# COMPLEX COMPOUNDS AS MODELS OF BIOLOGICALLY ACTIVE SYSTEMS

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## ABSTRACT

Transition metals are known to play an important role in many biological processes. This review describes work on synthetic complexes of cobalt, iron and copper which serve as models of the natural systems.

Reference is frequently made to the essential role of metal ions in connection with the functioning of living organisms. Metal ions contribute to biological processes, such as the formation and cleavage of chemical bonds, charge transfer, oxygen transfer, nitrogen fixation and photosynthesis.

The specific role and selectivity of action of a metal ion are connected with the ability to form various solvates and complexes, since these are associated with very fine changes in electrochemical potential.

This review deals with aspects of the activity of cobalt, iron and copper complexes because, of the transition metals, these have important roles in metabolic processes. We have concentrated our studies on the complexes of these metals which can serve as models of biological compounds.

## REVERSIBLE OXYGEN UPTAKE

The synthesis, structure and properties of complexes capable of taking-up molecular oxygen reversibly has been the subject of intensive studies over many years[1–5]. These complexes provide simple models of the respiratory pigments responsible for the transport of oxygen, or of the enzymes involved in oxygen transfer. The complexes with molecular oxygen, reversibly or irreversibly taken up, can be treated as intermediate states of oxidation. The natural respiratory pigments[6] myoglobin and haemoglobin, like some enzymes participating in the cellular oxidation chain, contain characteristic ferrous porphyrin derivatives. A similar ferrous porphyrin system is present as the active centre in chlorocruorin, the green respiratory pigment of certain marine worms. The active centres are surrounded by high-molecular weight proteins, which protect them. The essential role in the oxygen fixation process in these systems is played by the iron(II) coordinated by the imidazole group of histidine, at the fifth coordination position. Haemprotein respiratory pigments fix oxygen in a ratio $Fe:O_2 = 1:1$, forming monomeric adducts. Non-haem pigments take up molecular oxygen in the bridge (dimeric) way. Examples include (i) haemerythrin (present in some worms), which is an $Fe^{II}$

protein complex, in which the thiol group is essential, (ii) haemocyanin, found in the blood of certain arthropods, which has cuprous ions bonded to protein via sulphur and imidazole groups, and (iii) haemovanadin, present in erythrocytes, which is the protein salt of disulphovanady(III) acid.

Various enzymatic systems[6] carry oxygen directly to the substrates. Depending on the mechanism of oxygen transfer they can be divided into three groups. The first group comprises oxygen transferases, activated by ferrous ions. They may be compared to the oxyhaemoglobin system, since they contain the perferryl ion group $FeO_2^{2+}$, the so-called 'complex III'. Many of these enzymes contain thiol groups which are probably responsible for coordination of the ferrous ions. Their function is based upon the splitting of double bonds by means of activated oxygen. To this group belong: pyrocatechase, tryptophan oxidase which splits the pyrrole ring in tryptophan, and homogentisic acid oxidase. To the second group belong the enzymes associated with hydroxylation reactions, which are thought to involve both the $MO_2^{n+}$ group ('complex III') and the $MO^{n+}$ ('complex II'). At first, the enzyme fixes oxygen to form the 'complex III', and then, as the result of interaction with the reductor, 'complex II' is formed which causes hydroxylation of olefins and aromatic systems. To this group belongs the cuprous protein, the so-called tyrosinase. Mason *et al.* have proposed a dimeric structure for this enzyme, after combination with oxygen—$[CuO_2Cu^{2+}]$—the so-called cuprous complex II, corresponding to 'complex III' of ferroenzyme. To the third group belong the electronic transferases, cuprous proteins, which are carriers of electron pairs in living tissues, not of oxygen directly. In this case both oxygen atoms are reduced to water, and electrons are taken from the substrate (*Figure 1*). The mechanism of the enzyme action is most probably as follows: first, the cuprous ions are oxidized to cupric ions by molecular oxygen, and next the enzyme oxidizes the substrate.

$$4\,H^+ + 4\,Cu^{I}(\text{enzyme})^+ + O_2 \rightarrow 4\,Cu^{II}(\text{enzyme})^{+2} + 2H_2O$$
$$4Cu^{II}(\text{enzyme})^{+2} + 2RH_2 \rightarrow 2R + 4H^+ + 4Cu^{I}(\text{enzyme})^+$$

*Figure 1.*

Ascorbic acid oxidase, or laccase, containing four copper atoms per mol, which reacts with one oxygen molecule, provides an example of such an electronic transferase. To that group also belong the cytochrome coenzymes, that is the porphyrin systems, containing iron.

The many synthetic complexes which take up molecular oxygen reversibly[1–5, 7] can be divided into two groups: (1) those which fix molecular oxygen in the bridge, dimeric, way, M: $O_2$ = 2:1, and (2) in the monomeric way, where M: $O_2$ = 1:1 as in the natural systems. Many dimeric adducts with a bridge of the μ-peroxy type have been known for a long time, but monomeric adducts have only been obtained and examined recently. All the oxygen adducts can be divided into three groups according to the geometry of the molecule towards the metal atoms[7] (*Figure 2*). It must be emphasized that most of the models which closely resemble natural systems have been prepared from cobalt.

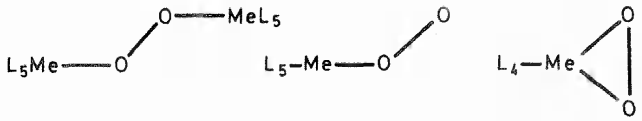



*Figure 2.*

Our studies on models of oxygen-carrying systems led to the discovery of new synthetic systems which take up the molecular oxygen reversibly aqueous and in dimethylformamide solutions[8,9]. These are the cobalt(II) complexes, containing amino acid molecules, and imidazole as ligands. Systems of the type Co–imidazole–amino acid, which are capable of taking up oxygen reversibly, have been obtained with the amino acids listed in *Figure 3*. Intensely dark brown coloured oxygen adducts arise on dissolving

| Neutral aliphatic amino acids | Amino acids with substituents |
|---|---|
| Glycine | Serine |
| Alanine | Homoserine |
| Sarcosine | Proline |
| α-Aminobutyric acid | Hydroxyproline |
| Valine | Methionine |
| Norvaline | Citrulline |
| Isoleucine | Lysine |
| | Glutamine |
| | Ornithine |
| | Arginine |
| | Asparagine |

