Published in final edited form as:

J Med Chem. 2009 September 24; 52(18): 5582–5585. doi:10.1021/jm900974p.

Beyond Thermodynamics: Drug Binding Kinetics Could Influence Epidermal Growth Factor Signaling

Mayank Goyal, Michael Rizzo, Frank Schumacher, and Chung F. Wong*

Department of Chemistry and Biochemistry, and the Center for Nanoscience, University of Missouri-Saint Louis, One University Boulevard, MO 63121

Abstract

We modeled the kinetics of drug binding to protein kinases in the EGF signaling pathway relevant to non-small cell lung cancer and found that binding kinetics could influence therapeutic potential, that fast binding kinetics was advantageous for most targets with a couple of exceptions, that targeting some protein kinases could enhance rather than attenuate the pathway, and that IC₅₀ could be sensitive to the kinetic parameters of drug binding.

Computer-aided drug design has mostly focused on considering binding strength or thermodynamics rather than kinetics. Yet, there is already evidence that drug binding kinetics can also play a role. In a recent paper, Tummino and Copeland proposed that the residence time, rather than the binding affinity, determined the therapeutic potential of drug candidates.

¹ They defined the residence time to be the inverse of k_{off} .

One study supporting this proposal concerned the design of peptide mimics to bind to the extracellular domain of the receptor tyrosine kinase Human Epidermal growth factor Receptor 2 (HER2), a protein kinase in the same family as Epidermal Growth Factor Receptor (EGFR).

² In this work, the authors found that the desired cellular interference correlated better with the dissociation constant than with the binding affinity of the peptide mimics. Another example came from studies correlating the response of T cells with the types of MHC/peptide complexes with which they interacted. Whether the presented peptide by MHC was an agonist, partial agonist, antagonist, or one that did not trigger any T-cell response appeared to correlate with the duration of interaction between a T cell and an MHC/peptide complex.³⁻⁸

On the other hand, some studies found that k_{on} , rather than k_{off} , associated better with therapeutic potential. For example, Wu et al showed that k_{on} of engineered antibodies linked better with the ability of the antibodies to neutralize the respiratory syncytial virus.⁹

To gain further insights into the role of drug-binding kinetics in drug discovery, we performed kinetic modeling on the Epidermal Growth Factor (EGF) signaling pathway, which is overly active in non-small cell lung cancer, for example. Modeling has the advantage that it can study theoretical compounds that may be difficult to make experimentally to help test a concept of drug design. For example, it is not easy to synthesize compounds with exactly the same binding affinity but different rate constants of drug-receptor association and dissociation to examine whether kinetics, in addition to thermodynamics, can be an important factor to consider in drug discovery. However, one can do this easily with a kinetic model.

*Department of Chemistry and Biochemistry, and the Center for Nanoscience, University of Missouri-Saint Louis, One University Boulevard, MO 63121, wongch@umsl.edu, phone: 314-516-5318.

Supporting Information Available: The two tables S1 and S2 give the species, rate laws, and kinetic parameters used in the pathway model based on the work of Wang et al.¹⁰. This material is available free of charge via the Internet at <http://pubs.acs.org>.

In kinetic modeling, it is also useful to examine a larger pathway instead of only analyzing the impact of a drug candidate on its direct target. In this work, we studied the effects of applying theoretical drugs to different protein kinases in the EGF signaling pathway constructed previously by Wang et al.¹⁰ We modified their pathway only slightly to include drug interactions. Fig.1 shows an example in which a drug is applied to the EGF-EGFR dimer denoted by (EGF:EGFR)₂.

In order to study whether drug-binding kinetics could attenuate the production of the doubly phosphorylated form of MAP kinase (ERK-PP depicted in Fig. 1) differently, we prepared theoretical drugs with exactly the same binding affinity (K_I) to its protein kinase target but with different kinetic constants of binding (k_a) and unbinding (k_d) such that $K_I=k_d/k_a$ remained constant. If these drugs with exactly the same binding affinity K_I affected the activity of ERK-PP differently, one could conclude that binding kinetics indeed played a role in drug design. (We monitored the concentration of ERK-PP because its level of activity is believed to control cell proliferation.)

We performed kinetics modeling by using Copasi 4.5 (build 30).¹¹ The model consisted of twenty-two molecules and involved solving twenty-two coupled differential equations. We used the same rate laws, kinetic constants and initial concentrations as in Wang et al's work,¹⁰ except when we modeled an over-activated EGF-EGFR dimer or when we mimicked an over-expression of EGFR. On the other hand, because our model introduced drug binding, we modified one kinetic equation accordingly and added one equation for drug binding (step R3 in Fig. 1). The equations and parameters of the kinetic model are reported in Tables S1 and S2 of Supplementary Materials.

Fig. 2 gives an example of the time course of the concentration of ERK-PP. The concentration rose to a maximum before decaying to zero. In this study, we monitored the change in the maximum concentration when different theoretical drugs were applied in order to calculate percent inhibition of ERK-PP, which was obtained by using the maximum concentration of ERK-PP before and after a drug was applied.

