Biochemical and Biophysical Research Communications 380 (2009) 514–519

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

S100A13-C2A binary complex structure—a key component in the acidic fibroblast growth factor for the non-classical pathway

Sepuru K. Mohan, Sandhya G. Rani, Sriramoju M. Kumar, Chin Yu *

Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan

ARTICLE INFO

Article history:
Received 11 January 2009
Available online 29 January 2009

Keywords:
Acidic fibroblast growth factor
S100A13
Synaptotagmin
Non-classical pathway
ARIA/CNS
HADDOCK
S100A13-C2A binary complex

ABSTRACT

Fibroblast growth factors (FGFs) are key regulators of cell proliferation, differentiation, tumor-induced angiogenesis and migration. FGFs are essential for early embryonic development, organ formation and angiogenesis. They play important roles in tumor formation, inflammation, wound healing and restenosis. The biological effects of FGFs are mediated through the activation of the four transmembrane phosphotyrosine kinase receptors (FGFRs) in the presence of heparin sulfate proteoglycans (HSPGs) and therefore require the release of FGFs into the extracellular space. However, FGF-1 lacks the signal peptide required for the releasing of these proteins through the classical endoplasmic reticulum (ER)-Golgi secretary pathway. Maciag et al. demonstrated that FGF-1 is exported through a non-classical release pathway involving the formation of a specific multiprotein complex [M. Landriscina, R. Soldi, C. Bagala, I. Micucci, S. Bellum, F. Tarantini, I. Prudovsky, T. Maciag, S100A13 participates in the release of fibroblast growth factor 1 in response to heat shock in vitro, J. Biol. Chem. 276 (2001) 22544–22552; C.M. Carreira, T.M. LaVallee, F. Tarantini, A. Jackson, J.T. Lathrop, B. Hampton, W.H. Burgess, T. Maciag, S100A13 is involved in the regulation of fibroblast growth factor-1 and p40 synaptotagmin-1 release in vitro, J. Biol. Chem. 273 (1998) 22224–22231; T.M. LaValle, F. Tarantini, S. Gamble, C.M. Carreira, A. Jackson, T. Maciag, Synaptotagmin-1 is required for fibroblast growth factor-1 release, J. Biol. Chem. 273 (1998) 22217–22223; C. Bagalá, V. Kolev, A. Mandinova, R. Soldi, C. Mouta, I. Graziani, I, Prudovsky, T. Maciag, The alternative translation of synaptotagmin 1 mediates the non-classical release of FGF1, Biochem. Biophys. Res. Commun. 310 (2003) 1041–1047]. The protein constituents of this complex include FGF-1, S100A13 (a Ca^{2+}-binding protein), and the p40 form of synaptotagmin 1 (Syt1). To understand the molecular events in the FGF-1 releasing pathway, we have studied the interactions of S100A13 with C2A by ^{1}H-^{15}N HSQC titration and 3D-filtered NOESY experiments. We characterized the binary complex structure of S100A13-C2A by using a variety of multi-dimensional NMR experiments. This complex acts as a template for FGF-1 dimerization and multiprotein complex formation.

Introduction

Soluble secretary proteins characteristically include N-terminal signal peptides that direct them to the translocation apparatus of the endoplasmic reticulum [5]. However, there are proteins such as acidic fibroblast growth factor (FGF-1), HIV-TAT, Annexin II, sphingosine kinase 1, interleukin 1α and 1β, gelectin-1, and the extra-vesicular p40 fragment of Syt1, which lack the classical signal sequence to export them from the cell in an ER/Golgi-dependent manner [6–10]. Most of these proteins are involved with key cellular processes like angiogenesis, inflammation, tumor growth, cell proliferation, and differentiation. Therefore, the releasing pathways of these proteins lacking signal peptides have recently been the subject of extensive exploration.

FGF-1 belongs to a large family of heparin-binding growth factors that have mitogenic activity and are the key activators of tumor-induced angiogenesis [11,12]. The majority of the members of the FGF family are exported by ER/Golgi-dependent secretary transport. However, FGF-1 has been shown to be secreted by an alternative pathway, which is activated by different forms of stress, such as heat shock [13], serum starvation or hypoxia [14]. While it was first assumed that angiogenic growth factors might be released from mechanically injured tissue to promote wound healing, a process that requires angiogenesis, various lines of evidence demonstrated that FGF-1 is exported from cultured cells in the absence of appropriate amounts of cell death [13].

