Cooperating molecules in biology

Cooperative phenomena, best known to physics, have an important function in biology that was first and most radically defined in an article published a quarter of a century ago.

COOPERATIVE phenomena are ill-defined, nowhere more so than in biology. The general idea is that the effect of the simultaneous action of separate entities may be greater than the sum of their separate effects. Order is one consequence. Physicists know cooperative phenomena best in phase transitions. In a ferromagnetic material below the Curie temperature, for example, the magnetization at a particular lattice site will be the more certainly identical with that of its immediate neighbours if there is such a degree of long-range order that other than immediate neighbours exert a similar effect. Order, and also disorder, are properties of the whole and not of the parts separately.

Usage among biologists is more casual. At one extreme, the word cooperative is wrongly used to describe circumstances when two molecules are necessary for the interaction of a third. Thus molecules of both an enzyme and a coenzyme may be needed to effect the transformation of a substrate molecule. Literally, the effect of the enzyme and coenzyme jointly is greater than the sum of their separate effects (in each case zero). But the reaction rate is determined simply by the product of the concentration of the two components, as the law of mass action would suggest. Happenings at one place do not help to determine what happens at another.

The classic case of cooperativity among the molecules of biology is that of the absorption of oxygen by haemoglobin, still something of a mystery despite the endless investigations since the discovery of the Bohr effect at the turn of the century. (The variation with pH of the affinity of haemoglobin for oxygen explains why haematologists are fond of measuring bicarbonate concentration in blood.) What does simpleton mass action say? The product of the haemoglobin and oxygen concentrations (with the partial pressure as a standard proxy) is equal to the concentration of the oxygenated form multiplied by an equilibrium constant.

So how does the proportion of oxygenated haemoglobin vary as the partial pressure of oxygen increases? Simple algebra says that the proportion of oxygenated haemoglobin will be $P/(K + P)$, where P is the pressure and K the equilibrium constant, which is known as the Michaelis–Henri equation. This gives a simple and plausible graph, increasing from zero at zero pressure to some plateau on which all the haemoglobin in oxygenated. The exact shape of the curve is determined only by the equilibrium constant. The chief interest of the Michaelis–Henri equation is that it hardly ever corresponds with the truth.

What happens instead is a consequence of cooperativity, for which purpose it is crucial that haemoglobin in the blood is not a single protein molecule, but a tetramer. For those not already familiar with the argument, the standard textbooks provide a better explanation than the telegraphese that follows, which has a different purpose — partly that of celebrating how it proved possible to figure out what really happens without much help from a knowledge of the detailed structure of haemoglobin. (The very best way to hear the tale is to seize the next opportunity to listen to Dr Max Perutz on the subject.)

Doubters should read the classic paper by Jacques Monod, Jeffries Wyman and Jean-Pierre Changeux (*J. molec. Biol.* **12**, 88–118; 1965), published exactly a quarter of a century ago. The observations show that the proportion of oxygenated haemoglobin usually increases from zero with increasing partial pressure less quickly than simple-minded mass action would suggest, but that the upward slope of the curve then increases more quickly than would be expected. (The shape of the curve is physiologically beneficial in concentrating the absorptive capacity of haemoglobin in a relatively narrow range of partial pressure.)

The qualitative explanation is that the attachment of a first molecule of oxygen to one component of a tetramer eases the attachment of other oxygen molecules to the remaining components. So the phenomenon is strictly cooperative; the condition of one component of the tetramer conditions the others in the same sense. As textbook readers know, this in turn leads to the doctrine of allostery — the notion that proteins can be stimulated to change their shape and, in the process, their activity.

The neatness of the argument by Monod *et al.*, which reads like pure reason, is its generality. Allosteric proteins (24 were known then) are multimeric molecules, so why not assume that their components occupy equivalent positions, which says something about their geometrical symmetry. On this view, normal human adult haemoglobin, which has two pairs of α globin chains and two β chains, is a dimer of components each with two absorbtion sites for oxygen.

The natural temptation would be to look for detailed mechanisms by which the attachment of a single oxygen molecule to one chain, with its characteristic haem iron atom, would influence the oxygen affinity of the others. Not for Monod *et al.*, who simply posit that allosteric change yields another tetramer in which the four units are again in equivalent positions. Moreover, although the function of the oscillation between one state and another may be crucially related to the attachment of oxygen molecules, the equilibrium between them must be definable without the intervention of oxygen. That makes one equilibrium constant. For haemoglobin, four others arise from the affinity for oxygen of the binding sites on the α and β chains in the two different allosteric states. Then it is simple calculation, as with the Michaelis–Henri equation, and simple demonstration that the more complicated relations that result do indeed correspond with reality.

All this is by way of introduction to what must be the most elaborate argument of this kind. The case is that of the oxygen-carrying haemocyanin with which evolution has endowed molluscs. The structure of the standard haemocyanin complex consists of ten linear protein units, each consisting of two protein monomers placed equivalently head to tail, which are arranged like the staves of a barrel to form a hollow drum. There may be 100 or more binding sites for molecular oxygen in each such hollow drum.

E. Di Cera of the Catholic University at Rome has now gleefully calculated the allosteric possibilities of these molecules (*Il Nuovo Cimento* **12D**, 61; 1990). It seems to be established that oxygen attachment to a binding site within a single barrel stave causes allosteric change in that stave alone, which can be catered for with the techniques of Monod *et al.*. Di Cera supposes that allosteric change in one stave of the barrel will influence its neighbours in its own image, and sets out to calculate the propagation of that influence around the barrel. He calculates the partition function and derives what thermodynamic properties he wishes. The model has the virtue that its parameters are physically defined as interaction energies. Observational data are well fitted. Who could ask for more? **John Maddox**