Available online at www.sciencedirect.com
ScienceDirect
Molecular Immunology 45 (2008) 1752–1760
Molecular Immunology
www.elsevier.com/locate/molimm

Revisiting the mechanism of the autoactivation of the complement protease C1r in the C1 complex: Structure of the active catalytic region of C1r

József Kardos [a,b,1], Veronika Harmat [c,d,1], Anna Palló [d,f], Orsolya Barabás [d], Katalin Szilágyi [b], László Gráf [a], Gábor Náray-Szabó [c,d], Yuji Goto [e], Péter Závodszky [b], Péter Gál [b,*]

[a] Department of Biochemistry, Institute of Biology, Eötvös Loránd University, Pázmány sétány 1/C, Budapest H-1117, Hungary
[b] Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Karolina út 29, Budapest H-1113, Hungary
[c] Protein Modelling Group, Hungarian Academy of Sciences-Eötvös Loránd University, Pázmány Péter sétány 1/A, Budapest H-1117, Hungary
[d] Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, Pázmány Péter sétány 1/A, Budapest H-1117, Hungary
[e] Institute for Protein Research, Osaka University and CREST, Japan Science and Technology Agency, Yamadaoka 3-2, Suita, Osaka 565-0871, Japan
[f] Department of Neurochemistry, Chemical Research Center, Hungarian Academy of Sciences, H-1025 Pusztaszeri ut 59-67, Hungary

Received 15 August 2007; received in revised form 25 September 2007; accepted 27 September 2007

Abstract

C1r is a modular serine protease which is the autoactivating component of the C1 complex of the classical pathway of the complement system. We have determined the first crystal structure of the entire active catalytic region of human C1r. This fragment contains the C-terminal serine protease (SP) domain and the preceding two complement control protein (CCP) modules. The activated CCP1–CCP2–SP fragment makes up a dimer in a head-to-tail fashion similarly to the previously characterized zymogen. The present structure shows an increased number of stabilizing interactions. Moreover, in the crystal lattice there is an enzyme–product relationship between the C1r molecules of neighboring dimers. This enzyme–product complex exhibits the crucial S1–P1 salt bridge between Asp631 and Arg446 residues, and intermolecular interaction between the CCP2 module and the SP domain. Based on these novel structural information we propose a new split-and-reassembly model for the autoactivation of the C1r. This model is consistent with experimental results that have not been explained adequately by previous models. It allows autoactivation of C1r without large-scale, directed movement of C1q arms. The model is concordant with the stability of the C1 complex during activation of the next complement components.

Keywords: Complement system; Mechanism of C1r autoactivation; C1r catalytic domain; Modular serine protease; Complement control protein

1. Introduction

Modular serine proteases consist of several distinct domains of various protein families of different size and fold (Lorand and Mann, 1993). The noncatalytic modules provide both high specificity and elasticity in the catalytic properties and are responsible for the regulation of the enzyme function regarding the localization, timing, or recognition of interacting partners. In such a context, the enzyme function can be interpreted only in the detailed knowledge of the structure and function of the individual regulatory domains even if the reaction catalyzed by the catalytic domain is well known. A representative example is the complement system, one of the proteolytic cascades found in the blood of vertebrates. It mediates several effector functions of the innate and adaptive immunity such as eliminating invading pathogens and altered host cells as well as triggering inflammatory reactions. The uncontrolled activation of the complement system, however, can result in self-tissue damage and/or pathologic inflammation. The main components of the complement system are modular serine proteases, which activate each other in a strictly ordered manner. There are three distinct pathways through which the complement system can be activated: the classical, the lectin and the alternative pathways. The first component of the classical pathway (C1) is a 790 kDa Ca^{2+}-dependent heteropentamer complex, which consists of a recognition subunit C1q and a tetramer of C1r and C1s serine proteases (Schumaker et al., 1987; Arlaud et al.,

* Corresponding author. Tel.: +36 1279 3135.
E-mail address: gal@enzim.hu (P. Gál).
[1] These authors contributed equally to the work.

