Of life and Death: Chronic Myeloid Leukemia and the Bcr-Abl/Imatinib Paradigm

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ABSTRACT

Bcr-Abl is a constitutively active tyrosine kinase that transforms cells and causes chronic myelogenous leukemia (CML). Imatinib mesylate produces complete hematological and cytogenetic response in a high percentage of CML patients; however, most patients in blast crisis either fail to respond or quickly relapse following an initial response to imatinib. Mutations within the Bcr-Abl kinase domain with less affinity towards imatinib are the most commonly identified mechanism associated with the relapse. All mutagenesis and biochemical studies supporting this hypothesis, however, were never paralleled by a thorough investigation at the computational biology level. In this work, we report the results obtained from the application of the MM/PBSA approach to the evaluation of the binding free energy of wild type and mutant Bcr-Abl core domains with Imatinib. Our data lead us to the conclusion that all analyzed mutations can be classified into two major groups, A and B:

- mutations in group A greatly decreases sensitivity of Abl toward Imatinib, and can be claimed to be the cause of resistance when identified in patients;
- mutations found in group B can be further subdivided into two categories:
  - ✓ those for which a dose escalation of the inhibitor would be expected to recapture a response, and
  - ✓ those which will indeed be unresponsive to dose escalation, as no significant interaction is involved with the inhibitor. For these substitutions, we propose that they confer drug resistance through alternative mechanisms.

According to our results, and the limited computational times required, this technique could be adopt in the pool of routine analyses in cancer research.

1. Introduction

Chronic myeloid leukemia (CML) is a clonal disease involving the pluripotent hematopoietic stem cell compartment, and is associated with the Philadelphia chromosome, a reciprocal translocation between chromosome 9 and 22. This translocation links the c-ABL tyrosine kinase oncogene on chromosome 9 to the 5' half of the BCR gene on chromosome 22, and originates the fusion gene BCR-ABL. The fusion gene produces a chimeric 8.5-kb transcript that codes for the $p210^{BCR\text{-}ABL}$ protein,[1] which possesses constitutive tyrosine kinase (TK) activity and is the pathogenic agent of CML.

From the therapeutic perspective, as CML arises from a single genetic lesion, a research goal has been to develop kinase specific inhibitors. The development of imatinib (also known as imatinib mesylate, Glivec®, Gleevec™) is a landmark achievement in this respect, as it has shown great promise in the chronic phase, and some expectations in the accelerated and blastic phase of CML, as well as in BCR-ABL-expressing acute lymphoblastic leukemias.[2,3]

Unfortunately, however, most responding blast phase patients relapse despite continued chemotherapy and, as frequently happens with cancer chemotherapeutics, resistance to imatinib has been reported in both Bcr-Abl expressing cell-lines and in patients with CML.

Mutations within the Bcr-Abl kinase domain (KD) are the most commonly identified mechanism associated with the relapse. These mutations are clustered in four regions:

- ✓ the first region is the phosphate-binding loop (P-loop), a highly conserved sequence responsible for phosphate binding;
- ✓ the second region is residue T315, a non-conserved amino acid that is in part responsible for the selective inhibition of the Abl kinase by imatinib;
- ✓ the third region is made up by residues M351 and E355;
- ✓ the fourth region is the activation loop (or A-loop).

In particular, the threonine-to-isoleucine replacement at position 315 (T315I) has been described in details. Mutation of this amino acid, along with residues 253, 255 and 351, was subsequently identified in 60% of patients with kinase domain mutations at the time of disease relapse, with an overall mutation frequency between 30% and 90%. The substantial decrease of sensitivity of these mutants toward imatinib definitely marks them as the likely cause of resistance.

