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Expression and purification of the active variant of recombinant murine Golli-interacting protein (GIP)—characterization of its phosphatase activity and interaction with Golli-BG21

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ABSTRACT

We have successfully expressed an active variant of recombinant murine GIP (rmGIP) with the N-terminal domain deletion (ΔN-rmGIP) in *E. coli* Rosetta(DE3)-RIPL cells. Whereas ΔN-rmGIP could be purified under native conditions, the purification of full-length rmGIP required denaturing conditions; and the yields were 31.4 mg and 7.4 mg per L of culture, respectively. Purity was at least 97% as assessed by HPLC. Both proteins exhibited a well-defined secondary structure composition as determined by circular dichroism spectroscopy, with a slightly higher ratio of helical and strand components in ΔN-rmGIP. The phosphatase activity of both proteins was Mg^{2+}-dependent, with a pK_{Mg} of activation being ~2.8 and non-cooperative binding. The Golli-myelin basic protein isoform rmBG21 (recombinant murine form) enhanced the phosphatase activity of ΔN-rmGIP below 6 μM, but significantly inhibited it at higher concentrations. Using glutaraldehyde cross-linking and gel shift assays, the rmBG21-ΔN-rmGIP interaction was shown to be equimolar and specific, but seemingly relatively weak, suggesting that a third interaction partner is required *in vivo*.

The myelin basic proteins (MBPs)[1] are a family that have long been known to be self-antigens in the central nervous system, as they can induce experimental autoimmune encephalomyelitis in mice, a model for the inflammatory component of diseases such as multiple sclerosis [1]. This family includes both the "classic" MBP isoforms (the 18.5 kDa isoform is the most abundant in adult myelin) that originate from the third transcription start site of the gene, and the Golli (genes of oligodendrocyte lineage) isoforms that arise from the first transcription start site [2]. All known members of this family possess a high net charge coupled with a low mean hydrophobicity, and thus belong to the class of intrinsically disordered proteins (IDPs) [3,4], the majority of which are multifunctional and participate in signal transduction, or as linkers in interaction networks.

One of the three Golli-MBPs is BG21, the protein that has a C-terminal segment in common with the "classic" 18.5 kDa isoform, and an N-terminal Golli-specific segment [2,5]. Though the function of the Golli proteins is not yet definitively established, it has been shown that BG21 is localized in the nucleus of many cell types, potentially participating in signal transduction or transcriptional regulation. Recently, it has been demonstrated that BG21 can be a substrate for protein kinase C (PKC), and inhibit its activation pathway in T cells [2].

The yeast two-hybrid system is a powerful tool to search for the interaction partners of proteins with unknown function. Recently, the yeast two-hybrid system was used to identify binding partners of BG21, particularly a protein called Golli-interacting protein (GIP, 261 amino acids) [5]. This GIP was shown to have almost complete identity (99%) to a human protein called nuclear LIM interactor-interacting factor (NLI-IF), and a high degree of sequence similarity to the proteins from a small family of RNA polymerase II C-terminal domain (CTD) phosphatases, particularly the small CTD phosphatase Scp1 [6]. The function of the NLI-IF has not yet been elucidated, but based on the fact that NLI-IF has been found transcribed in 15 human tissues examined, it was speculated that it may function as a coordinator of transcriptional activity via its interaction with NLI [7]. However, there is no direct evidence of the interaction between NLI-IF with NLI.

In contrast, the proteins from the second family of RNA polymerase II CTD phosphatases have been intensively studied, and all known members have been shown to exhibit phosphatase activity [6, 8–13]. All phosphatases from this family have a conserved sequence of DXDX(T/V) at their active site, and belong to a superfamily of the haloacid dehalogenases (HAD) [14–17]. The common denominator for enzymes from the family of RNA polymerase II CTD phosphatases is that they are all known to dephosphorylate

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[1] *Abbreviations used:* MBPs, myelin basic proteins; Golli, genes of oligodendrocyte lineage; IDPs, intrinsically disordered proteins; NLI-IF, nuclear LIM interactor-interacting factor; GIP, Golli-interacting protein; rmGIP, recombinant murine GIP.

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the C-terminus of RNA polymerase II [6,12]. The protein Scp1 (small RNA polymerase II CTD phosphatase) has 261 amino acids and 99% identity with murine GIP. Recently, Scp1 was shown to inhibit activated transcription from different promoter–reporter gene constructs, whereas its mutated isoform (that lacks phosphatase activity) enhanced transcription [6]. Therefore, Scp1 was suggested to play an important role in the regulation of gene expression.

In addition, murine GIP was shown to be co-expressed with Golli-MBP in the nuclei of granule cells and oligodendrocytes, and GIP mRNA expression followed the BG21 expression pattern in developing brains [5]. These two proteins were shown to co-immunoprecipitate from whole-cell lysates of PC12n and N19 cells. Moreover, the immunoprecipitation data suggested that there was no domain specificity to the interaction of BG21 with GIP, since both the N-terminal sequence of GIP (residues 1–89) and the more conserved region (residues 90–261) co-immunoprecipitated with BG21 [5]. Finally, GIP was co-immunoprecipitated with BG21 and NLI from lysates of N19 cells transfected with NLI. Based on these results, it was suggested that Golli proteins, particularly BG21, might play a role in NLI-associated regulation of gene expression/transcription in nerve cells [5].

Herein, we present a method (optimized with respect to several parameters) for the overexpression and purification of recombinant murine GIP (rmGIP), and particularly its truncated form representing the conserved region of the sequence (residues Q77-G260). We have characterized the properties of both full-length rmGIP and its truncated form ΔN-rmGIP, as Mg^{2+}-dependent phosphatases. Finally, we have investigated the direct interaction of GIP with BG21 using a gel shift assay, and have studied BG21's effect on the phosphatase activity of GIP.