Table I shows that binding kinetics did affect ERK-PP production when we applied theoretical drugs with different kinetic, but same thermodynamic, parameters to the EGF-EGFR dimer. When we used a drug concentration of 1 nM (equaled to K_I), there was no inhibition. But as we increased the concentration to 100 nM, appreciable inhibition appeared and the degree of inhibition depended on binding kinetics. Percent inhibition was low at slow binding kinetics but approached a maximum value with faster binding kinetics (note that as we increased the dissociation constant k_d , the association constant increased accordingly so as to keep K_I constant.) The last column in Table I gives the results for a truncated pathway in which only steps R1 to R5 in Fig. 1 were included. The purpose of this computational experiment was to examine whether a simpler assay could predict the behavior of a more complex assay including more downstream steps. We found that this truncated pathway gave similar results as the larger pathway in Fig. 1. The percent inhibition was nearly the same for the full and truncated pathways. (For the truncated pathway, we monitored the concentration of (EGF:EGFR)-P in Fig. 1 instead of ERK-PP.)

Fig. 3 shows similar qualitative dependence of percent inhibition on k_d when we decreased the binding affinity (K_I) of the drug to the EGF-EGFR dimer by a factor of ten, when we decreased the concentration of EGFR to 1 nM, when we increased the concentration of EGFR by a factor of ten to mimic an over-expression of the protein, and when we increased the rate constant of the phosphorylation of the EGF-EGFR dimer by twenty times to mimic an over-activated protein resulting from a diseased mutation, for example. (In this last case, we used a higher-affinity drug, with $K_I = 0.1$ nM, in order to obtain observable percent inhibition.)

Although applying drugs to most protein kinases in this pathway gave similar qualitative dependence of percent inhibition on k_d as those shown in Fig. 3, there were exceptions. For example, when we applied a drug to the activated form of Raf – denoted by Raf* in Fig. 1 – we observed that the percent inhibition peaked at an intermediate k_d approximately around $1 \times 10^{-3} \text{ s}^{-1}$ and decreased afterwards, instead of increasing monotonously with k_d to an asymptotic value as the cases illustrated in Fig. 3. (Fig. 4) On the other hand, applying drug to the un-activated form of Raf showed the common behavior discussed earlier. The larger percent inhibition observed for the un-activated form of Raf also suggested that targeting the un-activated form of Raf might be more effective in attenuating ERK-PP production than targeting the activated form, according to this kinetic model.

Fig. 5 shows yet another behavior when we applied drugs to the three different forms of MAP kinase kinase. Applying a drug to the un-phosphorylated form, MEK, increased the production of ERK-PP rather than attenuating it. On the other hand, applying drugs to either the mono- or doubly-phosphorylated forms of MEK both reduced ERK-PP production.

Using the singly phosphorylated form of MAP kinase kinase, MEK-P, as an example, Fig. 6 shows that IC50 obtained from a kinetic assay could depend on the kinetic constants of drug binding. The IC50 varied by about a factor of four for the range of k_d covered in the figure. Computational drug designers need to be aware of this as they often compare computed binding affinity – a thermodynamic rather than a kinetic quantity – with experimental $\ln(\text{IC}_{50})$.

In summary, our kinetic modeling of drug interference with protein kinases in the EGF signaling pathway showed that drug-binding kinetics could play a role in attenuating this pathway. Thus, tuning the kinetic parameters of drug binding can also be important in drug discovery. Although fast-binding kinetics seemed to be favored for most protein kinase targets in this pathway, there were exceptions. In the case of activated Raf, drug with intermediate kinetic constants appeared best. On the other hand, we found that applying a drug to a protein kinase such as the unphosphorylated form of MEK could activate rather than attenuate signaling. In addition, we found that IC50 could depend on drug binding kinetics when measured by kinetic assays. Although the reliability of our predictions on EGF signaling depends on how well this kinetic model reflects reality, the principles that drug-binding kinetics can play a role in drug discovery should be general. As kinetic models are continually being refined to fit experimental observations for specific pathways and networks, these models can play an important role in guiding drug discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research is supported in part by the National Institutes of Health.

References

1. Tummino PJ, Copeland RA. Residence time of receptor-ligand complexes and its effect on biological function. *Biochemistry* 2008;47:5481–5492. [PubMed: 18412369]
2. Berezov A, Zhang HT, Greene MI, Murali R. Disabling erbB receptors with rationally designed exocyclic mimetics of antibodies: structure-function analysis. *J Med Chem* 2001;44:2565–2574. [PubMed: 11472210]
3. van der Merwe PA, Davis SJ. Molecular interactions mediating T cell antigen recognition. *Annu Rev Immunol* 2003;21:659–684. [PubMed: 12615890]

