Plenary Lectures

A1

Inorganic Elements in Biological Space and Time

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Inorganic chemists studying biological systems have been fascinated by the structure and properties of isolated (dead) compounds. This is but an extracted part of the interaction of inorganic elements with biological materials. *In vivo* we need to uncover the different compartments into which the elements are distributed and the timing of the distribution and its re-distribution. To this end we must define the time dependence of the chemical potential of elements in different parts of space. The chemical potential of an element itself can be broken down into concentration terms, field dependencies, and chemical bondings including oxidation state changes. The compartments for an element can be due to limitations upon physical diffusion, membrane control or to kinetically stable chemical traps. For simplicity I shall divide the elements first into two extreme groups: those that are restricted by physical diffusion only *e.g.* Na^+, K^+, Cl^-, and to a lesser degree Mg^{2+}, Ca^{2+}, and those that are confined by chemical traps *e.g.* Mo, Cu, Zn. We then observe that there are other elements of an intermediate category which are controlled by both types of traps and are mobile to a limited degree. While movement through membrane limitation is controlled by pumps and the synthesis of proteins controls the chemical traps some elements such as P, Fe, Mn both move between chemical traps and cross physical, membrane, barriers in very specific ways. The movement from the chemical traps often requires (catalysed) chemical reaction. We shall look at the movements of K, Ca, P, Fe, Cu and Mo asking about their residence time at any site.

Integrated over a very long period of time we can describe an average, though highly energised, distribution of any element. Over shorter periods there are found to be variations within this energised state. In other words elements have patterns of concentration and patterns of flow which are not constant in living systems—even of the simplest cells. The patterns of flow cause changes in local chemical potentials and hence in patterns of spatial activity especially since element movements trigger gross biological changes. The simplest examples of time dependences are the flows of electrons and ions, electronic and electrolytic currents, which can be connected to both chemical and mechanical devices *e.g.* the cytochrome chain activation and the nerve impulse. In more complicated situations the flow of an element is connected to differentiation and growth. Incidental changes in a biological system such as the storage of information or cell division (reproduction) are interwoven with changes in the patterns in space of elements. Iron movements are particularly interesting. I shall begin to explore some of these possibilities which will surely lead inorganic chemists closer to the nature of living systems. Very many elements are not inorganic and much of organic chemistry is inorganic.

A2

Mechanistic Aspects of Coenzyme B_{12}-dependent Rearrangements: Organometallics as Free Radical Precursors

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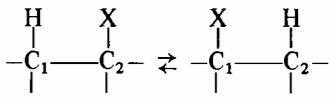
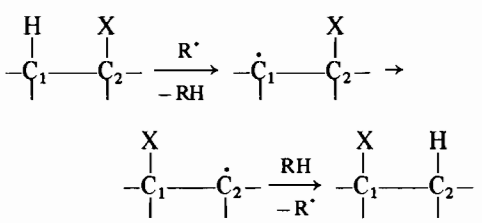
Coenzyme B_{12} (5′-deoxyadenosylcobalamin, abbreviated $[Co^{III}–R^-]$) serves as a cofactor for several enzymic reactions, a common feature of which involves the 1,2-interchange of a H atom and another group (X = OH, NH_2, $CH(NH_2)COOH$, *etc.*) on adjacent carbon atoms, *i.e.*,



(1)

A widely accepted mechanistic interpretation of these reactions, supported by a variety of evidence from studies on the enzymic processes as well as on model systems, is,

$$[Co^{III}–R^-] \overset{\text{enzyme}}{\rightleftharpoons} [Co^{II}] + R^{\cdot} \qquad (2)$$



(3)

At the present stage it appears that the principal, if not the only, role of coenzyme B_{12} is to serve as the precursor for an organic free radical which triggers the substrate rearrangement. This role implies and utilizes a very weak Co–alkyl bond, susceptible to homolytic cleavage under mild conditions, for which there has until recently been no convincing evidence or precedent.

