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published in

From Computational Biophysics to Systems Biology (CBSB08),
Proceedings of the NIC Workshop 2008,
Ulrich H. E. Hansmann, Jan H. Meinke, Sandipan Mohanty,
Walter Nadler, Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **40**, ISBN 978-3-9810843-6-8, pp. 297-300, 2008.

http://www.fz-juelich.de/nic-series/volume40

Modelling of Possible Binding Modes of Caffeic Acid Derivatives to JAK3 Kinase

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Janus kinases (JAKs) belong to a family of receptor-associated protein tyrosine kinases. They play a crucial role in the JAK/STAT signaling pathways which are responsible for transduction of growth factors and cytokine-mediated signals. Abnormal activation of these pathways is observed in many types of tumors and hematopoetic malignancies[1,2] In the current study we attempt to propose binding modes of caffeic acid derivatives to JAK3, These derivatives are most likely competitive inhibitors for tyrosine-containing protein substrates. Insulin Receptor Kinase (IRK) was used to model an active, open form of JAK3. Based on JAK2 and JAK3 sequence and structure similarity analyses, residues that are responsible for the specific binding were indicated. Leading compounds were docked and modified to get a better binding specificity. The designed inhibitors are being synthesized and their biological activity will be studied experimentally.

1 Introduction

JAKs are crucial enzymes, responsible for the signal transduction through the JAK/STAT pathways, whose abnormal activation is observed in many types of tumors and hematopoetic malignancies, making them an important, but still poorly explored target for therapeutic intervention. Our goal was to create substrate competitive inhibitors of JAK3, based on our knowledge of the JAK2 inhibitors. Caffeic acid derivatives, including AG490, are the potential, promising inhibitors[3]. Even though the sequence similarity between catalytic domains of JAK2 and JAK3 is about 60%, it is difficult to propose JAK3 specific inhibitors. This is because essential residues (those which form the ATP binding pocket or the peptide binding site) are mostly the same for both kinases. In addition, there are no experimental JAK3 open (active) conformations. There is, however, an IRK structure with a bound protein inhibitor in an open enzyme conformation. Even though sequence similarity between IRK and JAKs is relatively low, the three dimensional structure similarity is significant (Fig. 1). Structural information and JAK2 and JAK3 sequence similarity analysis allowed us to identify JAK3 potential binding sites and to design specific inhibitors.

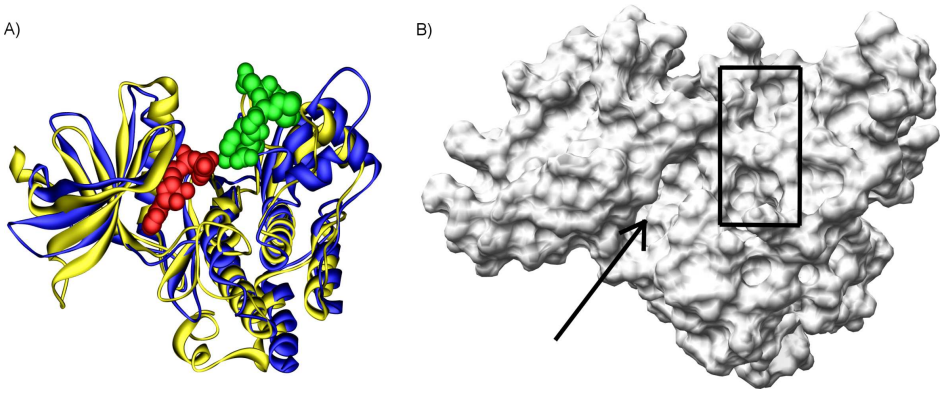

Figure 1. (A) Superposition of JAK3 on IRK. Yellow ribbon presents IRK, blue ribbon presents JAK3. The green spacefill atoms represent protein substrate atoms while the red spacefill atoms represent ANP (the ATP analog which is bound in the ATP binding cassette). (B) JAK3. The arrow indicates the nucleotide binding site, rectangle indicates the predicted docking sites.

2 Materials and Methods

Three dimensional structures of IRK, JAK3 as well as of JAK2 from the Protein Data Base were used (1IR3, 1YVJ and 2B7A structures, respectively). Because JAKs do not contain peptide substrates/inhibitors, the IRK structure in its open, active form was used for the identification of the JAK3 ligand binding site. Using MOE software we docked our potential inhibitors in the predicted peptide binding site as indicated by rectangle in Fig. 1. Because the site is relatively large, we identified two subsites that fit to our ligands. These two subsites are located in the top part and in the bottom part of the rectangle.

