

Citation Spotlight

Mechanism of PKR activation: dimerization and kinase activation in the absence of double-stranded RNA

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The kinase PKR is a central component of the interferon antiviral pathway. PKR is activated upon binding double-stranded (ds) RNA to undergo autophosphorylation. Although PKR is known to dimerize, the relationship between dimerization and activation remains unclear. Here, we directly characterize dimerization of PKR in free solution using analytical ultracentrifugation and correlate self-association with autophosphorylation activity. Latent, unphosphorylated PKR exists predominantly as a monomer at protein concentrations below 2 mg/ml. A monomer sedimentation coefficient of $s_{20,W}^0 = 3:58$ S and a frictional ratio of $f/f_0 = 1.62$ indicate an asymmetric shape. Sedimentation equilibrium measurements indicate that PKR undergoes a weak, reversible monomer-dimer equilibrium with $K_d = 450$ μ M. This dimerization reaction serves to initiate a

previously unrecognized dsRNA-independent autophosphorylation reaction. The resulting activated enzyme is phosphorylated on the two critical threonine residues present in the activation loop and is competent to phosphorylate the physiological substrate, eIF2 α . Dimer stability is enhanced by ~500-fold upon autophosphorylation. We propose a chain reaction model for PKR dsRNA-independent activation where dimerization of latent enzyme followed by intermolecular phosphorylation serves as the initiation step. Subsequent propagation steps likely involve phosphorylation of latent PKR monomers by activated enzyme within high-affinity heterodimers. Our results support a model whereby dsRNA functions by bringing PKR monomers into close proximity in a manner that is analogous to the dimerization of free PKR.

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To express active recombinant protein kinase R (PKR) and mutant K296R PKR, the authors transformed Rosetta™(DE3) Competent Cells with the appropriate open reading frames cloned into pET-11a vectors. They state that, "As previously observed (Barber 1991), PKR expresses very poorly in *Escherichia coli* BL21(DE3) cells and cannot be detected in Coomassie-stained SDS/polyacrylamide gels of whole lysates following induction." "This low expression level is due to codon bias, and both wild-type and a catalytically inactive K296R PKR mutant express to high levels using a host containing a plasmid encoding genes for tRNAs that are rare in *E. coli*." ■

REFERENCE

Barber, G. N. et al. 1991. *Biochemistry* 30, 10356.

Rosetta host strains are BL21 derivatives designed to enhance expression of eukaryotic proteins that contain codons rarely used in *E. coli*. The original Rosetta strains supply tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA on a chloramphenicol-resistant plasmid. Rosetta 2 strains supply an additional seventh rare codon, CGG.

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