*Figure 3.*

the polymeric $Co^{II}$ 'inner' complex of imidazole $[Co(imid)_2]_n$ in an aqueous solution of an amino acid or dipeptide through which oxygen or air is passed, at a temperature close to 0°C. A similar reaction is observed with $[Co(Himid)_2(H_2O)_2CO_3]$. The amino acid has to be in excess to shift the equilibrium in favour of the adduct (*Figure 4*). The 'active' $Co^{II}$ complex capable of intense oxygen absorption can be obtained in a similar way in an atmosphere of $N_2$ or Ar, or even $O_2$, but at higher temperatures (50–60°). The inert gas and temperature cause the displacement of oxygen from the oxygen adducts. On the formation of the oxygen adduct, or 'active' complex, one imidazole molecule is replaced by oxygen or a solvent molecule (*Figure 4*). The unusual ability for rapid oxygen uptake, higher even than the histidine complexes, must be emphasized. That system is also unusual because of the perfect reversibility of the process, which allows the oxygen uptake cycle to be repeated many times, without noticeable irreversible oxidation to $CO^{III}$. The resistance of solutions of the oxygen adducts towards molecular oxidation to give an inactive monomeric $Co^{III}$ complex depends on the nature of the amino acids. Generally, adducts formed by complexes with dipeptides are

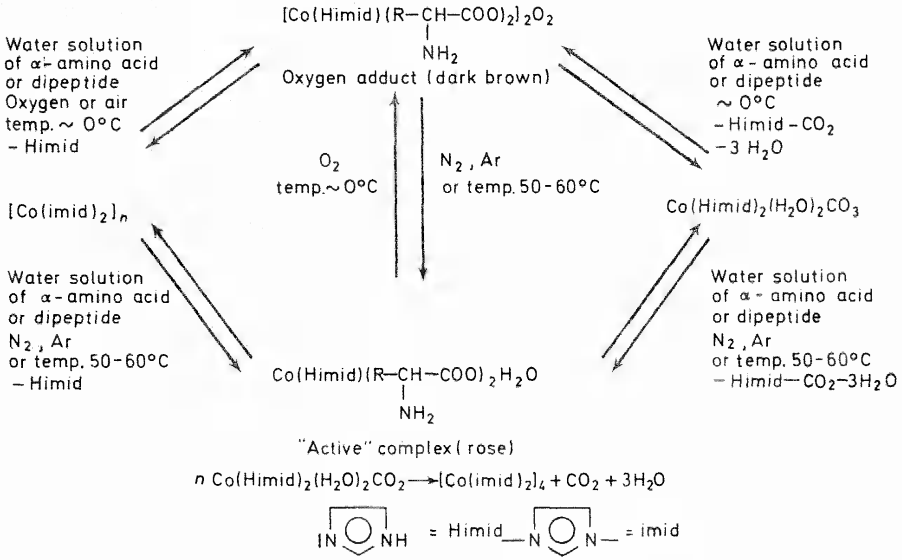



*Figure 4.* Scheme of reactions for forming 'active' and oxygenated complexes.

less stable. Only in the presence of an α-amino-carboxylic acid are complexes formed capable of taking up oxygen reversibly. A negative result is obtained with γ amino acids and with amino-dicarboxylic acids. The presence of an aromatic ring in the β-position, or of a heterocyclic ring, destroys the ability to take up oxygen. This is due most probably to the inductive effect of the ring on the electron density of the α-amino-carboxylic acid group. The basicity of the imidazole group is essential. Replacement of imidazole by weaker bases (pyrazole, benzimidazole, purine bases, pyrazine) results in complexes which have lost the ability to take up oxygen. The considerable reactivity of the oxygen adducts must be emphasized. The oxygen is activated, as in many

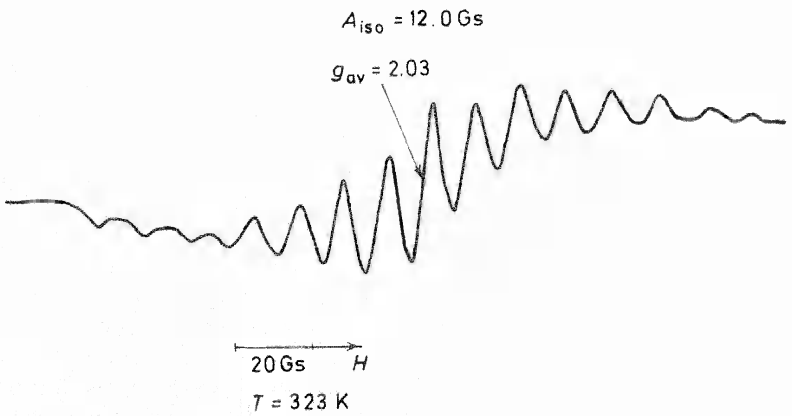



*Figure 5.* EPR spectrum of μ-superoxy species.

enzymatic systems. The adducts react immediately with L-ascorbic acid, hydroquinone, etc., yielding oxidation products. Spectroscopic studies at temperatures close to 0°C indicate two bands of the 'charge transfer' type (at 345 nm and 400 nm respectively, $\varepsilon$ about 7000 per dimer) characteristic of μ-peroxy-$Co^{III}$ dimers. Further oxidation [with cerium(IV)] causes the formation of the violet complexes of the μ-superoxy type, with characteristic bands at 700–730 nm. These complexes can probably be assigned a formula $[\{Co(Himid)(amino\ acid)_2\}_2O_2]NO_3$. After extraction with a mixture of isoamyl alcohol and acetone, they give an EPR spectrum corresponding to the superoxy bridge with 15 hyperfine structure lines resulting from the coupling of the unpaired electron on the bridge with two equivalent $^{59}Co$ nuclei (*Figure 5*). Polarographic measurements indicate the wave corresponding to the reduction of the μ-peroxy bridge, and the presence of cobalt(III) in the oxygen adducts. It follows that the dimer is the predominant form in solution (*Figure 6*). Recently Swen Berger *et al.*[10] have also shown that a

