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Allostery without conformational change

A plausible model

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Abstract. A general model is presented whereby ligand-induced changes in protein dynamics could produce allosteric communication between distinct binding sites, even in the absence of a macromolecular conformational change. Theoretical analysis, based on the statistical thermodynamics of ligand binding, shows that cooperative interaction free energies amounting to several kJ · mol^{-1} may be generated by this means. The effect arises out of the possible changes in frequencies and amplitudes of macromolecular thermal fluctuations in response to ligand attachment, and can involve all forms of dynamic behaviour, ranging from highly correlated, low-frequency normal mode vibrations to random local anharmonic motions of individual atoms or groups. Dynamic allostery of this form is primarily an entropy effect, and we derive approximate expressions which might allow the magnitude of the interaction in real systems to be calculated directly from experimental observations such as changes in normal mode frequencies and mean-square atomic displacements. Long-range influence of kinetic processes at different sites might also be mediated by a similar mechanism. We suggest that proteins and other biological macromolecules may have evolved to take functional advantage not only of mean conformational states but also of the inevitable thermal fluctuations about the mean.

Key words: Protein dynamics, fluctuations, allostery, cooperativity

Introduction

Allosteric effects, involving communication between distant ligand-binding sites on biological macromolecules, are central to many physiological control and receptor processes. Conventionally, these effects are ascribed to ligand-induced conformational changes transmitted through the macromolecule and across subunit boundaries. Monod et al. (1965) graphically demonstrated how this concept could explain quantitatively many of the observed cooperative and linkage phenomena in proteins – and yet even in this seminal paper it was emphasized that the concept of "conformational transition . . . should be understood in its widest connotation", and not solely in the strict stereochemical sense that we usually use today. Accordingly, we wish to develop here one of the alternative mechanisms for long-range site-site interaction (Cooper 1980; Salemme 1978) based on current thinking about the dynamic properties of proteins. We will show that it is possible to explain cooperative ligand binding in terms of the frequency and amplitude of atomic motions about fixed mean positions, i.e., without a conformational change in any sense that could be determined structurally.

The conformation of a macromolecule, as defined, for example, by X-ray crystallography, gives the mean atomic positions averaged over a large number of, supposedly, identical molecules and over times which are long compared to typical molecular motions. We now know, however, from fundamental theoretical considerations and from a wide variety of experiments, that individual macromolecules are dynamic objects undergoing various forms of intramolecular motion (for recent reviews, see: Cooper 1980; Gurd and Rothgeb 1979; McCammon and Karplus, 1983; Careri et al. 1979). These fluctuations have been variously described in terms of vibration, libration, or rotation of individual chemical groups, global oscillations of protein domains, "hinge bending", "breathing", "local unfolding", and so on, and can involve relative motion over several angstroms covering the entire time spectrum. Thermally excitable low-frequency vibrations ($\lesssim 200$ cm^{-1}) in globular proteins have been detected experimentally

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(Peticolas 1979; Jacrot et al. 1982; Middendorf 1984) and demonstrated by theoretical normal mode analysis to involve cooperative motions spanning entire molecules (Gō 1980, Gō et al. 1983; Brooks and Karplus 1983). Anharmonic and aperiodic motions are predicted by molecular dynamics simulations (McCammon and Karplus 1983; Levitt 1983a, b) and are also indicated by various experimental observations (Cooper 1980; Gurd and Rothgeb 1979). Such dynamic phenomena are not unique to biological macromolecules, being simply a manifestation of heat energy (Cooper 1976), but the thermodynamic fluctuations involved are quite large in these relatively small systems, and we might expect that, during evolution, any useful dynamic phenomena might become part of the repertoire of these systems. For example, in the present context of allostery, since the information content of a macromolecule consists not only of its average conformation but also of the frequencies and amplitudes of fluctuation about this conformation, communication across the molecule could go via changes in these dynamic frequencies and amplitudes, independently or even in the absence of conformation change.

