FOR THE RECORD

Arm–domain interactions can provide high binding cooperativity

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Abstract

Peptidyl arms extending from one protein domain to another protein domain mediate many important interactions in biology. A well-studied example of this type of protein–protein interaction occurs between the yeast homeodomain proteins, MAT α2 and MAT **a**1, which form a high-affinity heterodimer on DNA. The carboxyl-terminal arm extending from MAT α2 to MAT **a**1 has been proposed to produce an allosteric conformational change in the **a**1 protein that generates a very large increase in the DNA binding affinity of **a**1. Although early studies lent some support to this model, a more recent crystal structure determination of the free **a**1 protein argues against any allosteric change. This note presents a thermodynamic argument that accounts for the proteins' binding behavior, so that allosteric conformational changes are not required to explain the large affinity increase. The analysis presented here should be useful in analyzing binding behavior in other systems involving arm interactions.

Keywords: binding cooperativity; allostery; arm–domain; protein interactions; local concentration; mating type; peptidyl arm; peptidyl tail

Peptidyl arms or tails mediate interactions between proteins in a wide variety of processes, including DNA replication (Berdis et al. 1996; Sanders et al. 1997), signaling (Sano et al. 1997), vesicle transport (ter Haar et al. 2000), apoptosis (Shi 2002), and regulation by proteins like AraC (Saviola et al. 1997; Soisson et al. 1997; Seabold and Schleif 1998), and homeodomain proteins (Mak and Johnson 1993; Piper et al. 1999; LaRonde-LeBlanc and Wolberger 2003). An important paradigm for peptidyl arm–domain interactions is found in the interaction between the yeast homeodomain proteins, MAT α2 and MAT **a**1. The proteins bind to adjacent sites on the DNA and contacts between the proteins are mediated by a flexible carboxyl-terminal tail of α2 that becomes ordered upon binding to **a**1 (Wolberger et al. 1991; Mak and Johnson 1993). The binding of the carboxyl-terminal tail of MAT α2 to the globular protein domain of MAT **a**1 dramatically increases the DNA binding affinity of both proteins, and the large increase in binding affinity has been attributed to allosteric effects of the bound peptidyl tail that reshape the MAT **a**1 protein so as to increase its DNA binding affinity (Stark et al. 1999). Although structures of the two proteins have been interpreted to suggest the existence of such changes (Anderson et al. 2000; Ke et al. 2002), a more recent report of the structure of MAT **a**1 protein has raised the possibility that allosteric structural changes may not be required (Ke and Wolberger 2003). We show here that a very large cooperativity in DNA binding by two proteins interacting via an arm can result from the binding energetics of the arm, independent of any allosteric effects. Such a cooperative effect, whether mediated by an arm or not, can arise when the two interacting components are held near one another. This general mechanism for generating cooperativity has been well described (Creighton

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1993) and analyzed, and is termed the chelate effect (Page and Jencks 1971). We present here an application of this analysis that readily accounts for the contribution to cooperative binding by a flexible peptidyl arm.

Consider two proteins, P and Q, whose dissociation constants from their respective DNA binding sites are K_p and K_q, and whose binding sites on DNA are positioned such that, when P and Q are both bound, an arm extending from P can bind to Q and that the apparent solution binding affinity of the arm to Q is K_{arm}. Let P, Q, and D represent the concentrations of the free proteins and of DNA, PD and QD the concentrations of the individual complexes, $(PQ)D$ the complex of P and Q on DNA without the arm binding to Q, and $(P \sim Q)D$ the concentration of PQ complex on DNA with the arm from P bound to Q (Fig. 1). Directly from the definition of the dissociation constants,

$$PD = \frac{P \times D}{K_p},$$

and

$$QD = \frac{Q \times D}{K_q}$$

Similarly, $(PQ)D$ is obtained from the reaction of PD with Q, that is,

$$(PQ)D = \frac{PD \times Q}{K_q}$$

and hence

$$(PQ)D = \frac{P \times Q \times D}{K_p \times K_q}.$$

Binding of the arm to Q is governed by the effective concentration of the arm in the immediate vicinity of its binding site on Q, C_{eff}, and K_{arm}. In solution, the fraction of the protein molecules that bind a ligand is

$$\frac{C_{eff}}{K_{arm} + C_{eff}}$$

if the concentration of ligand is C_{eff} and it binds to the protein with a dissociation constant of K_{arm}. Equivalently, the fraction of time that an individual protein molecule in the solution binds the ligand is described by the same equation. Hence, $(P \sim Q)D$ is proportional to