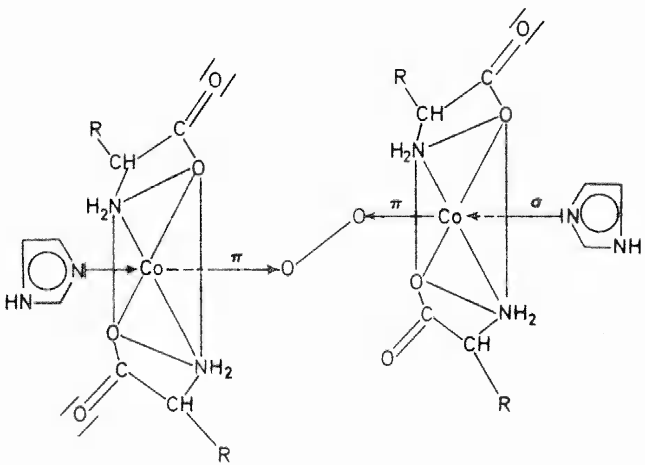



*Figure 6.* Proposed structure for dimer in solution.

μ-peroxy bridge is formed in the unstable Co salt–imidazole–alanine–$O_2$ system in solution. The oxygen adduct was also obtained by us in the solid state by precipitation from aqueous solutions with acetone. The solid liberates oxygen irreversibly to give the inactive $Co^{II}$ complex. A magnetic moment corresponding approximately to one unpaired electron per cobalt atom, and the presence of an oxygen-radical line in the EPR spectrum, $g_1 = 2.00$ and $g_2 = 2.04$ with eight poorly resolved hyperfine structure lines of $A = 13$ G, suggest the presence of monomer species in the solid (*Figure 7*). A mixture of three forms in equilibrium is probably involved: a dimeric form as in solution, a monomeric form corresponding to $L_5Co^{III}O_2^-$, the limiting superoxy species and an oxygen-free $CO^{II}$ complex.

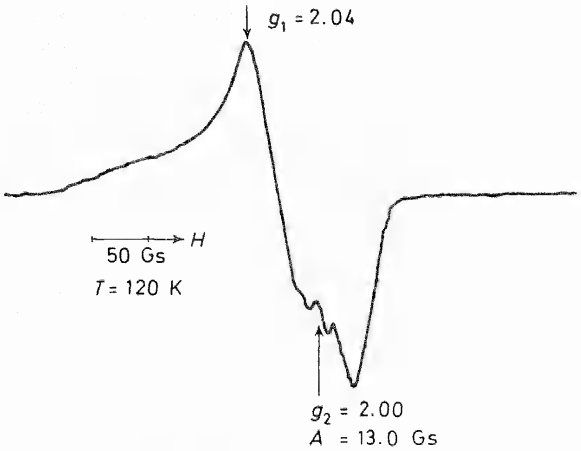



*Figure 7.* Spectrum of monomeric superoxy species in solid.

The schemes proposed for the oxygen take-up mechanisms, and the forms of adducts in both solutions and the solid state, are presented in *Figure 8*. It was assumed that the 'activation' of the dissolved oxygen, with simultaneous formation of the labile monomeric adduct, occurs in the first stage. Next the

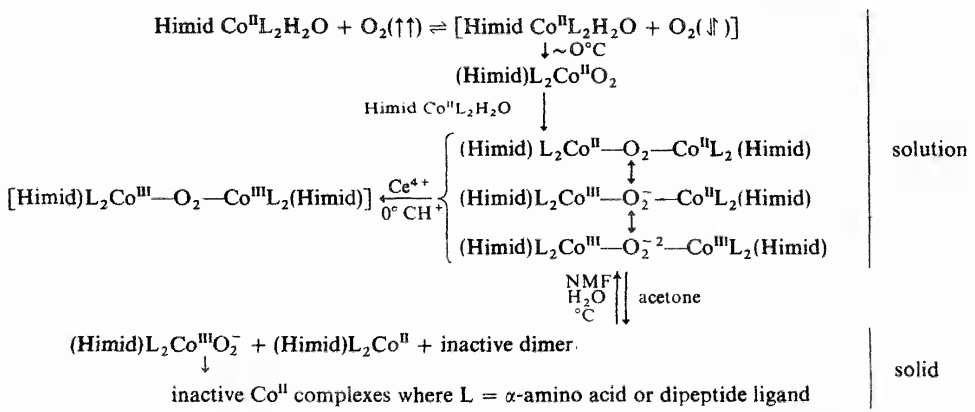



*Figure 8.*

adduct dimerizes to the bridge species described by three limiting resonance structures in which the species with the μ-peroxy bridge seems to be the most stable. Transition to the solid state leads to the partial decomposition of a dimer, with simultaneous formation of inactive $Co^{II}$ complexes and of the monomeric adduct of the superoxy type. To discuss the structure of both monomeric and dimeric adducts, the structure of the oxygen molecule must be mentioned (*Figure 9*). In the gaseous state it is in the triplet state, $^3\Sigma$, lower by 23 kcal $mol^{-1}$ than the singlet $^1\Delta$ state. It is paramagnetic with two unpaired electrons in the antibonding orbitals, viz. $\pi_z^*$ and $\pi_x^*$. In the case of dissolved oxygen some splitting of antibonding $\pi^*$ orbitals is observed, because of asymmetric interactions. That, however, does not lead to spin

pairing; only in ligand fields can the splitting be large enough to cause the spin pairing on one of the antibonding orbitals, while another orbital acts as the $\pi$-acceptor[11]. Both in the dimeric and monomeric forms, as well as in the 'active' complex, two amino acid molecules form the equatorial plane. The 'axial' position, '*trans*' towards the imidazole molecule, is occupied by oxygen or the solvent molecule. The similarity of the monomeric species to the haem respiratory pigments is evident (*Figure 10*).

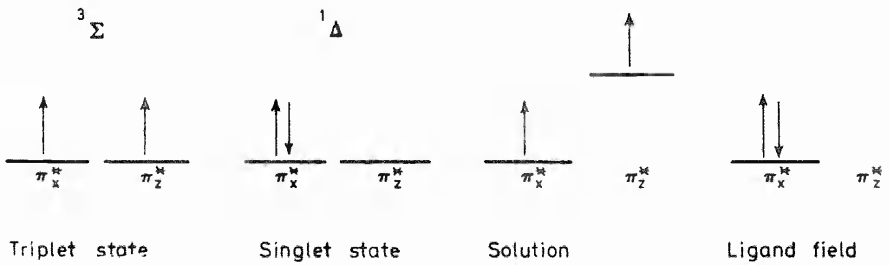



*Figure 9.*

The role of the porphyrin plane of the haem coordinating the iron(II) is played by the amino acid or dipeptide molecules chelating the cobalt atoms.