Upon heat shock, two intracellular proteins, which are cleavage products of the transmembrane protein synaptotagmin (p40Syt1) (its cytoplasmic domain) and the Ca^{2+}-binding protein S100A13, have been shown to associate with the latent FGF-1 homodimer in the cytoplasm [1–4,13]. Apparently, they are exported together

* Corresponding author. Fax: +886 3 5711082.
E-mail address: cyu.nthu@gmail.com (C. Yu).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.01.143

with FGF-1. A direct role of p40Syt1 and S100A13 in FGF-1 export has been proposed through the repression of p40Syt1 expression by the antisense technique and the expression of a dominant negative S100A13 mutant that attenuated FGF-1 export [2]. These results suggested that the FGF-1-p40Syt1-S100A13 complex formation is the first step in the FGF-1 export pathway, followed by the direct translocation of this protein complex across the plasma membrane. In addition, FGF-1 purified from ovine brain as a molecular weight aggregate exists as a component of the non-covalent heparin-binding complex with p40syt1 and S100A13. FGF-1 is released under temperature stress as a multiprotein complex consisting of FGF-1, S100A13 and p40Syt1. Maciag and coworkers demonstrated that S100A13 is involved in the regulation of the release of FGF-1 in response to stress, independent of the conventional ER Golgi pathway [4].

The extra-vesicular domain of Syt1 is a constituent of the extra cellular multiprotein complex. This multiprotein complex is released in response to heat shock. Based on the results of mutation analysis, it was suggested that the C2A domain (127 amino acids) of p40Syt1 provides the interface for the binding of FGF-1 and S100A13 [3]. Our findings demonstrated that the S100A13-C2A complex is the preliminary step in the multiprotein complex formation. This S100A13-C2A binary complex could act as template for the FGF-1-S100A13-C2A multiprotein complex formation.

Materials and methods

Ingredients for Luria Broth were obtained from AMRESCO. Aprotinin, pepstatin, leupeptin, phenylmethylsulfonyl fluoride, Triton X-100, and β-mercaptoethanol were obtained from Sigma Co. Heparin and glutathione sepharoses were obtained from Amersham Pharmacia Biotech. $^{15}NH_4Cl$, ^{13}C labeled glucose and D_2O were purchased from Cambridge Isotope Laboratories. All other chemicals used were of high quality analytical grade. Unless specified, all solutions were made in 10 mM sodium phosphate containing 100 mM NaCl and 2 mM $CaCl_2$.

Expression and purification of S100A13 and C2A domain. The cDNA of S100A13 and C2A was sub cloned into the pGEX expression vector, and the expression of the S100A13 and C2A proteins was carried out in the *E. coli* (BL21DE3) host system. The unlabeled protein was expressed in Luria Broth (LB) medium. Uniform ^{15}N and ^{15}N & ^{13}C labeling of S100A13 and C2A was achieved using M9 minimal medium containing $^{15}NH_4Cl$ or $^{15}NH_4Cl$ and ^{13}C glucose. The S100A13 and C2A over expression and purification were described elsewhere [15,16].

^1H–^{15}N HSQC titration. NMR data were recorded at 25 °C on Bruker AV 800, 600 and 500 MHz (at ^1H precession frequency) spectrometers equipped with cryogenic probes. All of the protein samples were prepared in phosphate buffer (in 90% H_2O, 10% D_2O) containing 100 mM sodium chloride and 2 mM $CaCl_2$. The spectra were recorded at 25 °C in pH ~6.0. The ^{15}N-labeled proteins were titrated with unlabeled proteins at 1:1 molar ratios. Amide proton exchange rates were monitored by acquiring a series of ^1H-^{15}N HSQC spectra of proteins in their free states and after the addition of its protein partner in the multiprotein release complex. These complexes were characterized before the advent of the TROSY technique [17].

