Chemistry & Biology, Vol. 10, December, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.chembiol.2003.12.006

Summing Up Ligand Binding Interactions

Artificial molecular recognition systems show negligible positive binding cooperativity compared to biological systems, possibly because rigid ligands cannot accommodate numerous partly bound states that comprise overall ligand binding affinity. This directly correlates with the phenomenon of enthalpy-entropy compensation.

There is something about the large substrate binding constants exhibited by proteins that just doesn't add up: specifically, the interactions between the individual functional groups. Molecular recognition systems that feature multipoint binding between the components usually display considerably higher binding affinities than one would expect from simply summing the association energies of all the respective parts [1]. This phenomenon, called "positive cooperativity," holds for many synthetic host-guest systems as well as providing vital additional binding energy for enzymes to use to stabilize transition states and catalyze reactions. The general entropic benefit of multipoint binding is well understood; the penalty associated with bringing two partners together is only paid once, by the first interaction formed, and subsequent ligand-substrate contacts are accordingly made "entropy free." However, the origins of some of the increased enthalpic stabilization associated with multipoint binding are rather less clear. Drawing on the behavior of enzymes and the enhanced substrate binding of vancomycin upon dimerization as examples [2–4], Williams has recently proposed that the enthalpy of binding events can be significantly weakened by the background thermal movements between a ligand and its substrate. Multipoint binding dampens such motions, and consequently all of the individual interactions are strengthened, providing a positive enthalpic enhancement to cooperative binding. Of course, biological systems are so complex that identifying the contribution of each functional group interaction to the overall strength of binding between a protein and a substrate is an inconceivable task. But now, Hunter and Tomas have probed the cooperativity phenomenon through a beautifully thought out series of experiments which accurately determine the strength of individual binding interactions on a well-defined synthetic system [5]. The investigative tool the researchers use is termed a "triple-mutant box" (Figure 1) [6]. By comparing the binding strengths of the structurally related complexes A, B, C, and D, the individual strength of the i-j interaction can be calculated. The strength of the same i-j interaction is then also measured under different circumstances, using complexes A′, B′, C′, and D′, in which there are more points of interaction between the ligands, and thus a measure of the cooperative effect can be directly determined.

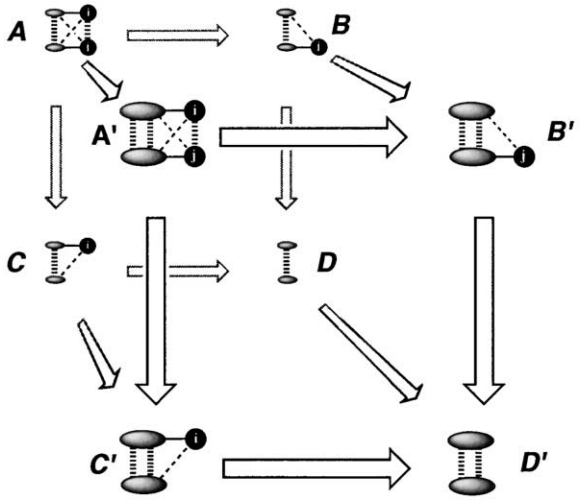

Figure 1. Measuring Cooperativity Using a Triple-Mutant Box

Determination of the binding constants of complexes A, B, C, and D (and A′, B′, C′, and D′, a similar system with an increased number of intermolecular contacts) allows the strength of the interaction between functional groups i and j to be calculated. By comparing the i-j binding strength value from A-D with that from A′-D′, the effect of cooperativity from the increased number of binding interactions can be established.

The results show a positive cooperativity of only 0.2 ± 0.4 kJ mol^{-1} for a system where the interaction energies vary from 8–13 kJ mol^{-1}, suggesting that the strong enthalpic chelate effect seen in biological systems is not a universal phenomenon. Why does the synthetic model not show the effect? One significant difference between the artificial and biological ligands is that the designed system is composed of rigid units so that there is no ambiguity in the interactions measured. Hunter and Tomas suggest that this could be what is responsible for the observed differences in behavior between the biological and model systems. Where a ligand or substrate has degrees of conformational freedom, in addition to the fully bound state in which all the possible ligand-substrate interactions are formed, there will also be numerous partially bound states where some of the interactions are missing. In these partially bound states, the loss of binding enthalpy will be accompanied by a corresponding increase in entropy. This explanation is particularly appealing because it provides a molecular level rationalization of the entropy-enthalpy compensation frequently observed in recognition events: increasing the strength of enthalpic binding comes at the expense of lowering the population of conformationally mobile partially bound states, thus decreasing the entropy of the system. The suggestion that in order to understand the properties of ligand-substrate complexes they need to be considered as populations of complexes where only some of the interactions are present at any one time is fascinating, and could have major

implications for structural and molecular biology far beyond simple recognition events.