The role of the imidazole group of the proximal histidine is played by the imidazole ligand. Imidazole as the $\sigma$ donor stronger than the solvent molecule,

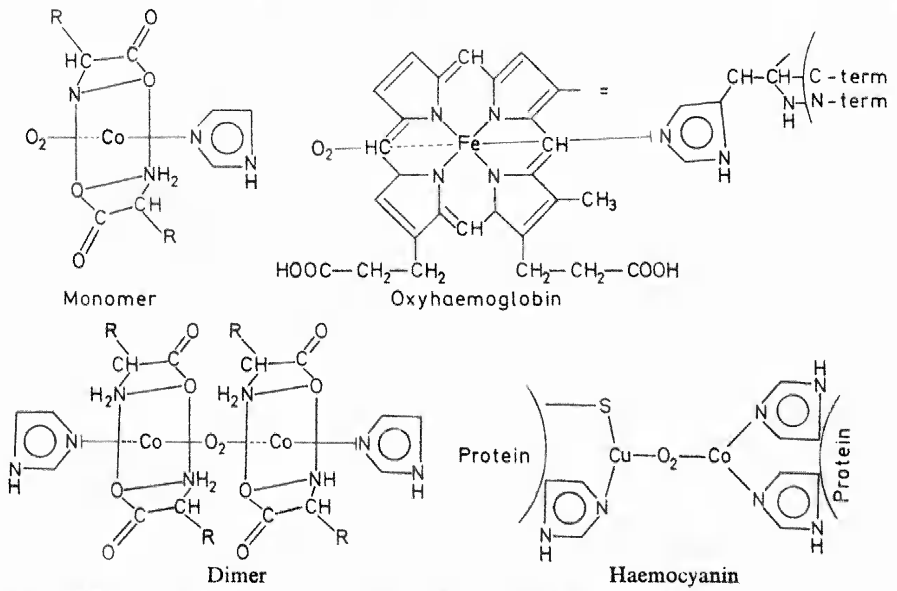
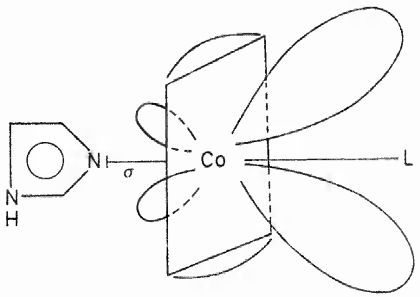


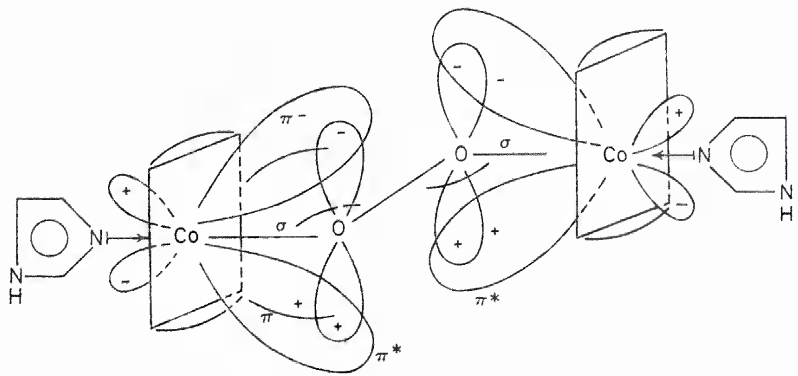

*Figure 10.* Comparison of proposed structure of monomeric and dimeric species with the structure of neutral respiratory pigments.

causes some distortion of filled *d* orbitals (e.g. $d_{xy}$ and $d_{yz}$) towards the weaker donor, because of electrostatic resistance (*Figure 11*). In this way, these

orbitals become the $\pi$-donor orbitals. The close activated oxygen molecules with empty $\pi$-acceptor, $\pi^*$ antibonding, orbitals can cause the delocalization of electrons, from $\pi$-donor cobalt orbitals to $\pi^*$ antibonding oxygen orbitals, with formation of the $\pi$-bonding. The solvent molecule is simultaneously removed, and the $\sigma$-bonding between the non-bonding $sp^2$ electron pair of oxygen and the $d_{z^2}$ orbital of cobalt is formed. Depending on the conditions,




'active complex'




dimer $Co \underset{\sigma}{\overset{\pi}{\rightleftarrows}} O_2 \underset{\sigma}{\overset{\pi}{\rightleftarrows}} Co$

where L = $H_2O$ or N-methylformamide

*Figure 11.* '*trans*' effect of imidazole molecule.

e.g. on temperature, the transfer of the charge back to the cobalt may be observed and oxygen will be replaced by a solvent molecule. In a case of monomeric oxygen adducts, two alternative geometries for the position of atoms towards the equatorial plane can be discussed (*Figure 12*): (a) the symmetric arrangement, according to Griffith's model[11], and (b) the angular, according to Pauling's model[12].