3D-NMR experiments. S100A13-C2A binary complex assignments were done by using various multi-dimensional NMR experiments. Resonance assignments for backbone ^1H, ^{13}C and ^{15}N nuclei of both S100A13 and C2A in the S100A13-C2A binary complexes were obtained through 3D HNCA and HNCOCA experiments. Side chain resonances were assigned using 3D ^{15}N-edited TOCSY-HSQC and HCCH-TOCSY data sets supplemented with CBCACONH and HBHACONH. The HNCO experiment was used to assign the carbonyl carbons in proteins. Aromatic resonances of the S100A13 and C2A were assigned using $^{13}C/^{15}N$-edited NOESY-HSQC spectra. Intermolecular distance constraints were derived from the 3D $^{13}C(\omega_2)$-edited $^{12}C(\omega_3)$-filtered NOESY-HSQC experiment [18] of 1:1 $^{15}N/^{13}C/^1H$ S100A13: $^{14}N/^{12}C/^1H$ C2A and $^{15}N/^{13}C/^1H$ C2A: $^{14}N/^{12}C/^1H$ S100A13 for the S100A13-C2A binary complex. Spectra were processed with Topspin and analyzed with Sparky [19].

Structure calculation. The structures of S100A13 and C2A in the S100A13-C2A binary complex were calculated by ARIA/CNS [20]. Preliminary structure calculations based on the intra molecular NOE data and TALOS [21] data established that the backbone folds of S100A13, and C2A are not substantially altered by the formation of the protein complex. HADDOCK [22] was applied to dock S100A13 and C2A for the S100A13-C2A complex, using the previously determined structures and 38 intermolecular NOEs. The structures were analyzed with PROCHECK [23].

Results and discussion

Chemical shift perturbation is the most widely used NMR method to map protein interfaces [24]. The ^1H-^{15}N HSQC spectrum of one protein is initially recorded. The unlabeled interaction partner is titrated into the system, and the chemical shift perturbations are monitored, which provide information on the residues involved in protein–protein association. An interaction results in chemical and environmental changes on the protein interfaces and, therefore affects the chemical shifts of the nuclei in that area. The ^1H-^{15}N chemical shifts perturbation technique has been used in many cases to map protein–protein interactions [24]. The overlaid ^1H-^{15}N HSQC spectrum of free S100A13 and C2A/S100A13 (^{15}N-labeled) complex in a 1:1 ratio showed that selected ^1H-^{15}N cross peaks in the spectrum are perturbed, suggesting that S100A13 specifically interacts with C2A. On the other hand, an ^1H-^{15}N HSQC spectrum of S100A13/C2A (^{15}N-labeled) showed that the selected ^1H-^{15}N cross peaks in the spectrum were either perturbed or disappeared, suggesting that C2A specifically interacts with S100A13. It was evident from these experiments that there was interaction between S100A13 and C2A. At an S100A13 (^{15}N-labeled):C2A (unlabeled) ratio of 1:1, cross peaks corresponding to 13 residues, including Leu46, His48, Leu49, Leu50, Val53, Gly54, Ser55, Leu56, Glu58, Lys91, Lys94, Ile95 and Arg96 are perturbed (Fig. 1A). These residues possibly constitute the C2A binding site(s) in S100A13. A plot of the ^1H-^{15}N weighted average of residues in S100A13 upon complex formation with C2A is represented in Fig. 1B. In the S100A13 molecule, the binding region is distributed in two regions: one is from Leu46 to Glu 58 and the second (most important) is the C-terminal end of the S100A13 from Lys91 to Arg97.

At a 1:1 ratio of C2A (^{15}N-labeled):S100A13 (unlabeled), cross peaks corresponding to 14 residues, including Ala31', Leu32', Asp33', Gly35', Thr37', Ser38', Arg60', Lys61', Phe92', Asp93', Arg94', Phe95', Ser96' and Lys97' (' is used for C2A residues) were perturbed (Fig. 1C). These residues possibly constitute the C2A binding site(s) in S100A13. A plot of the weighted average of the (^{15}N and ^1H) chemical shift perturbation in C2A upon complex formation with S100A13 is represented in Fig. 1D. These 14 residues are located in the three loop regions of the C2A domain. The binding interfaces in the S100A13-C2A binary complex were also confirmed by H–D exchange measurements. Triple resonance experiments were performed by mixing double-labeled (^{15}N and ^{13}C) protein with the unlabeled protein partner in binary complexes. We assigned the S100A13 and, C2A resonance assignments in the S100A13-C2A binary complex.

Assignments of S100A13 and C2A in complex

The S100A13 and C2A resonance assignments in the binary complex were obtained based on the triple resonance experiments. A to-