0161-5890/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2007.09.031

2001). The C1q protein has an overall shape of a bunch of six tulips with six C-terminal heterotrimeric globular heads connected to six collagen-like stems that merge into a stalk at the N-terminus. According to the current models of the C1 complex, the C1s–C1r–C1r–C1s tetramer is wrapped around the collagen-like arms of the C1q. The C1r and C1s serine proteases are present in zymogenic state until the C1 complex meets an activator structure. The binding of the globular C1q heads to the activator surface results in the activation of the zymogen proteases. The first enzymatic event in the classical pathway is the autoactivation of C1r, which involves the cleavage of the Arg446–Ile447 bond in the protease domain. Active C1r then cleaves and activates zymogen C1s that in turn cleaves C4 and C2, the subsequent components of the cascade. Activation and activity of C1 is controlled by the serpin C1-inhibitor, which inhibits both serine proteases (Beinrohr et al., 2007). C1r and C1s, together with the MBL-associated serine proteases (MASPs) of the lectin pathway, form a family of serine proteases with identical domain organization (Gál et al., 1989; Gál et al., 2007). The N-terminal CUB1-EGF-CUB2 region is followed by a tandem repeat of complement control protein (CCP) modules and a chymotrypsin-like serine protease (SP) domain. The CUB1-EGF-CUB2 region mediates the Ca^{2+}-dependent C1r–C1s as well as the $C1q–C1r_2C1s_2$ association. The C-terminal CCP1–CCP2–SP catalytic region of C1r shows the enzymatic properties characteristic of the full-length molecule and forms a homodimer providing the core of the C1s–C1r–C1r–C1s tetramer. Previously it was shown on synthetic substrates that the isolated SP domain itself is an active serine protease with trypsin-like specificity. However, on protein substrates its activity is highly restricted to a unique dual function of self-activating ability and the capability of cleaving zymogen C1s (Kardos et al., 2001).

During the recent years the 3D structures of several fragments of C1r, C1s and C1q have been solved and low resolution models for the C1 complex have been proposed (Bersch et al., 1998; Budayova-Spano et al., 2002a; Budayova-Spano et al., 2002b; Gaboriaud et al., 2000, 2003; Gregory et al., 2003). Despite all these achievements, some basic questions remain unanswered. The structure of zymogen catalytic CCP1–CCP2–SP fragment of C1r shows a dimer, which is stabilized by intermolecular CCP1–SP interactions (Budayova-Spano et al., 2002a; Lacroix et al., 2001). In this structure, the active site of one monomer and the cleavage site of the other are separated by a distance of 92 Å which prevents the physical contact that is necessary for autoactivation. On the other hand, we have shown earlier that the presence of the CCP2 module stabilizes the structure of the catalytic domain and greatly enhances the rate of cleavage of protein substrates (*i.e.* autoactivation and C1s cleavage) in solution phase (Kardos et al., 2001). It indicates that the CCP2 module mediates important protein–protein interactions during proteolysis. Here we report the first functional dimeric structure of the active C1r CCP1–CCP2–SP fragment. In the crystal lattice an enzyme–product complex is formed. The complex shows the S1–P1 salt bridge (characteristic to trypsin-like enzymes) and the CCP2–SP intermolecular interactions. Based on this novel structural information and careful consideration of former experimental data we propose an improved model for the autoactivation of C1r in the C1 complex.

2. Materials and methods

2.1. Protein expression, renaturation and purification

CCP1–CCP2–SP fragment of human C1r (corresponding to amino acids 292–688) was cloned from cDNA containing four extra amino acids (Ser-Thr-Gln-Ala) at their N-terminus and expressed in BL21(DE3)pLysS *E. coli* strain. After purification, inclusion bodies were solubilized in GdnHCl. The protein was renatured in GdnHCl using a glutathione redox system and further purified by ion exchange and gel filtration chromatography. The protocol was presented in full details previously (Kardos et al., 2001).

2.2. Functional characterization of the renatured C1r CCP1–CCP2–SP fragment

Recombinant, human C1s CCP2–SP fragment, which has been expressed in the same expression system as the C1r fragments, renatured similarly (Kardos et al., 2001), and purified by ion exchange and gel filtration chromatography was incubated with the C1r catalytic fragment using 1:50 enzyme–substrate molar ratio in 50 mM Hepes (pH 8), 100 mM NaCl. The activation (*i.e.* cleavage) was analyzed on a 12.5% SDS-PAGE, under reducing conditions.

2.3. Crystallization and data collection

Crystals were grown by the hanging-drop method at 15 °C. Crystals were obtained by mixing 8 μl of reservoir solution and 8 μl of protein solution. The reservoir solution contained 14% (w/v) PEG 6000, 0.2 M NaCl, 10% (v/v) glycerol and 0.1 M Tris–HCl (pH 7.4). The protein solution contained 0.2 mg/ml of the active C1r CCP1–CCP2–SP fragment in 20 mM Tris–HCl, 130 mM NaCl at pH 7.4. The low solubility and relatively high propensity for crystallization or precipitation of the protein required the use of large drops and low protein concentration. To obtain cryoprotective conditions crystals were soaked in solutions with glycerol content raised to 20%. Crystals were tested using the ID14 EH1 beam line at the European Synchrotron Radiation Facility. Data sets were collected at ID29 beam line at the European Synchrotron Radiation Facility at a wavelength of 0.979 Å at 100 K. A data set with improved resolution and mosaicity was collected at the BL41-XU in SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) using a wavelength of 1.000 Å at 100 K. This data set was used for structure determination. Data processing and data reduction were carried out with the MOSFLM (Leslie, 1992) and SCALA (Evans, 1993) programs of the CCP4 package (CCP4, 1994). More details of the data collection statistics are shown in Table 1.