Biochimie 146 (2018) 127–138

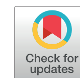
Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Chicken GRIFIN: Structural characterization in crystals and in solution



Federico M. Ruiz [a], Ulrich Gilles [b], Anna-Kristin Ludwig [c], Celia Sehad [d], Tze Chieh Shiao [d], Gabriel García Caballero [c], Herbert Kaltner [c], Ingo Lindner [b], René Roy [d, **], Dietmar Reusch [b, ***], Antonio Romero [a, ****], Hans-Joachim Gabius [c, *]

[a] *Chemical and Physical Biology, Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain*
[b] *Pharma Biotech Development Penzberg, Roche Diagnostics GmbH, 82377 Penzberg, Germany*
[c] *Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Veterinärstr. 13, 80539 Munich, Germany*
[d] *Pharmaqam and Nanoqam, Department of Chemistry, Université du Québec à Montréal, P.O. Box 8888, Succ. Centre-Ville, Montréal, Québec H3C 3P8, Canada*

A R T I C L E I N F O

Article history:
Received 31 August 2017
Accepted 11 December 2017
Available online 15 December 2017

Keywords:
Aggregation
Crystallin
Galectin
Lectin
Lens
Ultracentrifugation

A B S T R A C T

Despite its natural abundance in lenses of vertebrates the physiological function(s) of the galectin-related *i*nter-*fi*ber prote*in* (GRIFIN) is (are) still unclear. The same holds true for the significance of the unique interspecies (fish/birds vs mammals) variability in the capacity to bind lactose. In solution, ultracentrifugation and small angle X-ray scattering (at concentrations up to 9 mg/mL) characterize the protein as compact and stable homodimer without evidence for aggregation. The crystal structure of chicken (C-) GRIFIN at seven pH values from 4.2 to 8.5 is reported, revealing compelling stability. Binding of lactose despite the Arg71Val deviation from the sequence signature of galectins matched the otherwise canonical contact pattern with thermodynamics of an enthalpically driven process. Upon lactose accommodation, the side chain of Arg50 is shifted for hydrogen bonding to the 3-hydroxyl of glucose. No evidence for a further ligand-dependent structural alteration was obtained in solution by measuring hydrogen/deuterium exchange mass spectrometrically in peptic fingerprints. The introduction of the Asn48Lys mutation, characteristic for mammalian GRIFINs that have lost lectin activity, lets labeled C-GRIFIN maintain capacity to stain tissue sections. Binding is no longer inhibitable by lactose, as seen for the wild-type protein. These results establish the basis for detailed structure-activity considerations and are a step to complete the structural description of all seven members of the galectin network in chicken.
© 2017 Elsevier B.V. and Société Française de Biochimie et Biologie Moléculaire (SFBBM). All rights reserved.

1. Introduction

The emerging versatility of physiological functions of animal and human lectins gives ample reason to characterize their structures in detail. In overview, more than 12 folds have been identified that convey ability to bind glycans [1–4]. Sequence divergence after duplication events, starting from an ancestral gene, then led to forming families of homologous proteins. The case study on galectins (β-galactoside-binding proteins with β-sandwich fold and a sequence signature responsible for ligand contact [5]) is describing such a network with overlapping and distinct expression profiles [6–8]. This emerging evidence poses the challenge of a complete characterization of the galectins of an organism. It would be a step forward towards delineating rules of network design and providing insights into the functional meaning of sequence variations. In this respect, the galectin fold presents remarkable adaptability for accommodating ligands of different biochemical nature.