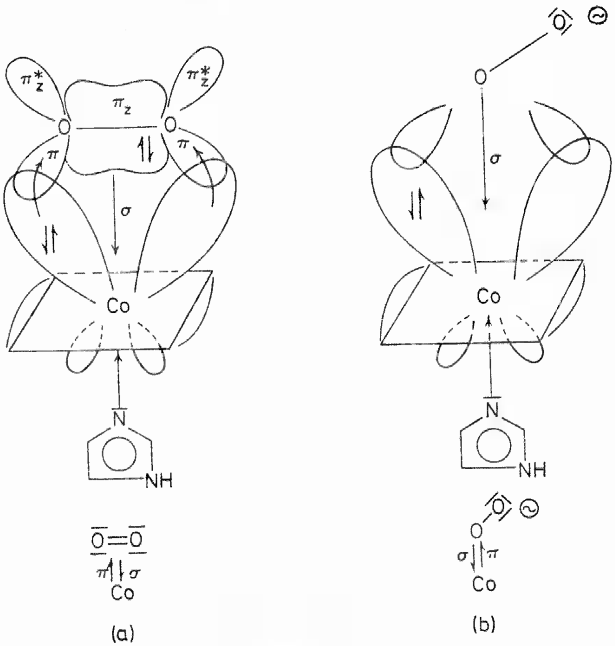



*Figure 12.*

## NITROGEN FIXATION

The biological fixation of nitrogen and of carbon dioxide are processes essential for life on earth. In nature the processes operate under mild conditions at normal pressure and temperature. Broad studies are now focused on providing an explanation of the catalytic activities of enzymes, on determining their structure, and on establishing a correlation between structure and catalytic activity. In living organisms nitrogen is present in the reduced form. Absorption of nitrogen by microorganisms as a food has to be connected with its reduction, the source of energy for this process coming from oxidation of organic compounds. Ability to perform the reduction process is associated with the presence of metalloenzymes—nitrogenase, hydrogenase—and of electron carriers of very low redox potential, such as ferredoxin, a protein containing iron in a non-haem form. The metal, besides providing a site for substrate binding, can also contribute to the electron transfer reaction. The nitrogenase enzyme from *Azotobacter vinelandi* has been separated in the pure form[13] (molecular weight 270 000–300 000) and has a molybdenum : iron : cysteine : SH groups ratio of 1 : 20 : 20 : 15. The characteristic feature of enzymatic nitrogen fixation is the contribution of the two metals, molybdenum and iron, present in nitrogenase to the coordination of the $N_2$ molecule and to its reduction to ammonia.

Hidai *et al.*[14] tried to obtain a system capable of fixing molecular nitrogen like the enzymatic model, from hydride and nitride molybdenum, iron and cobalt complexes. The mixing of the nitrogen molybdenum complex $[Mo(N_2)(PPh_3)_2(C_6H_5CH_3)]$ with the hydride complexes $[FeH_2(PPh_3Et)_3]$

or $[CoH_3(PPh_3)_3]$ results in decomposition of the complex with liberation of nitrogen and hydrogen, and not ammonia as was expected. Mixing of complexes $[Mo(N_2)_2(DPE)_2]$ and $[CoH_3(PPh_3)_3]$ in benzene or toluene leads to an exchange reaction of nitrogen and hydrogen between molybdenum and cobalt (*Figure 13*). van Tamelen[15] also tried to form a model for

$$\xrightarrow{\text{benzene}} \quad \textit{trans}\text{-}Mo(N_2)_2(DPE)_2 + CoH_3(PPh_3)_3$$
$$\xrightarrow{\text{toluene}} \quad \textit{trans}\text{-}MoH_2(DPE)_2 + CoH(N_2)(PPh_3)_3$$
$$[\textit{trans}\text{-}MoH_2(DPE)]_2\text{-u-}(DPE) + 2(DPE) + CoH(N_2)(PPh_3)_3$$
$$DPE = Ph_2PCH_2CH_2PPh_2$$

*Figure 13*

molecular nitrogen fixation by mixing the dinitrogen molybdenum complex $[Mo(N_2)_2(DPE)_2]$ with the iron–dithiolene, a compound of the ferredoxin type (*Figure 14*), but only a small amount of the fixed nitrogen was reduced to

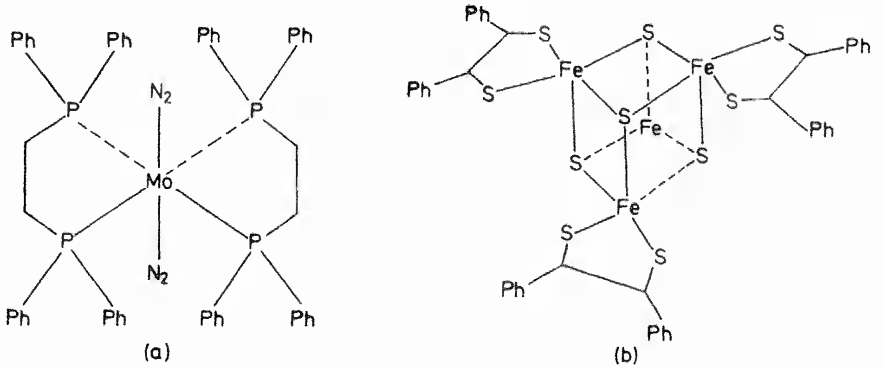



*Figure 14.* Structure of dinitrogen complex of molybdenum $[Mo(N_2)(DPE)_2]$ (a) and (b) structure of iron–dithiolene compound of ferrodoxin type.

$NH_3$*. We have also undertaken studies on molecular nitrogen fixation under mild conditions in systems using metals suggested by the natural system[16–18]: $FeCl_3 + Mg + THF + N_2$ and $MoOCl_3 2THF + Mg + THF + N_2$. We have reported[18] that magnesium causes the reduction of $Fe^{3+}$ and $Mo^{5+}$ ions to the low oxidation states, in which they are capable of molecular nitrogen fixation. The complex formula $[THF_{1.5}MgCl_3Fe]_2N_2$ in the system with $FeCl_3$ and with $MoOCl_3$ 2THF gives a product containing approximately 8.5% N with a Mo:N:Mg ratio of 1:4:5. The coordinated nitrogen undergoes further reduction; hence, after the hydrolysis of the reduction product in the system containing ferric chloride, hydrazine was obtained, and in the system containing the molybdenum compound both ammonia and hydrazine were formed. In our opinion, the real reducer of nitrogen is magnesium in the first oxidation state, which is generated in the solution and which may form the pseudo-Grignard systems Mo–MgCl or Fe–MgCl. Tetrahydrofuran, very often used in nitrogen fixation processes, though its contribution to the reaction has not been considered, plays a double role: it is the solvent, and at the same time it reacts with $FeCl_3$ or $MoOCl_3$ in the presence of

* Chatt found this system inactive [J. Chatt, C. M. Elson and R. L. Richards, *Chem. Commun.* 189 (1974)].

metallic magnesium yielded hydrocarbons[16,17], most probably with the simultaneous formation of the unusually active systems Fe–MgCl and Mo–MgCl, which are capable of coordinating and reducing $N_2$.

