [4]. Similar to hGBP-1, hGBP-2 is a monomer without the substrate and forms a dimer in the presence of GppNHp. hGBP-2 forms a mixture of monomer, dimer, and tetramer, with tetramer being the predominant form in the presence of GDP.AlF4, a transition–state analogue, which mimics GDP.Pi state, unlike hGBP-1 which forms only tetramer [14]. Contrary to our finding, another group suggested that hGBP-1 forms an extended dimer instead of a tetramer in solution [19]. This ambiguity could be due to the differences in experimental conditions (salt concentration) or techniques used for determining the molecular mass of the protein oligomer. Using cross-linking assay, we and the other group have shown the existence of hGBP-2 [14] and hGBP-1 [20] tetramer in cell-line experiments, suggesting that these proteins exist as a tetramer during the course of GTP hydrolysis. To simplify this issue, henceforth we describe the extended dimer or tetramer of hGBP-1 in the presence of GDP.AlF4 as GDP.AlF4-induced protein assembly.

Despite catalytic residues conservation, similar domain architecture, and oligomer forming propensity in these two homologs, a huge difference in the hydrolytic product formation is observed. GMP is the major product in hGBP-1 (~90%), whereas GDP predominates in hGBP-2 (~85%). In the absence of full-length hGBP-1 assembly structures, the mechanism of GTP hydrolysis is explained based on the structures of a truncated hGBP-1 (1-317 residues, lacking the helical domain) in the presence of various analogues. The protein is shown to form a head-to-head dimer involving the GTP-binding domain [21]. It was suggested that after first phosphate cleavage of GTP dimerization of the protein induces a movement of the nucleotide in such a manner that β-phosphate takes the position of ⊡-phosphate resulting in second phosphate cleavage i.e., GMP formation. Recently, we showed that after first phosphate cleavage, a stable H-bond between the indole moiety of Trp 79 (located near the catalytic site) and the main chain carbonyl of Lys 76 in Switch-I along with the movement of Trp 79 containing region repositions the catalytic machinery leading to stimulated GMP formation [22]. This makes hGBP-1 functionally distinct from other GTPases. We also showed that stimulated GMP formation is essential for antiviral activity against hepatitis C

[17]. hGBP-1 and hGBP-2 sequence comparison reveals that substrate-binding motifs, catalytic residues, and Trp 79 are conserved, thereby indicating a similar mechanism of GTP hydrolysis. However, in-depth sequence analysis reveals variations in certain regions and residues in these two proteins, which may be responsible for the lower GMP formation in hGBP-2. Variation in the G-cap is observed (10 out of total 19 residues show variations in hGBP-2) and this region is found close to the active site. Mutation of residues in the G-cap of hGBP-1 led to approximately 2- to 6-fold decrease in the GTPase activity [23]. Comparing the model structure of substrate-bound hGBP-2 with the crystal structure of hGBP-1 with GppNHp showed differences of structural change in the G-cap [14]. This may have resulted in a significantly less amount of GMP formation in hGBP-2. Another possibility is the absence of H-bond formation between the side chain of Trp 79 and main chain carbonyl of Lys 76, showing the lack of repositioning of catalytic machinery after the first phosphate cleavage. Besides these differences, variation in Region-II and Region-III of the C-terminal helical domain between these two proteins could play a role in reduced GMP formation.

Studies on hGBP-2 truncated variants suggested that Region-II (analogous to α 12 in hGBP-1) is critical for the GDP.AlF4-induced assembly formation [14]. This is true for hGBP-1 [24], and possibly for other hGBP homologs. Our study showed that the GDP.AlF4-induced assembly of hGBP-1 allosterically stimulates GTPase activity leading to enhanced GMP formation [17]. Although hGBP-2 forms GDP.AlF4-induced assembly, it has no role in GMP formation [14]. Disruption of salt-bridge contacts between the GTP-binding domain (Arg 227 and Lys 228) and helical domain (Glu in Region-II and Region-III) of hGBP-1 leads to a conformational change in the dimeric protein, a prerequisite condition for enhanced GMP formation [6,18]. Even though these residues are conserved in hGBP-2, some of their interactions are absent (unpublished data), which might have resulted in a conformationally altered GDP.AlF4-induced assembly as compared to hGBP-1. Our previous findings on hGBP-1 indicate that the intermediate region plays a significant role in GMP formation through dimerization [24,25]. However, a single residue variation in the intermediate region of hGBP-2 is not responsible for reduced GMP formation [14].

While the present review provides insight into lower GMP formation in hGBP-2, a detailed mechanistic understanding requires in-depth biochemical studies along with high-resolution protein structure determination and its extensive molecular dynamics simulations. Additionally, the biological functions of GDP.AlF4-induced assembly need to be explored.

## Discussion

### Future perspectives

The large GTPases superfamily consists of a variety of multi-domain proteins that differentiated from small GTPases on the basis of size and affinity for guanine nucleotides. Most of the proteins belonging to the large GTPases superfamily exhibit the property of self-assembly to form higher oligomers, essential for stimulated GTPase activity and their biological functions. These

proteins are primarily involved in membrane remodelling. However, within this superfamily there is a subset of proteins (Mx and GBPs), whose expression is induced by interferons, thereby providing protection against pathogens. The question that still remains, whether these proteins have the ability to remodel membranes? Do their anti-pathogenic activities depend on membrane remodelling property? In recent times, GBPs have emerged as vital proteins for their role in host defence against infectious diseases and cancer. Therefore, a detailed understanding into GTP hydrolysis mechanism and its effect on biological functions of each GBPs has become essential. Compared to hGBP-1, a comprehensive study of biochemical and biological functions of other hGBP homologs has been lacking. In this review, we described biochemical properties and mechanism of GTP hydrolysis of hGBP-2, and also illustrated how this protein differs from its close homolog hGBP-1. Additionally, we attempted to provide the underlying mechanism for lower GMP formation in hGBP-2.

## Conclusion

However, a detailed mechanistic understanding requires in-depth biochemical studies along with high-resolution protein structure determination and its extensive molecular dynamics simulations. The biological functions of GDP.AlF4-induced assembly also need to be explored.

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