$$\frac{C_{eff}}{K_{arm} + C_{eff}}$$

and $(PQ)D$ is proportional to

$$1 - \frac{C_{eff}}{K_{arm} + C_{eff}} = \frac{K_{arm}}{K_{arm} + C_{eff}},$$

making the ratio

$$\frac{(P \sim Q)D}{(PQ)D} = \frac{C_{eff}}{K_{arm}}.$$

Substituting from above,

$$(P \sim Q)D = (PQ)D \times \frac{C_{eff}}{K_{arm}} = \frac{P \times Q \times D}{K_p \times K_q} \times \frac{C_{eff}}{K_{arm}}.$$

Hence, the apparent binding affinity is increased by the ratio of

$$\frac{C_{eff}}{K_{arm}}.$$

In the case of **a**1–α2, NMR data (Phillips et al. 1994) and calculation of the interaction energies from the crystal structure coordinates (Li et al. 1995), both show that the length of the linker from the end of the structured region of α2 to the position of the first significant contact with **a**1 is two residues, about 10 Å. If we approximate the linker as being flexible, then the nearest binding site on the tail is confined

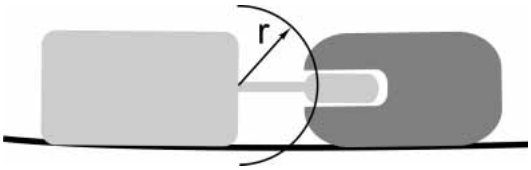

Figure 2. Representation of the hemisphere within which the first binding region of the carboxyl tail of α2 is confined.

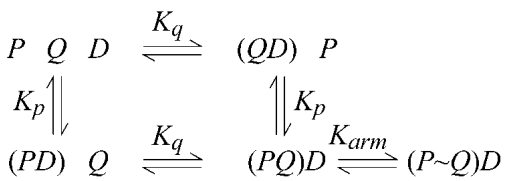

Figure 1. Pathways of complex formation. Each of these reactions are assumed to be in equilibrium. Only the $(PQ)D$ complex is considered in the formation of the arm-bound complex $(P\sim4Q)$ because the concentrations of P and Q are assumed to be much lower than C_{eff}, the effective concentration of the arm in the vicinity of its binding site on Q when both P and Q are bound to DNA.

to the volume within a hemisphere of 10 Å radius (Fig. 2). Hence, the binding target on **a**1 is exposed to the binding site at a concentration of 1 molecule in a 10 Å radius hemisphere, which is an effective solution concentration of about 1 M. Because the binding affinity of the arm is about 2×10^{-4} M (Phillips et al. 1994), the cooperativity from energetic effects can be as large as

$$\frac{C_{eff}}{K_{arm}} = \frac{1\ \mathrm{M}}{2 \times 10^{-4}\ \mathrm{M}} = 5 \times 10^{3}.$$

It has been found experimentally that $K_{a1} \geq 10^{-5}$ M, and $K_{\alpha 2} \approx 10^{-6}$ M (Phillips et al. 1994). Using the relationship derived above, the bimolecular dissociation constant can be estimated to be greater than

$$K_{\alpha 1} \times K_{\alpha 2} \times \frac{K_{arm}}{C_{eff}} = (10^{-5}\ \mathrm{M}) \times (10^{-6}\ \mathrm{M}) \times \frac{1}{5 \times 10^{3}} = 2 \times 10^{-15}\ \mathrm{M}^2.$$

This is consistent with experimental data showing that the constant is 10^{-14} M^2 to 10^{-15} M^2 (Phillips et al. 1994). Thus, binding energetics are capable of providing all of the cooperativity that is observed experimentally.

The above calculation, together with the recent crystallographic study of the **a**1 homeodomain in the absence of α2 or DNA (Ke and Wolberger 2003) supports that idea that binding energetics are sufficient to account for the observed ability of the α2 protein to raise the affinity of **a**1 for DNA. Because there are many other examples of macromolecular interactions that are mediated by flexible peptide arms, the estimation we present here based on the concept of local concentration (Crothers and Metzger 1972; Margossian and Lowey 1978; Klemm and Pabo 1996) should be instructive in evaluating interactions in other systems and determining whether it is necessary to invoke factors other than simple cooperativity to account for binding behavior.

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