4. Germain RN, Stefanova I. The dynamics of T cell receptor signaling: complex orchestration and the key roles of tempo and cooperation. *Annu Rev Immunol* 1999;17:467–522. [PubMed: 10358766]
5. Davis MM, Boniface JJ, Reich Z, Lyons D, Hampl J, Arden B, Chien Y. Ligand recognition by alpha beta T cell receptors. *Annu Rev Immunol* 1998;16:523–544. [PubMed: 9597140]
6. Lyons DS, Lieberman SA, Hampl J, Boniface JJ, Chien Y, Berg LJ, Davis MM. A TCR binds to antagonist ligands with lower affinities and faster dissociation rates than to agonists. *Immunity* 1996;5:53–61. [PubMed: 8758894]
7. Matsui K, Boniface JJ, Steffner P, Reay PA, Davis MM. Kinetics of T-cell receptor binding to peptide/I-Ek complexes: correlation of the dissociation rate with T-cell responsiveness. *Proc Natl Acad Sci U S A* 1994;91:12862–12866. [PubMed: 7809136]
8. Savage PA, Boniface JJ, Davis MM. A kinetic basis for T cell receptor repertoire selection during an immune response. *Immunity* 1999;10:485–492. [PubMed: 10229191]
9. Wu H, Pfarr DS, Tang Y, An LL, Patel NK, Watkins JD, Huse WD, Kiener PA, Young JF. Ultra-potent antibodies against respiratory syncytial virus: effects of binding kinetics and binding valence on viral neutralization. *J Mol Biol* 2005;350:126–144. [PubMed: 15907931]
10. Wang Z, Zhang L, Sagotsky J, Deisboeck TS. Simulating non-small cell lung cancer with a multiscale agent-based model. *Theor Biol Med Model* 2007;4:50. [PubMed: 18154660]
11. Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, Singhal M, Xu L, Mendes P, Kummer U. COPASI--a Complex PATHway SIMulator. *Bioinformatics* 2006;22:3067–3074. [PubMed: 17032683]

Abbreviations

EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
PLCγ	phospholipase c γ
PKC	protein kinase C
Raf	MAP kinase kinase kinase
MEK	MAP kinase kinase
ERK	MAP kinase

Figure 1.

Pathway model for studying the influence of drug-binding kinetics on EGF signaling in non-small cell lung cancer. A:B represents a complex between A and B. A-P denotes the phosphorylated form of A and A-PP represents the doubly phosphorylated form of A. A* indicates the activated form of A. (Note: The single-direction arrows do not imply all reactions are unidirectional – some represent reversible reactions.)

Figure 2.

Time evolution of the concentration of ERK-PP as a function of time. 10 nM of a theoretical drug, with $K_I=1\text{nM}$, was applied to $(\text{EGF:EGFR})_2$ shown in Fig. 1. The starting concentration of EGF and EGFR were 132.5 nM and 80nM respectively. The maximum concentration was used to compute percent inhibition in this study.

Figure 3.

Percent inhibition versus drug-receptor dissociation rate constant k_d . The reference simulation (solid line) started with [EGFR]=80nM, [EGF]=132.5nM, and 100 nM of a drug with $K_I=1$ nM applied to EGFR. Dashed line: K_I of the drug increased to 10 nM. Dot-dashed line: starting concentration of EGFR increased by a factor of ten to 800 nM. Dot-dot-dashed line: concentration of EGFR decreased to 1 nM. Dotted line: forward rate constant in step R5 in Fig. 1 increased by twenty times; K_I of drug decreased to 0.1 nM.

Fig. 4. Percent inhibition of ERK-PP when 100 nM of drug was applied to Raf (solid line) or Raf* (dashed line). The forward rate constant of step R5 in Fig. 1 was increased by a factor of twenty to mimick an over-activated EGF-EGFR dimer.

Fig. 5.

Applying drug to MEK elevated the production of ERK-PP rather than decreasing it (shortest dashed line). Solid line represents the reference state when no drug was applied. On the other hand, applying drugs to both the singly and doubly phosphorylated forms of MAP kinase kinase both attenuated the production of ERK-PP. (The two plots with longer dashed lines.) 10 nM of drug, with $K_I=1$ nM, was used in each case.

Fig. 6.

IC_{50} as a function of drug-receptor dissociation rate constant for MEK-P. $K_I=1$ nM and the forward rate constant for step R5 in Fig. 1 was increased by a factor of twenty to mimic an over-activated EGF-EGFR dimer.

Table I

Inhibition of ERK-PP as a function of kinetic parameters of drug binding

k_d (s^{-1})	% Inhibition ([drug]=1nM)	% Inhibition ([drug]=10nM)	% Inhibition ([drug]=100nM)	% Inhibition ([drug]=100nM)
0.0001	0.0008	0.0032	0.1953	1.9255
0.001	0.0008	0.0145	10.8051	13.0047
0.01	0.0008	0.0209	43.2716	45.3306
0.1	0.0008	0.0185	43.8325	45.3210
1	0.0008	0.0193	43.8399	45.3202
10	0.0008	0.0193	43.8389	45.3202

Theoretical drugs with different kinetic parameters and doses were applied to the EGF-EGFR dimer in the pathway shown in Fig. 1. The starting concentration of EGF and EGFR were 132.5 nM and 80nM respectively. The binding constant of the drugs to the dimer was fixed at $K_I=1$ nM. k_d was the dissociation rate constant for the drug-receptor complexes. Results in the last column were obtained by using a truncated pathway in which only steps up to R5 in Fig. 1 were included.