Several are, however, the amino acids involved in nonbonded interaction with imatinib

in the Abl complex, as derived from an inspection of the corresponding crystal structure (D381, H361, I360, M318, T315, E286, F382, A380, L370, R362, G321, F317, I313, V299, M290, V289, K271, A269, V256, Y253, and L248). On the basis of these evidences, it was postulated that the above mentioned point mutation T315I in the TK domain of Bcr-Abl both alters the three-dimensional structure of the ATP pocket, and does not features a fundamental hydrogen bond, critical for binding with the drug, thus decreasing the sensitivity to Bcr-Abl for imatinib. Further, from extensive mutational analyses of sites that might alter the sensitivity of the Abl kinase to imatinib it was found that some mutations resulted in kinase-inactive proteins, whilst other substitutions either paralleled the wild type protein response or rendered the Abl kinase less insensitive to imatinib, thus highlighting a broad range of possibilities for clinical resistance.[4-6] All mutagenesis and biochemical studies supporting the Abl/imatinib structural/interaction hypotheses, however, were never paralleled by a thorough investigation at the computational biology level. Accordingly, we present the results obtained from a detailed molecular simulation study of the wild type and the following mutated Abl kinase domains: M244V, G250E, Q252H, Y253F, E255K, F311L, T315I, F317L, M351T, E355G, F359V, V379I, L387M, and H396P, aiming at offering, for the first time, both a qualitative and a quantitative picture of the molecular mechanism of failure of the tyrosine kinase inhibitor binding to the mutated protein.[7]

The major purpose of this work is to propose a reliable and relatively fast computational recipe for the classification of different mutations in Abl kinase domain with respect to their affinity towards imatinib. This technique could be adopted as a routine analysis, and flanked to all other techniques already employed in cancer research to define a better therapeutic strategy.

2. Computational details

All mutations were introduced into the wild-type crystal structure of c-Abl/imatinib complex[8] by swapping the mutant residues into the specific site according to a well-validate procedure.[9-11] To estimate the free energy of the complex system, we adopted following ansatz, based on the so-called molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) scheme:[9,12]

- ✓ we carried out molecular dynamics (MD) simulations in a periodic box with water and counterions, and correct representation of long-range electrostatic effect using the particle mesh Ewald method, saving a set of representative structures;

- ✓ we then postprocessed these structures removing any solvent and counterion molecules, and calculate the binding free energy, ΔG_{bind}, according to the following equation:

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{solv} - T\Delta S$$

The MM/PBSA version used in this work took snapshots from a molecular dynamics (MD) trajectory of each drug/protein complex, with explicit water molecules and counterions, and averaged the internal MM energies and solvation free energies of the molecules. All calculations were carried out using the AMBER 7 platform[13] with the Cornell et al force field (*parm 94*)[14] on a cluster of Silicon Graphics Octane R12K.

3. Free energy of binding for wt and mutated c-Abl and imatinib

The results of our simulations are reported in Table 1, which lists the calculated free energy of binding, ΔG_{bind}, and the equivalent IC_{50} values of wild type and mutant Abl KD and imatinib. The corresponding experimental values are also reported for comparison. The outstanding correlation (r^2 = 0.96) with experiment indicates an impressively accurate reproduction of the absolute free energies of binding by our procedure.

The MM/PBSA approach also allows us to separate the total free energy of binding into electrostatic and van der Waals interactions, giving some physical insight into the complex association process. The role of electrostatics in the Abl/imatinib complexes formation can be examined further by considering the electrostatic component of the molecular mechanical together with the electrostatic contribution to solvation (data not shown). The electrostatic contribution (ΔE_{EL} + ΔG_{PB}) is always positive, because the unfavorable change of solvation is not fully compensated by the favorable electrostatics within the complex. This indicates that the electrostatics constantly disfavor the binding of both the wild type and the mutated Abl core domains with imatinib. The favorable formation of these complexes is then driven by the van der Waals contribution and the nonpolar contribution to solvation, in agreement with other numerous, analogous studies. Finally, our estimates show that the entropy term $-T\Delta S$ adds an approximately equal contribution of ~ 17 kcal/mol to the total binding energy for the wild type and the mutants. The short range of entropy variation is an expected result, as all systems in our study are just one residue different from the wild type. Interestingly, the changes in entropic term upon substitutions are favorable.

