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Free energy theoretical calculations of PKA–Kemptide complex formation, and effect of mutation of Kemptide arginines to homoarginines.

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Abstract: Protein kinases (PKs) constitute a large and diverse family of enzymes that modify other proteins (substrates) by phosphorylation, which is crucial for cell-division regulation, metabolic routes regulation, and many other cellular functions.[1] A malfunction in such regulation processes can lead to several diseases like inflammatory disorders, endocrine disorders, and cancer. It is well known that PKs are highly selective during the substrate recognition process, and this selectivity is mainly associated to the chemical forces between the binding site of the protein and the sequence in the surroundings of the local phosphorylation site of the protein substrate.

In this work, in order to understand the selectivity of PKs for their substrates, we studied the interactions between PKA, a PK whose enzymatic activity is dependent on cellular levels of cyclic AMP, and the substrate Kemptide.[2] This substrate is the short synthetic heptapeptide Leu-Arg-Arg-Ala-Ser-Leu-Gly which has good selectivity for PKA. The substitution of either of the two arginine residues by other residues lead to higher apparent K_M values than the parent peptide. For instance, when arginines are replaced by the non-natural amino acid homoarginine, which is a very similar residue, the affinity of Kemptide for PKA decreases significantly.

Our purpose was to replicate experimental evidences by using a theoretical atomistic protocol. Molecular dynamic (MD) simulations of PKA (Protein Kinase A) forming complexes with the substrate Kemptide and mutants containing homoarginine instead of typical arginines were carried out. The models used to develop this study contains PKA, Kemptide substrate or its mutants, the solvent media, and ionic environment. After MD simulations, computational free energy calculations were developed by using the free energy perturbation (FEP) method implemented in NAMD software. The free energies differences (less than1 kcal/mol) between our calculations and the experimental data indicate that this computational protocol could replicate the experimental thermodynamic differences at atomistic level.

Conflicts of Interest

The authors declare no conflict of interest.

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References and Notes

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