We should emphasize from the start that it is not our intention to deny the existence or significance of conformational changes in protein receptor and control functions – but, rather, to illustrate that equally plausible, quantifiable alternatives do exist.

Statistical thermodynamics of multiple ligand binding

Sturtevant (1977) has discussed the various factors which contribute to the thermodynamics of protein interactions, emphasizing the significance of dynamic (vibrational) contributions. In reviewing the available data, he notes the almost universal decrease in heat capacity (i.e., negative ΔC_p) associated with protein-ligand binding and points out how this could arise from the loss of many internal, vibrational degrees of freedom. Similar conclusions may be reached by more general treatment of thermodynamic fluctuations (Cooper 1976), which shows that a decrease in heat capacity of a system inevitably implies that the thermal energy fluctuations in the system are reduced. Thus, we can picture the usual effects of ligand-binding to be a "stiffening" of the protein structure, although cases may be imagined in which the reverse is true. We wish to analyse in more detail the thermodynamic consequences of this.

Textbook statistical thermodynamics (e.g., Hill 1960; McQuarrie 1976; Davidson 1962) gives the free energy of molecular association in terms of the canonical partition functions of the molecular species involved. Thus, for the ligand-binding equilibrium at constant volume:

$$E + L \overset{K_1}{\rightleftharpoons} EL_1$$

the dissociation constant is given by:

$$K_1 = e^{-\Delta\varepsilon_1/kT} \cdot \frac{Q_0 Q_L}{Q_1},$$

where $\Delta\varepsilon_1 = \varepsilon_E + \varepsilon_L - \varepsilon_{EL1}$ is the difference in ground state energies corresponding to the (hypothetical) energy of ligand binding at 0 K in the absence of thermal motion, and Q_0, Q_1, and Q_L are the partition functions for the free enzyme (E), the binary complex (EL_1) and the free ligand (L), respectively. In the absence of significant volume changes, the Gibbs free energy of reaction is

$$\Delta G_1 = -kT \ln K_1$$

$$= \Delta\varepsilon_1 - kT \cdot \ln\left(\frac{Q_0 Q_L}{Q_1}\right).$$

Subsequent binding steps (at different sites) can be treated similarly, thus:

$$EL_1 + L \rightleftharpoons EL_2, \quad \text{etc.}$$

$$\Delta G_2 = \Delta\varepsilon_2 - kT \cdot \ln\left(\frac{Q_1 Q_L}{Q_2}\right),$$

where $\Delta\varepsilon_2 = \varepsilon_{EL1} + \varepsilon_L - \varepsilon_{EL2}$; Q_2 refers to the ternary complex (EL_2); and so on.

The difference $\Delta\Delta G = \Delta G_2 - \Delta G_1$ in binding free energies is a measure of the cooperativity (allostery) and may be written:

$$\Delta\Delta G = \Delta\varepsilon_2 - \Delta\varepsilon_1 - kT \cdot \ln\left(\frac{Q_1^2}{Q_0 Q_2}\right),$$

If we restrict our attention to the binding of identical ligands to (formally) identical and physically distant binding sites then $\Delta\varepsilon_2 = \Delta\varepsilon_1$, since the same molecular contacts are involved in each site, and

$$\Delta\Delta G = -kT \cdot \ln\left(\frac{Q_1^2}{Q_0 Q_2}\right).$$

The canonical partition function of a system is defined as $Q = \Sigma_i e^{-E_i/kT}$, where the summation is taken over all possible states, i, of the system with energies E_i. These will include all allowed translational, rotational, vibrational, electronic, and con-

formational states of the protein, or complex, as appropriate. As is conventional, we may assume separability and write

$$Q = q_{\text{Trans}} \cdot q_{\text{Rot}} \cdot q_{\text{Vib}} \cdot q_{\text{Elect}} \cdot q_{\text{Conf}}$$

and examine each contribution in turn. (For simplicity, we will ignore other possible internal modes of motion, such as free rotation of chemical groups, and assume that, at least formally, they may be treated as internal vibrational modes or different conformational substates.) The electronic energy level contributions may be eliminated from the start since they are not significantly excited at normal temperatures, and any changes in ground-state levels due to bond formation in the EL complex have already been assumed in the $\Delta\varepsilon$ terms.