Table 1. Comparison between calculated and experimental free energy of binding ΔG_{bind} (kcal/mol) and IC_{50} (μM) values of wild type and mutant Abl kinase domains and Imatinib*

Mutation	ΔG_{bind} (calc.)	ΔG_{bind} (exp.)	IC_{50} (calc.)	IC_{50} (exp.)
Wild type	-10.39 ± 0.18	- 10.37	0.024	0.025
Group A				
G250E[#]	-6.81 ± 0.15	NA	10.2	NA
Y253F	-7.72 ± 0.06	- 7.84	2.2	1.8
E255K	-6.51 ± 0.27	< - 7.23	16.8	> 5
T315I	-6.55 ± 0.20	< - 7.23	15.7	> 5
Group B				
M244V	-10.32 ± 0.16	- 10.37	0.027	0.025
F311L	-10.26 ± 0.08	- 10.37	0.030	0.025
E355G	-10.45 ± 0.14	- 10.50	0.022	0.020
F359V	-9.86 ± 0.19	- 10.02	0.059	0.045
V379I	-10.67 ± 0.05	- 10.30	0.015	0.028
L387M	-9.70 ± 0.10	- 9.93	0.077	0.053
Q252H[#]	-9.44 ± 0.07	NA	0.12	NA
F317L	-8.84 ± 0.07	- 9.11	0.33	0.21
M351T[#]	-9.12 ± 0.18	NA	0.21	NA
H396P	-8.71 ± 0.13	- 8.94	0.41	0.28

*The experimental ΔG_{bind} and the calculated IC_{50} values were obtained from the corresponding experimental IC_{50} and calculated ΔG_{bind} using the following relationship: $\Delta G_{bind} = RT \ln K_{diss} = RT \ln (IC_{50} + 0.50 C_{enz}) \cong RT \ln IC_{50}$.

[#]No experimental IC_{50} values were available for these compounds. NA = not available.

Coupling the observations from the MD trajectories to the energetic data leads us to the conclusion that all analyzed mutations can be classified into two major groups: group A gathers all those mutations that play a major role in imatinib binding, whilst group B collects all mutations that play a modest role in imatinib binding. Mutations belonging to group A greatly decreases sensitivity of Abl toward imatinib, and can be claimed to be the cause of resistance when identified in patients. Mutations found in group B can be further subdivided into two categories. The former includes those mutations for which the relevance in causing the resistant phenotype is questionable, and for which a dose escalation of the inhibitor would be expected to recapture a response. The latter category groups all those mutations which will indeed be unresponsive to dose escalation, as no significant interaction is involved with the inhibitor. For these substitutions, we propose that they confer drug resistance by interfering with intermolecular regulatory interactions, thus shifting the equilibrium of the kinase to the phosphorylated state in cells.

3.1. Group A: the T315I example

The detailed analysis[9] of the free energy of binding components reveals that both the van der Waals and the electrostatic interactions drastically decrease in the case of the isoleucine mutant. The absence of the hydrogen bond between the OH side chain of T315 and the amino group of imatinib correctly reflects in a decrease of the electrostatic stabilizing contribution of 2.20 kcal/mol. Obviously, isoleucine side chain is larger than threonine (73.0 $Å^3$ VS. 48.6 $Å^3$, respectively); from the model it appears, however, that it can be accommodated without too severe van der Waals repulsions ($\Delta\Delta E_{vdW}$= -3.55 kcal/mol). Accordingly, the only possible reason for the threonine to isoleucine reason to be destabilizing can be the difference in Gibbs free energy of hydration. Indeed, the calculated difference in ΔG_{solv} of threonine versus isoleucine is equal to 7.19 kcal/mol.

In a sort of a domino effect, however, the substitution of a single residue in this position induces several, sometimes substantial modifications in the conformation of other residues, both belonging to the binding site and the surrounding areas, as a direct consequence of the different position assumed by imatinib in the binding site. The residues which register the most consistent loss of favorable interactions with the inhibitor in the presence of the I315 substitutions are K271, E286, R362, and D381.