## SCHIFF BASE COMPLEXES WITH COPPER (II)

Transamination reactions are essential in protein metabolism. It has been stated that, in all known cases, the initial activation of the amino acid is the result of aldimine formation (Schiff base) by condensation of the amino acid with pyridoxal (vitamin $B_6$), or, in enzymatic transamination processes, with an enzyme containing pyridoxal[19]. The reactions satisfy the general scheme shown in *Figure 15*. In the case of non-enzymatic transaminations,

$$\begin{array}{l}\text{Amino acid}_1 + \text{pyridoxal} \leftrightharpoons \text{pyridoxamine} + \text{keto acid}_1 \\ \text{Keto acid}_2 + \text{pyridoxamine} \rightleftharpoons \text{pyridoxal} + \text{amino acid}_2 \\ \hline \text{Amino acid}_1 + \text{keto acid}_2 \rightleftharpoons \text{amino acid}_2 + \text{keto acid}_1\end{array}$$

*Figure 15.* Scheme of enzymatic transamination.

the reaction between pyridoxal and amino acids (in aqueous solution) is promoted by the presence of such metal ions as $Cu^{2+}$, $Fe^{3+}$[19–21]. For glutamic acid the rate is increased twentyfold. The electronic shifts consequent upon the formation of the chelate complex increase the lability of the α-hydrogen in the amino acid residue, and this promotes the conversion into pyridoxamine[19] (*Figure 16*).

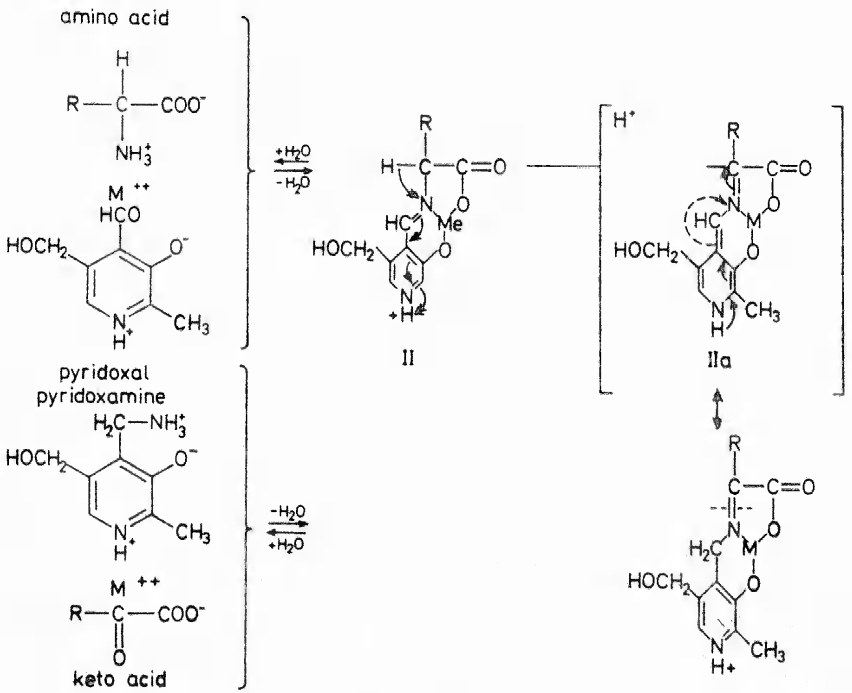



*Figure 16.* Mechanism of the pyridoxal and metal ion catalysed non-enzymatic transamination reaction.

The aldimine formed as the result of the condensation of pyridoxal with the amino acid can undergo decarboxylation to give a product which hydrolyses to the appropriate amine and pyridoxal. It has been stated that in the non-enzymatic decarboxylation reaction, catalysed by pyridoxal, metal plays the role of an inhibitor[22]. The formation of the chelate and of the metal–ligand bond enhances the electrophilic character of the carbonyl group and the lability of the α-hydrogen atom. This reduces the tendency towards decarboxylation and makes the transamination easier. In both transamination and decarboxylation processes, the structure of the complex formed is the important factor in determining the contribution of the metal ion. Our studies were focused on $Cu^{II}$ complexes with, as ligands, the products of condensing salicylaldehyde with primary amines and were aimed at determining the effect of amine structure on the character of the metal–closest coordination sphere bonding.

The spin Hamiltonian parameters for the series of copper compounds with aldimines were determined by the electron paramagnetic resonance method. This allowed the estimation of the anisotropic magnetic properties of the complexes, and the quantitative calculation of the parameters of the chemical bonding. We have determined the influence of substituents at the imine nitrogen on the symmetry of a complex, e.g. bulky alkyl-groups (tert-butyl, isopropyl) result in tetrahedral symmetry, whilst most copper complexes with aldimines are square. The conclusions drawn from the experimental determination of the molecular orbital coefficients were the most important[23]. We have stated that $\alpha^2$ coefficients, which are a measure of the σ bonding, do not depend on the initial amine and its basicity. For all compounds examined they were 0.80, approximately (*Figure 17*).

| Complex | $\beta_1^2$ | $E_{\frac{1}{2}}$ | $\alpha^2$ |
|---|---|---|---|
| *N,N'-o*-phenylenebis(salicylaldimino)copper(II) | 0.64 | − 0.76 | 0.82 |
| *N,N'*-ethylenebis(salicylaldimino)copper(II) | 0.66 | − 0.75 | 0.82 |
| bis(*N*-amylsalicylaldimino)copper(II) | 0.79 | − 0.40 | 0.79 |
| bis(*N*-benzylsalicylaldimino)copper(II) | 0.81 | − 0.30 | 0.81 |
| bis(*N*-ethanolsalicylaldimino)copper(II) | 0.86 | − 0.18 | 0.79 |
| bis(*N*-phenylsalicylaldimino)copper(II) | 0.88 | − 0.12 | 0.75 |
| bis(*N-p*-methylphenylsalicylaldimino)copper(II) | 0.90 | − 0.12 | 0.74 |
| bis(*N-p*-nitrophenylsalicylaldimino)copper(II) | 0.92 | + 0.03 | 0.80 |

*Figure 17.* Comparison of bonding parameters with half-wave potentials.