We have developed the first reliable systematic methods for the determination of transition metal–alkyl bond dissociation energies (BDE) and have applied these to the determination of such BDE's in a variety of organocobalt compounds (notably containing dioxime or Schiff base ligands) related to coenzyme B_{12}. The Co–C BDE's yielded by such measurements are in the range 15–25 kcal/mol, *i.e.*, low enough to be consistent with the proposed role of coenzyme B_{12} depicted in Eqn. (1). The influences of electronic and steric factors on Co–C bond dissociation energies, revealed by these studies, are such as to suggest that coenzyme B_{12} is an ideal candidate for such a role and that steric factors probably are responsible for the enzyme-induced Co–C bond weakening and dissociation.

An analogy is developed between the role of coenzyme B_{12} in biological systems as a reversible 'free radical carrier' (*i.e.*, according to Eqn. (1)) and the role of hemes as reversible dioxygen carriers, *i.e.*,

$$[Fe^{III}–O_2^-] \rightleftarrows [Fe^{II}] + O_2 \quad (4)$$

A3

Atomic Structures of Oxymyoglobin and Oxyhaemoglobin and the Cooperative Mechanism of Oxygen Binding

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Until very recently, it had not been possible to determine the structures of oxymyoglobin and oxyhaemoglobin, because the crystals autoxidized. My colleagues S. E. V. Phillips and B. Shaanan have been able to overcome this problem. Phillips did an X-ray analysis of sperm whale oxymyoglobin at 1.6 Å resolution, followed by a neutron diffraction analysis at 1.7 Å resolution in collaboration with B. Schoenborn. Shaanan solved the structure of human oxyhaemoglobin at 2.1 Å resolution. These analyses have provided firm stereochemical data about the conformation of the haem and the geometry of the Fe–O_2 bond. They have also revealed the role of the distal histidine in discriminating between O_2 and CO.

Bent geometry for the Fe–O_2 bond with an Fe–O–O angle of 114° was predicted by Pauling. This prediction was confirmed by X-ray studies of J. P. Collman's oxygenated picket fence complexes, except that the Fe–O–O angle there was 130°. Intuitively, one would have expected that angle to be determined by the nature of the Fe–O_2 bond, and to remain the same in all oxygenated haem derivatives, but this is not true. The Fe–O–O angle is 115(±5)° in oxymyoglobin and 156(±10)° in the α and β oxyhaemoglobin. The difference is due to the different constraints imposed by the distal residues in the haem pocket (His E7, Val E11 and Phe CD1).

Collman's picket fence complexed with dimethylimidazole has a partition coefficient between O_2 and CO of 4280; the same complex with a covalently attached imidazole has a partition coefficient of 26,600. By contrast, the partition coefficients of myoglobin and haemoglobin are 150 and 250 respectively. If they were as high as in the picket fence complex, respiratory transport by haem proteins would not be possible, since CO is produced endogenously in the breakdown of porphyrin (one mol CO per mol porphyrin). How do the two proteins discriminate between O_2 and CO? The electronic structures of the two ligands ensure that O_2 binds preferentially in the bent conformation, while CO prefers to bind linearly with Fe–C–O on the haem axis. The distal pockets in the myoglobin and haemoglobin are tailored so as to fit the bent oxygen, but to oppose the binding of the linear CO which is forced off the haem axis by steric hindrance. This appears to be one of the discriminating devices. The other consists of hydrogen bonding by the distal histidine. Solvent effects and spectroscopic evidence suggested that the Fe–O_2 bond is polar, with transfer of negative charge from the iron to the oxygen, while the Fe–CO bond is purely covalent. Pauling first suggested that the distal histidine could form a hydrogen bond to the terminal oxygen which carries a formal negative charge in his view of the FeO_2 complex. Evidence suggesting such a bond comes from electron paramagnetic resonance and oxygen affinity data on cobalt-substituted haemoglobins and myoglobins. Phillips and Schoenborn have now proved the existence of that hydrogen bond by a neutron diffraction analysis of oxymyoglobin. In D_2O at pH 8.4 the distal histidine carries one exchangeable deuteron which can bind either to N_ϵ facing the bound haem ligand or to N_δ, facing the external solvent. The neutron maps showed that in oxymyoglobin the deuteron is on N_ϵ, forming a hydrogen bond with the bound oxygen, while in carbonmonoxymyoglobin it is on N_δ, facing the solvent, and the histidine is further removed from the ligand. This result confirms the polar character of the FeO_2 bond and the non-polar character of the Fe–CO bond. It also tells us that nature employs not one but two methods to discriminate between the two ligands.