The partition functions representing global translation and rotation of the entire protein molecule, q_{Trans} and q_{Rot}, are given by standard expressions which depend on the mass and moments of inertia of the molecule, respectively (Hill 1960; Davidson 1962; McQuarrie 1976). If we assume that the ligands are small compared to the enzyme, then these terms are numerically very similar for the different ligated states and effectively cancel in the expression for $\Delta\Delta G$.

This leaves:

$$\Delta\Delta G = -\text{kT} \cdot \left\{ \ln \left(\frac{q_1^2}{q_0 q_2} \right)_{\text{Vib}} + \ln \left(\frac{q_1^2}{q_0 q_2} \right)_{\text{Conf}} \right\}$$

which identifies two possible sources of cooperativity between the two binding sites. The first term gives rise to a finite $\Delta\Delta G$ if there are changes induced in the vibrational spectrum of the system by ligand binding. The second term in the equation expresses any effects of conformational change in the conventional sense, plus more subtle dynamic effects which we shall examine later.

The vibrational contribution

The vibrational partition function of the ith. normal mode of a system, with frequency ν_i, taking quantized energy levels $nh\nu_i$ ($n = 0, 1, 2 \ldots$) and incorporating the zeropoint energy ($^1/_2 h\nu_i$) into the ground state energy term, is given by

$$q_{\text{Vib}}(\nu_i) = (1 - e^{-h\nu_i/\text{kT}})^{-1}$$

which, in the classical limit $\text{kT} \gg h\nu_i$, becomes

$$q(\nu_i)_{\text{Class.}} = \frac{\text{kT}}{h\nu}$$

(Hill 1960; Davidson 1962; McQuarrie 1976).

For the complete spectrum of normal modes in the system the total vibrational partition function is given by the product

$$q = \prod_i q(\nu_i)^{g(\nu_i)}$$

where $g(\nu_i)$ is the spectral density of normal modes and represents the degeneracy (multiplicity) at each frequency.

Thus, in our simple case of sequential binding of two ligands, the vibrational contribution to any differences in site-binding affinity is

$$\Delta\Delta G_{\text{Vib}} = -\text{kT} \sum_\nu [g_0(\nu) + g_2(\nu) - 2g_1(\nu)] \ln q(\nu),$$

where, as before, the subscripts refer to the different states of ligation of the enzyme and the term $[g_0(\nu) + g_2(\nu) - 2g_1(\nu)]$ represents any ligand-induced changes in the normal mode spectrum.

In the simplest case where binding of ligands has no effect on normal mode spectra, $g_0 = g_1 = g_2$ at all frequencies, and there is no difference in binding free energies at each site. Similarly, if only high frequency modes are affected ($h\nu \gg \text{kT}$) the (quantum) partition function is essentially unity (i.e., high frequency modes are not thermally excited) and again $\Delta\Delta G_{\text{Vib}} = 0$.

More interesting, however, is the case of low-frequency modes. Imagine the situation in which a single thermally excited mode of the free enzyme, ν_0, undergoes frequency shifts $\nu_0 \rightarrow \nu_1 \rightarrow \nu_2$ during the sequential binding process $E \rightarrow EL_1 \rightarrow EL_2$. In this case

$$\Delta\Delta G_{\text{Vib}} = -\text{kT} \{\ln q(\nu_0) + \ln q(\nu_2) - 2 \ln q(\nu_1)\}.$$