Materials and methods

Materials

Electrophoresis grade acrylamide, ultra-pure TRIS base, and ultra-pure Na_2EDTA were purchased from ICN Biomedicals (Costa Mesa, CA). Most other chemicals were reagent grade and acquired from Fisher Scientific (Unionville, ON) or Sigma–Aldrich (Oakville, ON). Electrophoresis grade sodium dodecyl sulphate (SDS) was obtained from Bio-Rad Laboratories (Mississauga, ON). The Ni^{2+}–NTA (nitrilotriacetic acid) agarose beads were purchased from Qiagen (Mississauga, ON). Acetonitrile and TFA (tri-fluoroacetic acid), both HPLC grade, were obtained from Fisher Scientific and Sigma–Aldrich, respectively.

Purification of rmBG21

Recombinant murine BG21 (rmBG21) was expressed in *Escherichia coli* (*E. coli*), grown in M9 minimal media supplemented with glucose and NH_4Cl, and purified as previously described [18].

Protein preparations were evaluated by discontinuous SDS-PAGE (5% stacking, 14% separating) along with low-range molecular mass markers (Bio-Rad Laboratories, Mississauga, Ont., Canada) and staining with Coomassie Brilliant Blue R-250 (Fisher Scientific, Whitby, Ont., Canada). For further evaluation of purity, reversed-phase HPLC was performed using a Waters apparatus with a Symmetry 300 C18, 5 μm, 4.6 × 250 mm column as previously described [18]. Protein concentration was determined by measuring the absorbance at 280 nm, using the extinction coefficient $\varepsilon = 6970\,M^{-1}\,cm^{-1}$.

Expression and purification of rmGIP and its derivative

The open reading frame of the murine GIP was cloned into the NdeI/XhoI sites of a pET 28b(+) vector (which was kindly provided by Drs. Anthony and Celia Campagnoni, Neuropsychiatric Institute, U.C.L.A.). The recombinant protein had a sequence that included residues 1–260 of the murine GIP and a C-terminal LE-H_6 tag that was used for purification using nickel nitrilo-triacetic acid resin (Ni–NTA) agarose (Qiagen, Valencia, CA). Competent *E. coli* BL21-CodonPlus(DE3)-RIP, and/or Rosetta(DE3)-RIPL cells (Stratagene, La Jolla, CA), were transformed with the above vector. Different expression and lysis conditions were screened to optimize the expression level and solubility of the protein (Table 1). Of those various conditions screened, only one protocol was found to yield the soluble and at least partially biologically active protein. Briefly, *E. coli* Rosetta(DE3)-RIPL cells were grown at 37 °C in 500 mL LB kanamycin $(kan)^+$-chloramphenicol $(cam)^+$ (25 μg/mL and 33 μg/mL, respectively) medium until A_{600} ~0.8. The culture was then induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and grown for a further 4 h at 37 °C. Cells were harvested by centrifugation and stored at −20 °C until used.

The frozen pellet was suspended in 30 mL native lysis buffer (Buffer A: 10 mM HEPES–NaOH, pH 8.0, 0.15 M NaCl, 3 mM DTT, 1 mM PMSF), 30 mg lysozyme was added and the lysate was stirred vigorously on ice for 1 h, followed by sonication on ice six times for 10 s, with 10 s cooling between bursts. This material was centrifuged at 10,000*g* for 30 min at 4 °C to pellet cell debris, and the supernatant was sampled by SDS–PAGE for the presence of soluble rmGIP. Cell debris was resuspended in 40 mL denaturing buffer (50 mM Tris–HCl, pH 8.0, 8 M urea, 0.5 M NaCl, 5 mM DTT, 10 mM

Table 1
Screening of the expression conditions for rmGIP

Expression conditions					Lysis buffers	Detergent	rmGIP solubility
OD_{600} before induction	T (growth), °C	[IPTG], mM	T (post-induction), °C	Incubation time after induction, h			
0.4	37	1	37	5	A, B	±Nonionic	Insoluble
0.6	37	1	37	5	A, B	±Nonionic	Insoluble
0.8	37	1	37	5	A, B	±Nonionic	Insoluble
0.8	37	0.5	37	5	A, B	±Nonionic	Insoluble
0.8	20	1	20	5	A, B	±Nonionic	Insoluble
0.8	20	1	20	O/N	A, B	±Nonionic	Insoluble
0.8	20	0.5	20	O/N	A, B	±Nonionic	Insoluble
0.8	37	1	37	5	A, B	+Anionic	Soluble
0.8	37	1	37	5	Denaturing	No	Soluble

The BL21-CodonPlus(DE3)-RIP and/or Rosetta(DE3)-RIPL cells were grown in LB-rich medium.
The buffers that were used for the lysis under native conditions consisted of: (Buffer A) 10 mM HEPES–NaOH, pH 8.0, 0.15 M NaCl, 3 mM DTT and 1 mM PMSF; (Buffer B) 300 mM NaCl, 50 mM Tris–HCl, pH 8.0, 5% glycerol and 10 mM β-mercaptoethanol.
Cells were lysed by sonication or alternatively by addition of lysozyme to 1 mg/mL. An addition of 1% of non-ionic detergent (either Tween 20, Triton X-100, or NP-40) had no effect on the solubility of rmGIP.
Only 0.3% of anionic detergent (either sarkosyl or SDS) caused rmGIP to appear in the soluble matter after the lysis.
For the denaturing lysis condition, we used the buffer that consisted of 50 mM Tris–HCl, pH 8.0, 8 M urea, 0.5 M NaCl, 5 mM DTT and 10 mM imidazole.