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Review

Peptide inhibitors of protein kinases—discovery, characterisation and use

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Abstract

Protein kinases are now the second largest group of drug targets, and most protein kinase inhibitors in clinical development are directed towards the ATP-binding site. However, these inhibitors must compete with high intracellular ATP concentrations and they must discriminate between the ATP-binding sites of all protein kinases as well the other proteins that also utilise ATP. It would therefore be beneficial to target sites on protein kinases other than the ATP-binding site. This review describes the discovery, characterisation and use of peptide inhibitors of protein kinases. In many cases, the development of these peptides has resulted from an understanding of the specific protein-binding partners for a particular protein kinase. In addition, novel peptide sequences have been discovered in library screening approaches and have provided new leads in the discovery and/or design of peptide inhibitors of protein kinases. These approaches are therefore providing exciting new opportunities in the development of ATP non-competitive inhibitors of protein kinases.

Keywords: Peptide inhibitor; Endogenous inhibitor; Pseudosubstrate; Library screening; Peptide design; Small molecule ATP non-competitive inhibitor

1. Introduction

Protein kinases comprise a large family of enzymes that catalyse the transfer of the terminal phosphoryl group of ATP to their specific protein substrates. When the sequences of protein kinases have been aligned, multiple conserved motifs have been identified [1]. This information has lead to the development of a standard nomenclature that defines critical subdomains or motifs within the protein kinase fold. These motifs have provided a powerful predictive tool in the identification of new protein kinases, and have revealed the presence of ~500 protein kinases within the human or mouse genomes [2,3].

It has been recognised for more than 50 years that protein phosphorylation regulates many aspects of cellular function such as metabolism, division, movement, survival and death. Thus, any disruption of normal phosphorylation can alter cell function and cause disease [4]. Protein kinases are now the second largest group of drug targets, coming after only the G-protein-coupled receptors [4]. To date, most protein kinase inhibitors in clinical development have been directed towards the ATP-binding site. However, one drawback with this current strategy is that these inhibitors must compete with high intracellular ATP concentrations. Furthermore, if they are to be specific, these inhibitors must discriminate between the ATP-binding sites of all protein kinases as well as >200 other human proteins that also utilise ATP (see review [5]).

For these reasons, it would be beneficial to target sites on protein kinases other than the ATP-binding site. However, protein–protein interaction interfaces have generally been considered as difficult targets for small molecule drug discovery. This is largely because the interactions appear to involve larger and frequently less well-defined contact areas when compared with classical drug targets such as enzyme active sites and ligand-binding sites on receptors. There have been some notable successes. For example, small molecule

Abbreviations: AKAP, A-Kinase Anchoring Protein; EGF-R, Epidermal Growth Factor-Receptor; GSK, Glycogen Synthase Kinase; IC_{50}, concentration of compound to achieve 50% inhibition; JIP, JNK Interacting Protein; JNK, c-Jun N-terminal Kinase; K_i, inhibition constant; MLCK, Myosin Light Chain Kinase; PKC, Protein Kinase C; PKI, Protein Kinase A Inhibitor; RACK, Receptor for Activated C-Kinase; SOCS, Suppressor of Cytokine Signalling; TI-JIP, truncated inhibitory region of JIP

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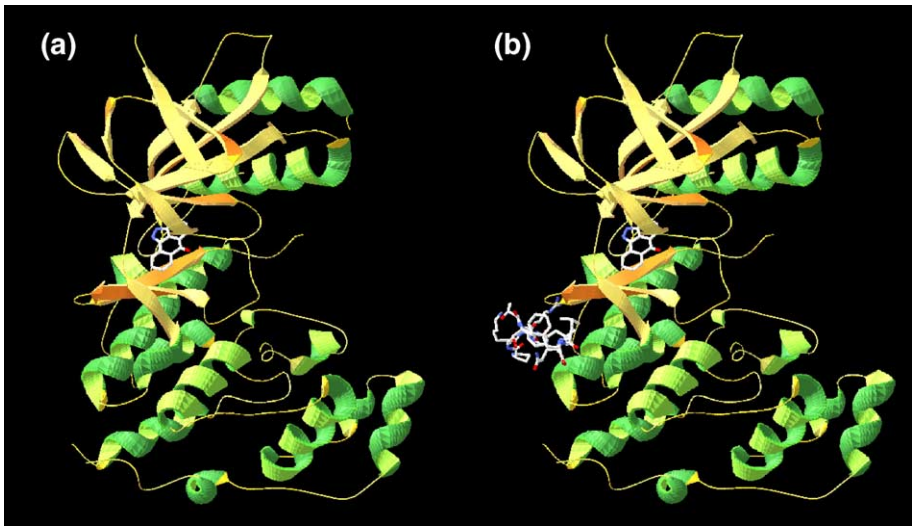

