Abstract

Protein-protein interactions are critical for most physiological and pathophysiological processes. Detailed characterization of these interactions is therefore essential not only to understand the nature of these events, but also to design strategies to target these interactions. This work focuses on the study of the structure and interactions of several proteins and their complexes. Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein kinase kinase kinase (MAP3K) that activates the p38/JNK protein kinase pathways, thereby directing cells toward an inflammatory response or apoptosis. ASK1 interacts with thioredoxin (TRX), a small dithiol oxidoreductase, which inhibits ASK1, but the mechanism of this inhibition has not been clarified. CaMKK1 and CaMKK2 are Ca^{2+}/calmodulin (CaM)-dependent protein kinases that regulate cellular energy balance, memory, and inflammation, among others. Both are inhibited by 14-3-3 proteins, but despite their domain and sequence similarities, the extent of 14-3-3 protein-mediated inhibition is different. Estrogen receptor alpha (ERα) is a nuclear receptor involved in breast cancer. Tamoxifen, an ERα antagonist, is used to treat this disease, but resistance often develops. 14-3-3 proteins interact with ERα and inhibit its transcriptional activity, but the nature of this interaction remains unclear. To better understand these protein-protein interactions, the proteins and their complexes were biophysically and structurally characterized. The cryo-EM structure of ASK1, together with analysis by analytical ultracentrifugation, shows that ASK1 forms a compact dimer with large intra- and inter-chain interactions that stabilize the active conformation of the kinase domain. Hydrogen-deuterium exchange coupled to mass spectrometry shows that TRX binding affects not only the TRX-binding domain, but also all other domains, the activation segment in the kinase domain included. Models of the pCaMKK1:14-3-3γ and pCaMKK2:14-3-3γ complexes obtained from the analysis of small angle X-ray scattering data suggest pCaMKK1 interacts with 14-3-3γ directly through its N-lobe and active site region, whereas pCaMKK2 forms minimal contacts with 14-3-3γ. Biophysical analysis of the ERα/14-3-3ζ complex shows that this complex forms with a stoichiometry of 2:2 and that neither the ERα-Y537S mutation nor ligand binding disrupts or prevents the formation of the complex. These results explain some aspects of the regulation of selected protein complexes and suggest possible ways to intervene in case of their dysregulation.