3 Results and Discussion

We attempted to design inhibitors that form hydrogen bonds with the backbone as well as with proteins side chains. The H-bonds with the backbone are responsible for the binding efficacy, and the H-bonds with the side chains are responsible for the substrate specificity. We indentified four potential binding sites, two of them appeared to be very promising. These are the second (Site2) and the fourth (Site4) largest pockets detected on the JAK3 surface. The most important residues which form the Site2 are: ALA952 Cys1024, ASP1025, CYS1028 and SER1031. In turn, ARG984, ASN1002 and LEU1047 form the Site4. The first binding mode accounts for interactions with the backbone of SER1029 and Ser1031. In JAK2 these positions are occupied by SER1056 and PRO1058 respectively. The second binding mode in JAK3 accounts for interactions with the backbone of ASN1002, which in JAK2 is occupied by SER1029 and SER 998. These two binding modes (Fig. 2) appeared to be the most promising. Concluding, it was possible to identify those JAK3 residues that are potentially responsible for the substrate/ligand specificity.

In order to get sufficiently high specificity of the potential inhibitors one can generate and visualize sequence variability profiles for a given protein family. Regarding protein

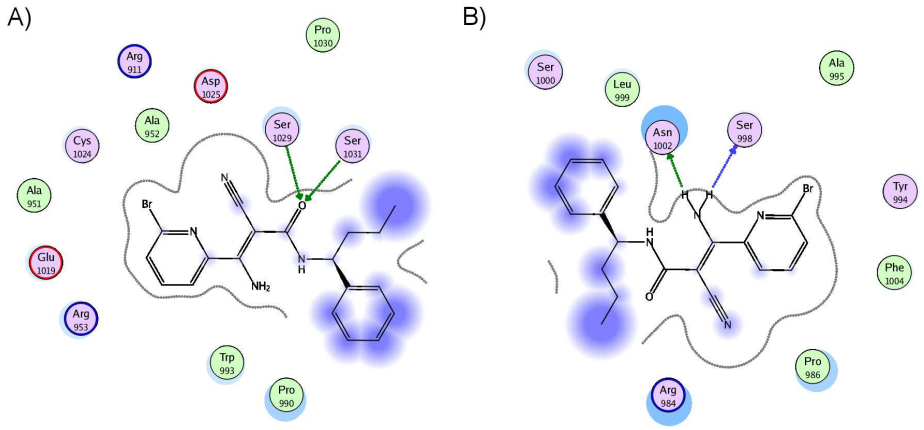

Figure 2. Binding of the caffeic acid derivatives to two subsites (A and B) in JAK3. The arrows represent the modelled hydrogen bonds that link JAK3 with added/modified atoms of the ligand.

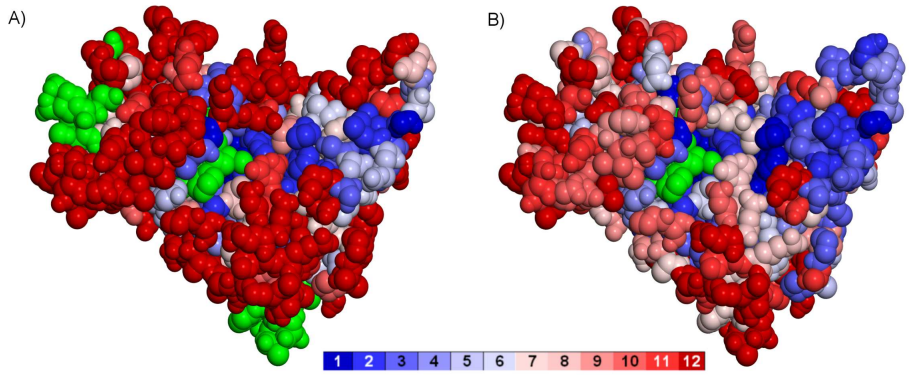

Figure 3. (A) Variability profiles resulting from alignment of 152 protein sequences from the PFAM database for the JAK family, using the JAK3 structure for visualization. (B) Visualization of 50 protein Homo sapiens sequences homologous to JAK3 acquired from the SWISS-PROT/TREMBL database. Each color represents a number of different amino acids which occupy a particular position in the multiple sequence alignment. In addition, the green color represents ATP and the protein C and N ends.

kinases, sequences from PFAM[4] and from Swiss-Prot/Trembl[5] data bases were used. A new application[6] processes these sequences and visualizes variability profiles in 3D on a 2D surface of the studied enzyme here JAK3, presented in Fig. 3. The mentioned above analysis can account for hundreds of protein sequences, it is very fast and brings a lot of essential data.

Acknowledgments

These studies were supported by the PBZ-MIN-014/P05/2005 grant as well as by Centre of Excellence BioExploratorium.

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