Fig. 1. Protein Kinase Interactions with ATP-competitive and ATP-noncompetitive inhibitors: c-Jun N-terminal Kinase. (a) In a recent example, structural analysis has shown that the ATP-binding site of the protein kinase c-Jun N-terminal Kinase (JNK) is occupied by an ATP-competitive inhibitor of JNKs, SP600125 [9]. (b) In this same study, the structure of the complex between JNK1 and the peptide inhibitor derived from the JNK pathway scaffold protein, JIP1, was solved. This shows the interaction of the JNK1 protein with the peptide inhibitor at a site remote from the ATP-binding pocket.

inhibitors of the interaction of p53 with its suppressor protein HDM2 have been designed, synthesised, tested in vitro and shown to have in vivo efficacy (see review [6]).

In this review, the discovery, characterisation and use of peptide inhibitors of protein kinases are considered. As will be shown, the development of many of these peptides has resulted from an understanding of the specific protein-binding partners for a particular protein kinase. In addition, novel peptide sequences as discovered in library screening approaches have provided new leads in the discovery and/or design of peptide inhibitors of protein kinases. Lastly, how information on peptide inhibitors can aid the development of new non-peptide small molecule inhibitors is considered alongside a number of examples of small molecule ATP-noncompetitive inhibitors of protein kinases. In combination, these approaches provide exciting new opportunities in the development of ATP non-competitive inhibitors of protein kinases.

2. Protein kinase inhibitors derived from biologically-relevant protein partners

The crystal structure of cAMP-dependent protein kinase [7] has provided insights into the organization of the catalytic core of serine/threonine kinases. Striking similarities have been

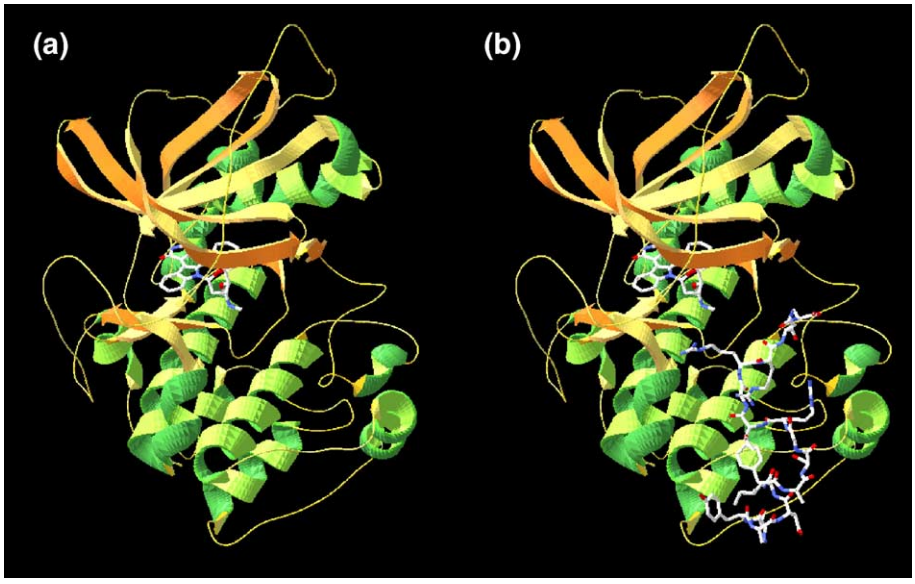

Fig. 2. Protein Kinase Interactions with ATP-competitive and ATP-noncompetitive inhibitors: cAMP-dependent Protein Kinase. (a) Structural analysis has shown that the ATP-binding site of cAMP-dependent protein kinase is occupied by an ATP-competitive inhibitor of protein kinases, staurosporine. (b) The structure of the complex between cAMP-dependent protein kinase and the peptide inhibitor derived from the Protein Kinase Inhibitor (PKI) protein. Again, this shows the interaction of cAMP-dependent protein kinase with the peptide inhibitor at a site remote from the ATP-binding pocket.