If the frequency shifts are small, such that they may all be treated classicially, then substitution of the appropriate partition functions give

$$\Delta\Delta G_{\text{Vib}} \simeq -\text{kT} \cdot \ln \left(\frac{\nu_1^2}{\nu_0 \nu_2} \right)$$

so that if, as we anticipate, ligand binding induces a "stiffening" in the protein to give higher normal mode frequencies, then $\Delta\Delta G$ will indeed be finite and negative – indicating positive cooperativity in ligand binding. (Strictly speaking, the condition for negative $\Delta\Delta G$ in this classical limit is $\nu_1^2 > \nu_0 \nu_2$. It is also feasible that the reverse is true and that $\Delta\Delta G$ is positive, i.e., negative cooperativity.) The magnitude of the effect can only be guessed at in the absence of detailed normal mode analysis of an appropriate system, but even a modest 10% increase in frequency at each binding step would give $\Delta\Delta G$ of order -0.01 kT per mode. Bearing in mind that there are several hundred low-frequency modes in any protein of

reasonable size (Sturtevant 1977; Gō et al. 1983; Brooks and Karplus 1983), which might all be affected, it is not difficult to arrive at cooperative free energies of the order of a few kJ · mol^{-1} in this classical limit. (kT ≃ 2.5 kJ · mol^{-1} at room temperature.)

But, in addition to small frequency shifts resulting from an overall stiffening of the protein structure, there are likely to be much larger ligand-induced effects on specific modes of vibration. For example, a soft "hinge-bending" mode involving collective motions of lobes or domains of polypeptide about an active site, such as has been described for lysozyme (McCammon et al. 1976), might well become "frozen" or converted to higher frequency modes by ligand binding at the hinge region. This intuitive picture is supported by the inelastic neutron scattering analysis of lysozyme (Middendorf 1984) and hexokinase (Jacrot et al. 1982) – both of which show an apparent loss of low-frequency modes on ligand binding (though details of the hexokinase experiment are proving difficult to reproduce: S. Cusack, personal communication).

Analysis of simple molecular models (unpublished work) also indicates that thermally excited collective modes strongly coupled to ligand binding sites can be effectively suppressed and converted to non-excited high frequency vibrations by the attachment of small ligands to the equilibrium conformation.

Thermodynamic analysis of this case requires the use of the fully quantized partition function, and gives

$$\Delta\Delta G_{\mathrm{Vib}} = -\mathrm{kT} \cdot \ln\left(\frac{U_1^2}{U_0 U_2}\right)$$

where

$$U_i = 1 - \mathrm{e}^{-h\nu_i/\mathrm{kT}}.$$

A typical low-frequency global mode, with $\nu_0 \simeq 50$ cm^{-1}, converted to higher frequencies (ν_1, $\nu_2 > 500$ cm^{-1}) on ligation, would provide $\Delta\Delta G_{\mathrm{Vib}} \lesssim -1.4$ kT, for each such mode affected. This corresponds to cooperative interactions of about 2.1 kJ · mol^{-1} at room temperature. One or two such modes would amply describe the magnitudes of typical cooperative interactions.

Separating the interaction free energy into its enthalpy and entropy components

$$\Delta\Delta G = \Delta\Delta H - T \cdot \Delta\Delta S$$

we obtain:

$$\Delta\Delta H = \frac{h\nu_0}{U_0} \cdot \mathrm{e}^{-h\nu_0/\mathrm{kT}} + \frac{\mathrm{h}\nu_2}{U_2} \cdot \mathrm{e}^{-h\nu_2/\mathrm{kT}} - \frac{2h\nu_1}{U_1} \cdot \mathrm{e}^{-h\nu_1/\mathrm{kT}}$$

$$\Delta\Delta S = k \cdot \ln\left(\frac{U_1^2}{U_0 U_2}\right) + \frac{\Delta\Delta H}{T}$$