3.2. Group B - Subgroup 1: the M244V example

As recently reported by Corbin et al.,[5] the M244V mutation shows no biochemical difference in sensitivity to imatinib relative to wild type (see Figure 1a). Further, it also has a low cellular IC_{50} value compared with other mutants. Accordingly, the authors have postulated that clinical response could be regained with higher inhibitor doses if this mutation was the sole abnormality. This finding was supported by the clinical response of a patient with the M244V mutation who achieved a major cytogenic response (MCR) with an increased dose to 600 mg of imatinib after failure to obtain a response to the initial dose of 400 mg.[11] In this respect, our simulation results clearly sustain all these experimental evidence. With respect to valine, the total electrostatics contribution is not very different from that of methionine. Therefore, the difference of the van der Waals energy is that major term that discriminates the binding energy towards imatinib of the two protein domains ($\Delta\Delta E_{vdW}$ = -1.84 kcal/mol). This may be expected because M244V is a conservative substitution. However, the consequences of a conservative mutation from methionine to valine are minimal, both in terms of

chemico-physical parameters and structural-energetical features, as none of the two residues contact imatinib. In fact, the two amino acids have comparable surface areas, and almost equal calculated values of the nonpolar part of the residue surface area (160 $Å^2$ and 142 $Å^2$, respectively). As the side chains of both residues point into a cavity lined by nonpolar residues, the slighter smaller dimensions of valine results in less extensive dispersion and hydrophobic forces, accounted for by the slightly less favorable ΔE_{vdW} term.

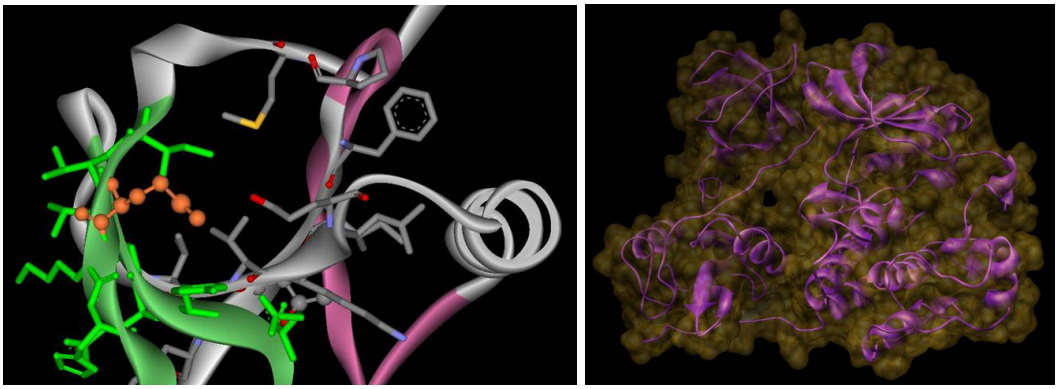

Figure 1. Details of the M244V substitution in the c-Abl KD (a, left) and cartoon of the kinase domain interface of c-Abl with SH2 domain (b, right).

3.3. Group B - Subgroup 2: the M351T example

SH2 is important for binding phosphotyrosine-containing peptides, enhancing phosphorylation of specific substrates and positively modulating Bcr-Abl transformation activity. By homology to c-Scr, the kinase domain interface of c-Abl with SH2 is made by part of the long α3 helix, within the large C lobe, which includes the variant M351T, already reported in patients.

The energetic analysis reveals that the difference in free energy of binding between c-Abl M351T and wild-type c-Abl core domain is confined to $\Delta\Delta G_{bind}$ = -1.24 kcal/mol. The energetic decomposition of ΔG_{bind} of this variant to imatinib on a per residue basis shows that the contribution to inhibitor binding of each imatinib contact point is poorly affected by the presence of the considered mutation. Accordingly, the relevance of this mutation in causing the "classical" resistant phenotype is questionable.

The results obtained from our simulations, based on a model obtained by homology starting from the c-Scr protein, reveal that the side chain of M351 faces SH2 and, therefore, concurs to determine the specificity of contacts along this surface. Therefore,

we propose that the M351T mutation may confer drug resistance by affecting c-Abl intramolecular regulatory interactions. All other mutations belonging to subgroup 2 may act in harmony, or might be epiphenomena, driving resistance only in the context of other simultaneous changes.

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Acknowledgments

Authors gratefully acknowledge the generous financial support from AIRC (Italian Association for Cancer Research), the Italian Ministry of Health, and the Italian Ministry for University and Scientific Research.