3D features of proteins and their structural changes related to phosphorylation

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The reversible phosphorylation of proteins is a regulatory mechanism widespread in live systems. Enzyme-catalyzed attachment of the phosphate moieties causes the changes in molecular structures providing the cell signaling transduction, metabolic reactions, protein-protein interaction, etc.

In accordance to the prevalent opinion, the region of protein-substrate adopts the extended conformation being complexed with a protein kinase. In this assumption, continuous sequence segments surrounding the phosphorylated residues aligned at modified positions can be described by the linear phosphorylation motif. The linear model is implemented in the most programs for phosphosite prediction [1]. Finding the functionally significant phosphosites in structurally ordered regions does not also fit into the disorder concept [2]. It is proposed that randomly phosphorylated sites, which have no functional significance, are more frequently located in disordered regions [3].

Using the data on phosphosites found in the amino acid sequences (with the known modifying kinase) we selected about 1500 phosphosites, which were mapped onto the 3D protein structures. Thus, the same sites were present in multiple structures. We studied differences in structural features per the site. The solvent accessibility of residues (non-modified in 3D) significantly changed for some sites. The several ones were explained as buried in all considered structure that can be estimated by existence of the short-lived conformations, in which these sites can be accessible. By mapping the secondary structures, we revealed that the most sites were found in the coil regions. However, the significant portion of ones was located in the structurally stable ordered regions (mainly alpha-helices), which kept the conformation in all considered structures.

Comparison of structures with the same sites in the modified and non-modified states (fig. 1) showed that the site-surrounding regions can be significantly shifted due to phosphorylation.

Additionally, paired comparisons of non-modified vs. non-modified structures and modified vs. modified structures suggests that phosphorylation of a movable site stabilizes one of the possible conformations.

Our work stresses the importance of structurally stable ordered regions as a location of phosphorylation sites. The significant structural shift due to phosphorylation is rather performed when the site shows structural mobility in the unmodified state. In the other situation, phosphorylation creates the interface for the intermolecular or intramolecular interactions without the remarkable shifts of the site surrounding.

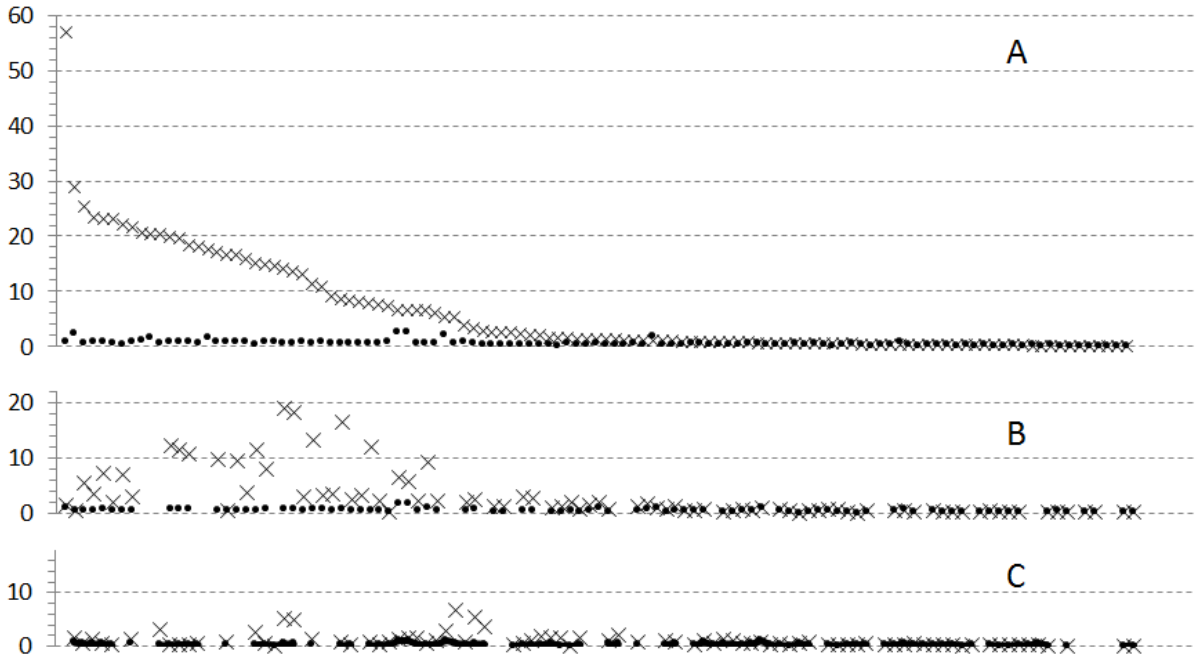

Figure1. Global (dots) and local (crosses) RMSD values (Y-axis) averaging for all structural comparisons per each phosphosite (X-axis). The calculations were performed for pairs of structures containing the same site in phosphorylated and non-phosphorylated forms (A), both site items without (B) and both site items with phosphate (C). The sites are ranked by decreasing the local RMSD values for phosphorylated / non-phosphorylated comparisons.

Accounting the secondary structure is required for revealing the most accurate patterns of phosphorylation, specified for the certain protein kinase types. It is necessary for explaining

the structural changes as a basis of the regulatory processes.

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