showing that the cooperative interaction is primarily an entropy effect. $\Delta\Delta H$ is normally positive (~ 0.6 kT with the parameters used above), but is offset by the larger, positive entropy contribution. This implies, interestingly, that binding of the first ligand is more exothermic, despite the stronger binding of the second ligand. (Note that in the classical limit $\Delta\Delta H = 0$, and the cooperative effect is entirely entropic. This is a consequence of the equipartition theorem in which, without quantization, all oscillators have the same mean internal energy, kT, regardless of frequency.) The origin of this vibrational contribution to cooperative ligand binding can thus be seen as follows: the free enzyme has a multiplicity of thermally excited, low-frequency vibrational modes, many of which involve motions spanning the entire macromolecule and coupling distant ligand binding sites. On introduction of the first ligand to one of the sites, enzyme-ligand contacts are formed which stabilize the complex and may, or may not, induce a change in conformation of the polypeptide. Concomitantly, the protein structure is stiffened so that some vibrational modes are shifted to higher frequencies where they are less thermally excited. The consequent release of thermal energy is, however, more than cancelled by the loss of vibrational entropy in these modes, and the net effect is to reduce the overall ligand binding free energy. Because of the non-linear nature of the thermodynamics (i.e., the exponential Boltzmann factor) these effects are significantly less for the binding of the second ligand which, therefore, has a higher thermodynamic affinity for its site.

In concluding this section, we should make some mention of the effect of damping since, it might be argued, in the presence of solvent and internal viscosity a protein does not vibrate perpetually like a tuning fork. This is true. But the solvent, as well as acting as a damper on motion, also acts as a source of fluctuations which excite motion by molecular collisions, Brownian motion, and the like. Thus, viewed classically, although harmonic oscillations may be rapidly damped out, they are equally rapidly being excited by solvent collisions, and the actual motion consists of perpetual random excitation and decay of different levels of the different vibrational modes (McCammon et al. 1976). It is precisely the average of this motion which is calculated by statistical thermodynamics. The quantum mechanical treatment of damped harmonic oscillators (Greenberger 1979a, b) leads to essentially the same picture.

The dynamic conformational contribution

Harmonic oscillations are not, of course, the only form that fluctuations in protein structure might take. The more general view, supported by molecular dynamics simulations (Levitt 1983a, b) is of the protein wandering in a haphazard and non-periodic fashion amongst a multitude of possible conformational states, with any harmonic motions superimposed. The width of the probability distribution of these conformational substates, and the associated partition function, can be viewed as a measure of the "flexibility" of the protein and will determine its average observed properties. The response to ligand binding might be two-fold: firstly, the presence of a ligand may stabilise certain of the conformational substates over others and result in a shift in the mean of the probability distribution, i.e., a conformational change in the conventional sense. Secondly, the *shape* of the distribution might be affected – a narrower distribution representing a "stiffening" of the protein structure due to ligand binding. Both of these effects would be reflected in the thermodynamics of ligand attachment, and the second, due to the change in conformational dynamics, could occur even in the absence of a gross conformational change.

To estimate the magnitude of such effects and to relate them to observable properties of the protein structure, we need to consider the contribution

$$\Delta\Delta G_{\mathrm{Conf}} = -\mathrm{kT} \cdot \ln \left\{ \frac{q_1^2}{q_0 q_2} \right\}_{\mathrm{Conf}} ,$$

where each partition function is of the form of a summation

$$q = \sum_{\text{all R}} \mathrm{e}^{-E(R)/\mathrm{kT}}$$

and $E(R)$ represents the generalized potential energy of the protein (n atoms) as a function of the $3n$-dimensional conformation $R \equiv \{x_1,y_1,z_1; x_2,y_2,z_2; \ldots . x_n,y_n,z_n\}$. (We shall assume classical dynamics so that the kinetic energy contributions are identical for each state of ligation and cancel in the expression for $\Delta\Delta G$). An exact calculation would require evaluation of the partition function $q_0,q_1,q_2 \ldots$ for each liganded state, which is beyond our present capabilities. However, we may proceed with the aid of two simplifying assumptions.