The $\beta_1^2$ coefficients, a measure of the π-bonds in the plane, change within broad limits. They are lowest for complexes with tetradentate Schiff bases (derived from aldehyde and diamine), being about 0.65. For the strongly basic aliphatic amine derivatives they are about 0.80. Their values increase for aromatic amines of lower basicity up to 0.92 (for the *p*-nitrophenyl substituent at imine nitrogen). Thus the $\beta_1^2$ coefficients are a better indication of covalent bonding character than $\alpha^2$.

The thermodynamic properties (stability) and reactivity of these complexes are related to the $\beta_1^2$ coefficient. Hence, both the chelation and the effects connected with changes in electron density within the molecule due to substituents remote from the central ion, can affect profoundly the bonding character in a complex. And, hence, its chemical and biological activity.

## NITROSYL IRON COMPLEXES

Iron plays a quite universal role in living organisms. Iron forms iron nitrosyl complexes. It has been stated that Roussin salts, where iron is coordinated by NO and sulphur, are inhibitors of respiration and of fermentation of yeasts, and that these salts are also inhibitors of carboxylases, alcohol and milk dehydrogenase, ribonuclease and other enzymes[24]. These salts are active at very low concentration ($10^{-5}$–$10^{-7}$ M) and combine reversibly with enzymes, being especially active with enzymes rich with SH groups or some heterocyclic rings.

Iron nitrosyl complexes are also formed in living organisms, e.g. in yeasts cultivated anaerobically in nitrate or nitrite medium, and in rat liver infected with a neoplasm cancer[25–27, 31]. In a liver cancer, instead of the free-radical EPR signals of $g = 2.002$–$2.005$, the signal characteristic of the nitrosyl iron complex of $g = 2.03$ was observed[26].

After separation of this compound it was found to be neither the known haem–NO complex nor Fe–NO–cytochrome–C[26]. Since the nitrosyl compounds in liver are easily formed (contact of the tissues with $NO_2^-$, or $NO_3^-$ ions, or with hydroxylamine, is sufficient) they are involved in some enzymatic reaction of the diseased liver, and studies on the structure of these complexes have become essential[26]. With natural FeNO–protein structures resolution of the superhyperfine structure in the EPR spectra is very poor, in contrast to the situation with simple models which produce well resolved superhyperfine structure.

The $Fe(NO)_2$–cysteine, nitrosyl mercaptan or iron nitrosyl thiourea complexes are good models of iron nitrosyl complexes with sulphur proteins[28]. We have examined a series of such models. With alkyl mercaptans $g_{av} = 2.028$, no matter which mercaptan was applied. The anisotropic spectra correspond to axial symmetry with $g_\perp = 2.038$ and $g_\parallel = 2.012$. The unpaired electron is shifted towards iron and occupies its $a_1(d_{z^2})$ orbital. The superhyperfine structure of the isotropic spectra represents the splitting of both $^{14}N$ nitrosyl groups and of the hydrogens of the alkyl group. The complexes formed are always dinitrosyl and possess two coordinated mercaptan molecules[29].

The parameters obtained for alkyl mercaptans are identical with those of one of the nitrosyl–cysteine complexes and of iron nitrosyl complexes with sulphur proteins[28]. The $Fe(NO)_2$–(thiourea derivatives) system exhibits similar properties[30]. The complexes described above are good models of iron complexes with sulphur proteins, e.g. actomiosine, albumin, aldolase, dehydrogenase, which are active in important redox processes in mitochondria, photosynthesis and other metabolic processes[25, 27, 31].

Proteins without SH groups can also react with Fe and NO. A series of iron complexes with amino acids, polypeptides and nucleic acid bases has been obtained[25]. The coordination is thought to be via nitrogen, but the spectra were poorly resolved and did not provide much information. Iron coordination by natural proteins containing SH groups and nitrogen groups capable of coordination proved to be a difficult problem which was examined with models. We have obtained a series of nitrosyl–azole–iron complexes which are good models of biological systems[29, 32]. The complexes with 1,3-diazoles (imidazole, benzimidazole, *N*-alkylimidazole) gave well resolved isotropic spectra (after purification by extraction). The azole and nitrosyl nitrogens are nearly equivalent (with respect to EPR) in these complexes, but non-equivalent for complexes with 1,2-diazoles and with symmetric and vicinal triazoles. That is due to the electron properties of the nitrogens of these rings. The anisotropic spectra are always nearly axial with $g_{\|} = 2.04$, $g_{\perp} \cong 2.01$, and the rhombic distortion is small ($g_2 - g_3 = 0.008$). The spectra correspond to those of the Fe nitrosyl–polyhistidine compounds[25]. The systems with 2-mercaptoazoles[29, 32] proved to be good models of proteins containing SH groups as well as azoles. 2-Mercapto-1,3-diazoles proved to be capable of coordination via nitrogen or SH groups. The studies showed the complexes with coordinated SH groups to be the more stable, and to be formed first. The relation $g_{\|} > g_{\perp}$ and the unpaired electron on the $b_1$ orbital corresponds to all compounds in which iron is coordinated by the azole nitrogen. Dinitrosyl compounds, which provide models of natural compounds, are given in *Figure 18*.

| Ligand | Coord. by | $A_{iso}$[Gs] | $g_{\|}$ | $g_{\perp}(g_2, g_3)$ | Ref. |
|---|---|---|---|---|---|
| Thiourea derivatives, xanthogenates, thiosemicarbazide derivatives | S | 2.5 | 2.01 | 2.03 | 30 |
| Alkyl mercaptans | S | 2.6 | 2.01 | 2.04 | 29 |
| 1,3-diazoles | N | 2.5 | 2.04 | 2.02; 2.01 | 29, 32 |
| 1,2-diazoles or triazoles | N | 3.1 | 2.04 | 2.02; 2.01 | |

*Figure 18.* Some e.s.r. parameters for iron dinitrosyl complexes as models of natural compounds.

The EPR signals of the livers of carcinogen-fed rats correspond to the $Fe(NO)_2$–protein system, with free thiol groups. It is well known that chemicals which react with free thiol groups are usually anti-carcinogens. Probably, the nitrosyl compounds formed in the cancer tissues of liver, because of the presence of nitrates in a food, contribute to the inactivation of the carcinogen[26].

