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Dynamic Flexibility of Double-stranded RNA Activated PKR in Solution

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PKR, an interferon-induced double-stranded RNA activated serine–threonine kinase, is a component of signal transduction pathways mediating cell growth control and responses to stress and viral infection. Analysis of separate PKR functional domains by NMR and X-ray crystallography has revealed details of PKR RNA binding domains and kinase domain, respectively. Here, we report the structural characteristics, calculated from biochemical and neutron scattering data, of a native PKR fraction with a high level of autophosphorylation and constitutive kinase activity. The experiments reveal association of the protein monomer into dimers and tetramers, in the absence of double-stranded RNA or other activators. Low-resolution structures of the association states were obtained from the large angle neutron scattering data and reveal the relative orientation of all protein domains in the activated kinase dimer. Low-resolution structures were also obtained for a PKR tetramer–monoclonal antibody complex. Taken together, this information leads to a new model for the structure of the functioning unit of the enzyme, highlights the flexibility of PKR and sheds light on the mechanism of PKR activation. The results of this study emphasize the usefulness of low-resolution structural studies in solution on large flexible multiple domain proteins.

Keywords: small angle neutron scattering; protein kinase R; solution structure

Introduction

PKR is an interferon (IFN)-induced double-stranded RNA (dsRNA) serine–threonine kinase, initially identified as a translational inhibitor in an antiviral pathway regulated by IFNs.[1] Cellular targets of PKR phosphorylation include the α subunit of translation initiation factor eIF-2 and B56 α, the regulatory subunit of phosphatase PP2A. More recently, it has become clear that PKR is a component of signal transduction pathways mediating cell growth control and responses to

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Abbreviations used: IFN, interferon; IRF-1, interferon regulatory factor-1; dsRBD, double-stranded RNA binding domain; dsRBM, dsRNA binding motif; SANS, small angle neutron scattering; JNK, c-Jun NH_2-terminal kinase; SAXS, small angle X-ray scattering; TAR, *trans*-activating region; MCA, monoclonal antibody; IEF, isoelectric focusing.

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stress†. For example, the transcription factor nuclear factor-κB (NF-κB) is activated by different stimuli *via* a PKR-modulated pathway. PKR is also involved in signal transduction mediated by the stress-activated protein kinase p38 and by c-Jun NH_2-terminal kinase (JNK). The activation of the transcription factor interferon regulatory factor-1 (IRF-1) by dsRNA and IFN-γ in fibroblasts is PKR-dependent and the activity of the tumor suppressor p53 may also be subject to PKR regulation. The transcriptional activity of the signal transduction and transcriptional activators, STAT1 and STAT3, can be modulated by PKR-dependent serine phosphorylation. PKR is also implicated in the control of splicing of tumor necrosis factor-α mRNA†.

Human PKR is a protein of 551 amino acid residues consisting of two functionally distinct domains: an N-terminal dsRNA binding domain and a C-terminal kinase catalytic domain. The dsRNA binding domain (dsRBD), residues 1–170, contains two binding motifs (dsRBM), of approximately 70 residues each. Sequence similarity between them is 49%, with 29% of identical residues. All dsRBM-containing proteins tested, including PKR, bind dsRNA in a sequence-independent manner. The N-terminal structure of PKR has been determined using NMR.[2] Both dsRBMs have identical secondary structure. A 20 amino acid residue linker between the two dsRBMs assumes a random coil conformation allowing the dsRBD to wrap around an *A*-form dsRNA helix, offering optimal interactions of each dsRBM with the RNA. The kinase domain of PKR contains the conserved protein kinase sub-domains and the homology with sub-domain VI identifies PKR as a serine–threonine kinase.[3] Recently, the X-ray crystal structure of the catalytic domain of PKR in a complex with eIF2α has been determined.[4,5] This reveals an eIF2α recognition mechanism conserved among the eIF2α protein kinase family.

Although activation or inhibition of PKR can occur in response to different agents in addition to dsRNA including polyanionic compounds such as heparin, and proteins such as protein activator of PKR (PACT), dsRNA-mediated activation is the most well-characterized. Point mutation and domain swapping experiments in the dsRBD have indicated that dsRBM1 is more important for dsRNA binding than RBM2,[6–10] although both motifs are required for optimal binding.[6] A cooperative mechanism for dsRNA binding is favored where this causes a major conformational change in PKR. Gel analyses of protein–RNA complexes,[11] tryptophan fluorescence quenching and neutron scattering of the PKR–dsRNA complex in solution,[12] and NMR dynamics experiments[2] support this mechanism. The change in conformation likely leads to the exposure of a catalytic site(s) for autophosphorylation, or the shift of domains within the protein molecule or between protein molecules in a complex, thus facilitating protein–protein interaction between PKR and substrate. Activation of PKR by dsRNA results in PKR autophosphorylation and the subsequent activation of substrate phosphorylation activity. In autophosphorylation activation, second-order kinetics suggests the reaction is intermolecular.[13] Dimerization is also critical for kinase activation[3,11,14–20] though dimerization alone is not sufficient for PKR activity.[17] However, it appears that dimerization serves to initiate a previously unrecognized dsRNA-independent autophosphorylation reaction, resulting in the phosphorylation of the two critical threonine residues in the activation loop.[21] Dimerization is mediated by the C-terminal region located outside of the dsRBD.[20,21] Moreover, the inactive mutant PKR (K296R) forms a dimer *in vitro*.[12]

Once activated, PKR phosphorylates downstream substrates, eIF-2α and B56α, resulting in the inhibition of translation.[4] dsRNA activated PKR likewise mediates NF-κB activation promoting IFN gene transcription. In addition to the IKK signalsome, PKR can be also activated by upstream kinases in other signaling pathways including p38 mitogen activated protein kinase (MAPK), and JNK.[22,23] While the roles played by PKR in different signaling pathways require different PKR interactions with stimuli, substrates, and upstream or downstream effector kinases, the primary interactions best characterized are between dsRNA and protein or protein and protein. The complexes so formed can be stable or transient intermediate states. In the absence of high-resolution structures of activating complexes, low-resolution information about the interactions can be obtained using a combination of biochemical and biophysical methods.[11,12] The interaction between kinase inactive PKR mutant K297R and a 57mer HIV TAR RNA in solution was characterized by a combination of biochemical methods plus small angle neutron scattering (SANS).[12] A major conformational change in PKR was detected, likely indicating the exposure of the catalytic site(s) for autophosphorylation. The ratio of PKR to TAR RNA in the complex was given as 2:1 and a functional model was built based on this information†. However, the first SANS experiments concentrated on the very low angle region, from which stoichiometry and radius of gyration information was extracted, but did not provide low-resolution structures for the protein or the complex, due to the lack of large angle scattering data.

Here we report the in solution structural characterization by SANS of a wild-type autophosphorylated PKR preparation. Structural models of PKR dimer and tetramer as well as the complex of PKR with monoclonal antibody were obtained. This is the first study to propose structural models for different association states and conformations of PKR in solution. The information obtained refines the proposed model for the PKR dimer, the activating unit,[3,11,14–21]† and sheds light on

† Williams, B. R. G. (2001). www.stke.org/cgi/content/full/OC_sigtrans;2001/89/re2