The crystallographic or NMR spectroscopical study of such domains of protozoan (*Toxoplasma gondii*) micronemal protein 1 and

Abbreviations: Å, Ångström; Arg, arginine; Asn, asparagine; Asp, aspartate; CG, chicken galectin; C-GRIFIN, chicken galectin-related *i*nter-*fi*ber prote*in*; Gln, glutamine; Glu, glutamate; HDX, hydrogen/deuterium exchange; His, histidine; ITC, isothermal titration calorimetry; Leu, leucine; Lys, lysine; SAXS, small angle X-ray scattering; Trp, tryptophan; Val, valine.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
**** Corresponding author.
E-mail addresses: roy.rene@uqam.ca (R. Roy), dietmar.reusch@roche.com (D. Reusch), romero@cib.csic.es (A. Romero), gabius@tiph.vetmed.uni-muenchen.de, gabius@lectins.de (H.-J. Gabius).

https://doi.org/10.1016/j.biochi.2017.12.003
0300-9084/© 2017 Elsevier B.V. and Société Française de Biochimie et Biologie Moléculaire (SFBBM). All rights reserved.

protein 2-associated protein [9,10] as well as the N-terminal modules of bovine and murine coronavirus spike proteins [11,12] have taught the following instructive lesson: conversion of the concave topology of the carbohydrate-binding pocket established by surrounding loops to a predominantly hydrophobic surface shifts specificity from glycans to distinct proteins. Looking at the assumedly crucial sequence signature for contact to a β-galactoside, a less dramatic deviation than just described may not necessarily impair glycan binding. For example, the change of a seemingly essential Asn (in position 46 in human galectin-1 (Gal-1)) to Ala at the equivalent position 64 in a fungal (*Agrocybe cylindracea*) galectin is not detrimental. It is neutralized by a five residue insertion at positions 42–46 (with the inserted Asn46 taking the place of the Asn lost at position 64) [13,14]. The structural way how to maintain affinity for lactosides in the conger eel (*Conger myriaster*) galectin from peritoneal cells (Con-P), although even seven from eight conserved amino acids are replaced as reported in [15], has not yet been characterized. Alternatively, sequence deviation(s) can alter carbohydrate specificity. The Trp81Arg change implemented binding to bi-N-acetylated disaccharides (chitobiose, LacdiNAc) in the third galectin protein (CGL3) from the inky cap mushroom *Coprinopsis cinerea* [16]. A unique situation, i.e. a species-dependent loss of lectin activity, is encountered for the *g*alectin-*r*elated *i*nter-*fi*ber prote*in*, termed GRIFIN.

This protein has first been described in rat as lens-specific protein [17,18]. It was found in the insoluble fraction of nuclear fiber cells and localized at the interface between adjacent fiber cells, representing about 0.5% of total protein in adult lens. Its gene with an elaborate promoter region to facilitate lens-specific expression is a common constituent of vertebrate genomes [19]. Given this site-specific occurrence and conserved presence among vertebrates, it is exceptional and thus intriguing to see sequence deviations at canonical positions between mammalian and bird/fish GRIFINs. Especially, the equivalent of the already noted Asn46 (in human Gal-1) is turned to Lys in mammalian GRIFINs [17]. As consequence, a bead (lactosylated Sepharose beads) assay revealed no lectin activity for rat GRIFIN [17]. In contrast, GRIFINs from zebrafish [20] and chicken [19] were bona fide lectins. Two reasons prompted us to initiate structural analysis of GRIFINs by studying chicken (C)-GRIFIN: the mentioned plasticity within the galectin fold and our long-term interest to achieve complete crystallographic documentation of galectin structures in an organism. Towards this end, chicken with its (only) seven family members is a favorable model. Since C-GRIFIN shares a deviation from the canonical sequence for lactose binding with mammalian GRIFINs, i.e. the Arg71Val exchange, defining the resulting contact pattern to the ligand will enable a comparison to common features. We here combine crystallographical analysis, reaching atomic resolution, with studies of C-GRIFIN in solution. They were started by determining its quaternary structure, a key feature of proto-type galectin functionality [21].

Quaternary structure and tendency for aggregation were first examined in solution, up to a concentration of 9 mg/mL, by ultracentrifugation and small angle X-ray scattering (SAXS). Crystallographically, respective specimen from solutions at seven pH values ranging from 4.2 to 8.5 could be processed to monitor stability of structural features. The interaction with lactose was monitored in the crystals and also in solution. Here, thermodynamic parameters (by isothermal titration calorimetry (ITC)) and profiles of hydrogen/deuterium exchange (HDX) in the absence and presence of the ligand were measured. Faced with the conundrum that re-establishing the sequence signature in rat GRIFIN with the Lys-to-Asn reconstitution did not repair the loss of lectin activity [17], we finally probed into the effects of site-specific mutations on C-GRIFIN's carbohydrate-binding activity by a histochemical assay.