Firstly, we will assume that each q may be written as a product of the $3n$ individual atomic coordinate partition functions.

$$q \equiv \prod_{i=1}^{3n} q_i .$$

This is equivalent to assuming that the motion of individual atoms, or groups, is uncorrelated, with each moving in a mean field generated by all the others. Although this is unrealistic, it has the virtue of being at the opposite extreme to the highly correlated motions assumed in the analysis of the normal mode vibrational contributions, and allows us to write

$$\Delta\Delta G_{\mathrm{Conf}} = -\mathrm{kT} \sum_i \ln \left(\frac{q_1^2}{q_0 q_2} \right)_i ,$$

where the summation is over all coordinates and the term in brackets now represents the contribution from each atomic coordinate.

Many of these terms in the summation might cancel because of molecular symmetry. For example, in the case of a system with two identical ligand binding sites (i.e., usually a dimer of symmetry-related monomers), for each atom i there will be an equivalent atom i' in an identical molecular environment, (e.g., on the opposite subunit). We must consider the combined effect of symmetry-related pairs of coordinates

$$\Delta\Delta G_i = -\mathrm{kT} \ln \left\{ \left(\frac{q_1^2}{q_0 q_2} \right)_i \left(\frac{q_1^2}{q_0 q_2} \right)_{i'} \right\} .$$

From symmetry:

$$q_0(i) = q_0(i')$$

and

$$q_2(i) = q_2(i') .$$

If the conformational effects of ligand binding are only short range, then no cooperativity occurs, i.e., binding of the first ligand might affect atom i [$q_0(i) \rightarrow q_1(i)$], but not i'[$q_0(i') = q_1(i')$]. Similarly, binding of the second ligand would affect i'[$q_1(i') \rightarrow q_2(i')$] but not i [$q_2(i) = q_1(i)$]; all the terms cancel and $\Delta\Delta G_i = 0$. This is merely a mathematical statement of what is intuitively expected: that in order to mediate communication between distant ligand sites, any atom must in some way "feel" the presence of ligand at each of the sites. But this effect depends on the thermodynamic partition functions and may result not only from a conformational change in the position of the atom but also from a change in the dynamic fluctuations about its mean position.

To see this, we make our second simplifying assumption: that the fluctuations in atomic coordinates are approximately Gaussian, of width σ (which will be different for each atom). For a Gaussian probability distribution about a fixed mean position (i.e., without conformational change) the atomic

partition function is proportional to the width, σ (see Hill 1960, for example). Thus, using the symmetry arguments for two identical sites:

$$\Delta\Delta G_i = -2\,\mathrm{kT} \cdot \ln\left[\frac{\sigma_1(i) \cdot \sigma_1(i')}{\sigma_0 \cdot \sigma_2}\right],$$

where σ_0, σ_2 are the root-mean-square fluctuations of coordinate i in the unliganded and fully liganded states, respectively; $\sigma_1(i)$ and $\sigma_1(i')$ represent the rms fluctuations at i and i' when only one ligand site is occupied. This gives the purely dynamic contribution to the interaction between the ligand sites in terms of quantities that may be visualized and, in principle, measured experimentally.

In practice, ligand-induced changes in conformational fluctuations may be small, e.g.,

$$\sigma_1(i) \simeq \sigma_0(1 - \delta_1)$$

$$\sigma_1(i') \simeq \sigma_0(1 - \delta_1')$$

$$\sigma_2 \simeq \sigma_0(1 - \delta_1 - \delta_1')$$

so that, to approximate first order in the fractional shifts, δ,

$$\Delta\Delta G \simeq -2\ \mathrm{kT}\ \delta_1\delta_1'$$

for each atomic coordinate affected.

Even rms shifts of the order of 1% per atom, scarcely observable with current techniques, if summed over much of the molecule would give cooperative free energies of the order of kT. Again, in this classical treatment, the effect is entirely entropic.