David A. Leigh
School of Chemistry
University of Edinburgh
The King's Buildings
West Mains Road
Edinburgh EH9 3JJ
United Kingdom

Selected Reading

1. Jenks, W.P. (1981). Proc. Natl. Acad. Sci. USA *78*, 4046–4050.
2. Williams, D.H., Maguire, A.J., Tsuzuki, W., and Westwell, M.S. (1998). Science *280*, 711–714.
3. Shiozawa, H., Chia, B.C.S., Davies, N.L., Zerella, R., and Williams, D.H. (2002). J. Am. Chem. Soc. *124*, 3490–3491.
4. Williams, D.H., Stephens, E., and Zhou, M. (2003). Chem. Commun. 1973–1976.
5. Hunter, C.A., and Tomas, S. (2003). Chem. Biol. *10*, 1023–1032.
6. Hunter, C.A., Jones, P.S., Tiger, P., and Tomas, S. (2002). Chemistry *8*, 5435–5446.

Chemistry & Biology, Vol. 10, December, 2003, DOI 10.1016/j.chembiol.2003.12.009

CDK versus GSK-3 Inhibition: a Purple Haze No Longer?

The ubiquitous ATP binding site offers a global target for protein kinase inhibitors. The corollary is that molecular selectivity with such agents may be difficult to achieve and ascertain. A relevant example is discussed in terms of design and biomedical rationale.

Over the last two decades, the structural biology of protein kinases as well as the design of their inhibitors has become of great interest to both academic and pharmaceutical scientists. Because the ATP binding site in kinases is inherently amenable to blocking with drug-like small molecules, the discovery and development of kinase inhibitors is now being pursued for every imaginable therapeutic indication [1]. Furthermore, since the protein kinases, of which some 500 have been identified in the human genome [2], are the key players in the regulation of all biochemical pathways, they appear to represent excellent targets for a new generation of mechanism-based drugs. However, unpredictable pharmacology and, ultimately, unforeseen patient side effects might be expected with therapeutic agents targeting any conserved recognition site, i.e. the ATP binding pocket, which is highly conserved not only in kinases but also in many other mononucleotide binding proteins. The challenge with kinase inhibitors is thus how to achieve selectivity. A paper in this issue of *Chemistry & Biology* by Meijer et al. addresses this very point [3].

The authors take us on an historical excursion regarding the discovery of indirubins (Figure 1, **1**). These compounds confer the characteristic purple color to natural indigo dyes. According to legend, purple dye was first discovered by Herakles, when he observed that his dog's mouth was stained purple after chewing on snails along the Levantine coast. King Phoenix of Tyre is believed to have received a purple-dyed robe from Herakles and decreed that the rulers of Phoenicia should wear this color as a royal symbol, hence the name Tyrian purple. Pharmacological properties of indirubins have also long been known, and indirubin (Figure 1, **1a**) is a constituent of a traditional Chinese cancer medicine [8]. Their antiproliferative properties may in part be due to the fact that indirubins inhibit the phylogenetically and structurally closely related cyclin-dependent kinases (CDK) and the glycogen synthase kinases-3 (GSK-3). In fact, many compounds discovered as CDK inhibitors are known to block GSK-3 function as well [9].

Meijer et al. present crystal structures of indirubin-3′-oxime (Figure 1, **1b**) and its 6-bromo derivative Figure 1, **1c**) in complex with CDK5 and GSK-3β, respectively [3]. The latter compound is >16-fold selective for GSK-3 compared to CDKs 2 and 5 (which are structurally extremely similar [10]). The des-bromo compound (Figure 1, **1b**), on the other hand, is only about 5-fold selective for GSK-3, and the 5-sulfonate analog (Figure 1, **1d**) is about 4-fold selective for CDKs [11]. The structures presented show that the selectivity gain associated with the bromo substituent on C6 of the indirubin scaffold correlates with one of the main structural differences between GSK-3 and CDK2/5, viz. the so-called gatekeeper residue often exploited for the design of kinase selectivity in ligands [12]. In nine of the ten known CDK isoforms, this residue is Phe (F80 in CDK2), whereas Leu (L132) is found in GSK-3. The authors argue that because of the smaller aliphatic side chain in that position, GSK-3 is better able to accommodate the 6-bromo group and that indirubin C5/6 substitution studies may give rise to future analogs with increased selectivity [3].

In fact, another very recently published study with the thiazole-methoxybenzyl-thiourea compound **2** (Figure 1), which is truly selective for GSK-3 versus CDKs [13], confirms this hypothesis. In that case, it was found that the thiazole nitro substituent occupies the subsite adjacent to L132 in GSK-3, which is not accessible in CDKs because of the larger aromatic Phe side chain. Additionally, the anisole system in compound **2** was observed to bind at the entrance to the ATP binding site in GSK-3 in the vicinity of R141, which is salt bridged with E137. In CDK2 and probably also in CDK5, a corresponding