2. Material and methods

2.1. Protein production

The wild-type protein was obtained after recombinant production directed by a pGEMEX-1 vector with the respective cDNA insert and purified by affinity chromatography using lactose-bearing resin as described [19]. cDNAs of the mutants of C-GRIFIN, i.e. the Trp66Lys and the Asn48Lys single-site mutants, the Asn48Lys/Arg50Val double mutant and the Asn48Lys/Arg50Val/Tyr66Leu triple mutant, were prepared by using the QuikChange™ Site-Directed Mutagenesis protocol (Agilent Technologies, Munich, Germany). The following primer pairs were used: 5′ C CTG GCC AAC CAC CTG GGG AAG GAG GAG G 3′ and 5′ C CTC CTC CTT CCC CAG GTG GTT GGC CAG G 3′ (Trp66Leu), 5′ C GCC TTC CAC TT T AAG CCC CGC TTT GCC AGC 3′ and 5′ GCT GGC AAA GCG GGG CTT AAA GTG GAA GGC G 3′ (Asn48Lys), 5′ GCT GGC AAA GAC GGG CTT AAA GTG GAA GGC G 3′ and 5′ C GCC TTC CAC TTT AAG CCC GTC TTT GCC AGC 3′ (Asn48Lys/Arg50Val) (exchanged base pairs are underlined). The cDNA of the triple mutant was generated by further altering the cDNA of the double mutant (Asn48Lys/Arg50Val) by respective processing with the primers for the Trp66Leu mutant. Successful implementation of the intended changes was checked by DNA sequencing (Sequiserve, Vaterstetten, Germany).

Mutant proteins of C-GRIFIN were designed as fusion proteins with a glutathione S-transferase (GST) part in a pGEX-6p-2 vector (GE Healthcare, München, Germany), they were purified after recombinant production by affinity chromatography using glutathione-presenting Sepharose 4B (GE Healthcare). Thereafter, the linkage between both proteins was cleaved by GST-tagged human rhinovirus 3C protease (at a ratio of 1:100 (w/w)), then the C-GRIFIN part was separated from released GST and the tagged protease by a second round of affinity chromatography as described [19]. Protein was either precipitated by adding $(NH_4)_2SO_4$ or labeled by biotinylation for the histochemical analysis, as described for human Gal-1 [22].

2.2. Analytical ultracentrifugation

Protein samples were diluted to final concentrations of 0.5 and 1.0 mg/mL in 5 mM phosphate buffer containing 150 mM NaCl and 4 mM β-mercaptoethanol, and solutions were pre-cleared at 16,000 × g. Sedimentation-velocity experiments were run at 293 K in an Optima KL-I analytical ultracentrifuge (Beckman Coulter, Indianapolis, USA) with an An50-Ti rotor and standard double-sector Epon-charcoal center pieces (1.2 cm optical path length). Measurements were performed at 48,000 rpm, registering the course of protein migration every minute at 280 nm. Rayleigh interferometric detection was used to monitor the course of development of the concentration gradient as a function of time and radial position, and the data were analyzed using the SedFit software (Version 14.7).

2.3. Small angle X-ray scattering (SAXS)

SAXS data were collected at the BM29 beamline (ESRF Synchrotron, Grenoble, France) using the BioSAXS robot and a Pilatus 1 M detector (Dectris, Baden-Daettwill, Switzerland) with synchrotron radiation at a wavelength of λ = 1.0 Å and a sample-detector distance of 2.867 m [23]. Each measurement consisted of 10 frames, each of 1 s exposure of a 100 μL sample flowing continuously through a 1 mm diameter capillary during exposure to X-rays. Buffer scattering was measured immediately before each measurement of the corresponding protein sample at 277 K. The obtained scattering profiles were spherically averaged, and the

Download English Version:

https://daneshyari.com/en/article/8304228

Download Persian Version:

https://daneshyari.com/article/8304228

Daneshyari.com