Discussion

We have shown how long-range interactions between ligand binding sites on a macromolecule might be produced by purely dynamic processes, over and above any additional effects due to conformational change. With plausible and experimentally verifiable assumptions about the magnitudes of ligand-induced changes in vibrational frequencies or thermal fluctuation amplitudes, allosteric interaction free energies amounting to several $\mathrm{kJ} \cdot \mathrm{mol}^{-1}$ can be estimated. Moreover, the effect can arise both from highly correlated global oscillations in the protein and from uncorrelated random motion of individual atoms or groups. Although we have concentrated on the simplest case of cooperative interactions between two identical sites in order to simplify the algebra, it is clear that similar arguments apply in the more general cases of allosteric communication and, by appropriate adjustment of dynamic amplitudes and frequencies, all the familiar phenomena of activation, inhibition, positive and negative cooperativity might be reproduced.

Furthermore, the effects might not be limited solely to equilibrium binding parameters. Rate processes such as enzymic catalysis or ligand attachment and dissociation rates, which depend on relatively rare thermal fluctuations ("activation steps"), might also be subject to control via the dynamic processes we have been describing. Such rare fluctuations would not contribute significantly to the thermodynamic ligand-binding affinities, but could give rise to the sort of kinetic allosteric effects seen in some systems (Dixon and Webb, 1979). For example, the rates of attachment or release of a ligand requiring the transient opening of the jaws of the active site (or a "gate" or "channel") might well be increased, or suppressed, if that particular mode of motion were coupled to similarly transient events at other ligand sites. The ramifications in such areas as transmembrane communication and translocation remain to be explored.

Although the concept of dynamically mediated allosteric interaction might appear unfamiliar and hard to visualize, at first, the molecular mechanism is fundamentally the same as in the more familiar process of conformational change. The basic requirement for long-range inter-site communication is the existence of atoms or structural groups dispersed throughout the protein molecule which, directly or indirectly, experience the presence of ligands at each of the sites concerned, and these effects could be either static or dynamic.

In practice ligand-induced changes in *both* the mean conformation and dynamics are to be expected, and even in cases where a gross conformational change can be demonstrated the associated dynamic changes may in fact be the real source of allosteric effects. Experimentally, the situation will be difficult to resolve especially as, given the finite resolution of structural methods, it will always be difficult to rule out "small" (i.e., not observed) conformational changes. But, one advantage of our dynamic formalism is that the interactive free energies are, within the approximations, expressed in terms of quantities which are, in principle, measurable – i.e., changes in normal mode frequencies and/or mean square amplitudes of coordinate fluctuations. Thus quantitative estimates might be made independent of any model of the molecular potential energy surface and the attendant problems of solvation, etc., which would be required to analyse the conformational contribution.

We have shown that dynamically mediated cooperativity should be entropy driven: that is, binding of a second ligand is made thermodynamically more

favourable because of a less negative $\Delta S°$. Similarly, with the failure of equipartition due to quantum effects, the enthalpy changes are in the opposite direction, i.e., more exothermic for binding the first ligand. It is also straightforward to show that heat capacity changes (Δc_p) on ligand binding are expected to be negative (Sturtevant 1977), and more so in this case for the first ligand. Reliable experimental data on relevant systems are, unfortunately, scarce and we are aware of only one detailed study, involving calorimetric measurements of cooperative binding of NAD to glyceraldehyde phosphate dehydrogenase (Niekamp et al. 1977). It is gratifying that the results are in accord with our expectations. But, even with such painstaking experiments, estimation of individual site binding parameters is not trivial and can be influenced by the choice of binding model (Niekamp et al. 1977), and data on other systems are sorely needed.

In conclusion, it is worth drawing attention to recent experiments on the appearance of allosteric effects in non-biological systems, for which dynamic conformational interpretations similar to those presented here are now receiving some consideration (Onan et al. 1983).

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