Development of the first model of a phosphorylated, ATP/Mg$^{2+}$-containing B-Raf monomer by molecular dynamics simulations: a tool for structure-based design†

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A model of phosphorylated and ATP-containing B-Raf protein kinase is needed as a tool for the structure-based design of new allosteric inhibitors, since no crystal structure of such a system has been resolved. Here, we present the development of such a model as well as a thorough analysis of its structural features. This model was prepared using a systematic molecular dynamics approach considering the presence or absence of both the phosphate group at the Thr599 site and the ATP molecule. Then, different structural features (i.e. DFG motif, Mg$^{2+}$ binding loop, activation loop, phosphorylation site and $\alpha$C-helix region) were analysed for each trajectory to validate the aimed 2pBRAF_ATP model. Moreover, the structure and activating interactions of this 2pBRAF_ATP model were found to be in agreement with previously reported information. Finally, the model was further validated by means of a molecular docking study with our previously developed lead compound I confirming that this ATP-containing, phosphorylated protein model is suitable for further structure-based design studies.

Introduction

Targeted-therapy, one of the most successful approaches to cancer treatment,$^1$ consists of selectively blocking enzymatic pathways upregulated in cancer cells such as those of protein kinases.$^2,3$ Kinases play an important role in cell growth, metabolism, differentiation, and apoptosis by catalysing phosphorylation of the next protein in the pathway. Moreover, it has been found that upregulation of protein kinases is implicated not only in cancer but also in other pathogenicities such as diabetes and inflammation.$^4$

In particular, about 30% of human tumours are triggered by the Ras oncogene and other proteins in its pathway that cause uncontrolled cell division.$^5,6$ The Ras-Raf-MEK-ERK (MAPK) signal transduction pathway regulates different aspects of cell biology in response to growth factors, cytokines and hormones.$^7$

Since Raf proteins were found to be key points in this MAPK pathway, characterisation of their complex regulation has attracted ample attention.$^8$ Thus, it is accepted that Raf activation is initiated by the association with Ras-GTP; then, recruitment to the cell membrane, conformational changes, phosphorylation and dimerization promote its serine/threonine kinase activity phosphorylating MEK.$^7,9$ There are three Raf isoforms (A-, B- and C-Raf), which differ in some details of their activity regulation,$^{10}$ and B-Raf is considered to be the most active of the three.$^11$

Taking advantage of the large amount of crystallographic information on protein kinases reported recently,$^{12-16}$ the understanding of their mechanism of regulation and activation has been advanced by the use of computational techniques such as molecular dynamics (MD) simulations. For example, recently published research has shown how phosphorylation and ATP binding to protein kinases results in different structural changes.$^{17}$ Furthermore, Gosu et al. analysed the dynamic structural change of apo and ATP-bound IRAK4 kinase$^{18}$ and recent MD studies on B-Raf gave insights into previously unexplored relationships between types of B-Raf inhibitors.$^{19,20}$ Very recently, Kuzmanic et al. have reported enhanced molecular dynamics simulations to study the canonical activation of p38a MAP kinase.$^{21}$

During the last few years, Rozas and coworkers have reported a series of diaryl 3,4'-bis-guanidinium derivatives as potential inhibitors of the Ras-Raf-MEK-ERK pathway. In particular, one of