## IRON BRIDGE COMPLEXES

Iron polymers contribute to the storage regulation, control and transport processes of the iron content in living organisms. The formation of soluble complexes of relatively low molecular weight is the main role of the chelating

agents. In the case of EDTA no proof has been found that the higher polymers are soluble; hence only dimers play a role in this case[34, 35]. While polymers with an oxygen bridge are essential in the storage of iron (ferritin) and its transport (transferrin), the non-haem systems with a sulphur bridge contribute directly to metabolism as electron carriers. Formation of polynuclear complexes can make the electron transfer by the polymer much easier, as the polymers are restricted by the cell membranes to the cells in which the biological processes occur. The formation of polymers containing $d^5$ and $d^6$ ions of iron leads to exchange interactions which affect the physical and chemical properties of the systems[33–35]. For a better understanding of biophysical and biochemical properties of iron we have undertaken studies on exchange interactions between iron ions and their influence on the electronic structure of the polymer. The binuclear complex of $Fe^{III}$ with EDTA of formula $Na_4$(EDTA–Fe–O–Fe–EDTA). $2H_2O$ was examined by the magnetic susceptibility measurements method.

We have calculated the isotropic interaction energy presented by the Heisenberg Hamiltonian $H_{ex} = JS_1 \cdot S_2$, and we have obtained the value of $J = 160\ cm^{-1}$.

Calculation of anisotropic exchange interaction energy and of dipole spin–spin interactions is possible only by the EPR method. This method was applied to the study of single crystals of the [Fe(salen)$_2$]O complex[35–38] which were obtained from chloroform solution and from dichloromethane

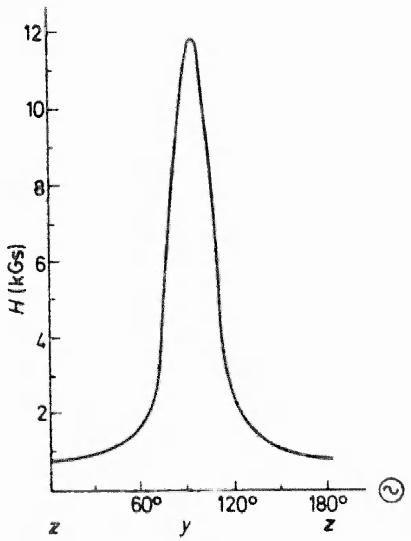



*Figure 19.* The angular relation of e.s.r. spectrum with rotation around the x axis.

solution. At the microwave frequency of approx. 10 GHz the EPR spectrum was observed only for the compound crystallized from chloroform[38]. The angular relations are presented in *Figures 19* and *20*. The EPR spectrum was

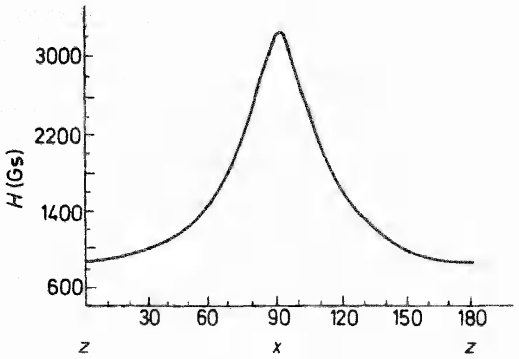



*Figure 20.* The angular relation of the e.s.r. spectrum with rotation around the *y* axis.

assumed to derive from the $S = 1$ state. That was confirmed by temperature dependence studies of the EPR line intensity[38]. Using the intensity line equation the exchange integral $J$ (corresponding to the Hamiltonian $H_{ex} = JS_1 \cdot S_2$) was calculated (*Figure 21a*).

$$R = \frac{\exp\left[-\dfrac{J(s+1)}{2kT}\right]}{\sum_{s=0}^{s_{max}=5}(2s+1)\exp\left[-\dfrac{J(s+1)}{2kT}\right]} \qquad \text{(a)}$$

$$J = 170\ \text{cm}^{-1}$$

$$H = \beta H \cdot \hat{\hat{g}} \cdot S + D[S_z^2 - \tfrac{1}{3}S(S+1)] + E(S_x^2 - S_y^2) \qquad \text{(b)}$$

$$g_{xx} = g_{yy} = g_{zz} = 2$$
$$D = 1.87\ \text{cm}^{-1} \qquad \text{(c)}$$
$$E = 0.13\ \text{cm}^{-1}$$

*Figure 21.* (a) Expression for the temperature dependence of intensity of the line; (b) spin Hamiltonian; (c) spin Hamiltonian parameters.

The value obtained of $J = 170\ \text{cm}^{-1}$ was in agreement with that deduced from magnetic susceptibility measurements by Coggon *et al.*[39]. The angular relations of the EPR spectrum (*Figures 19* and *20*) could be described by the spin Hamiltonian (*Figure 21b*) on the assumption that the $Z$ axis is the FeOFe angle bisector, and the $X$ axis is the straight line bonding the iron ions. The spin-Hamiltonian parameters shown in *Figure 21c* were obtained.

The energy dependences of the triplet state levels ($S = 1$) on the magnetic field, for the field directed along the main $X$, $Y$, $Z$ axes, are given in *Figures 22–24*. The spin-Hamiltonian parameters obtained explain the relatively high intensity of the 'forbidden' transition when the magnetic field is parallel to the $Z$ axis.

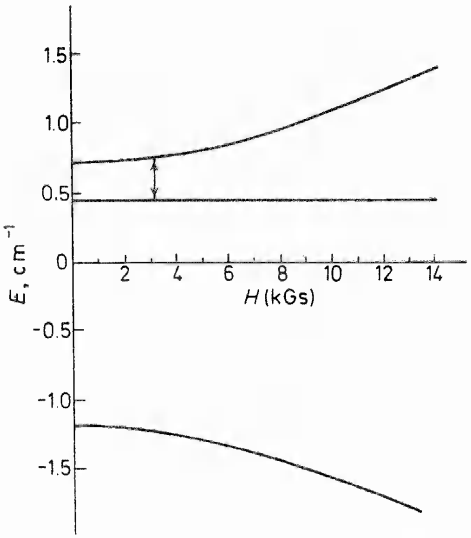



*Figure 22.* Triplet state levels at $H \| X$.

The large value of the $E$ parameter causes the mixing of states $|+1\rangle$ and $|-1\rangle$. Hence, two new states are formed $|+\rangle$ and $|-\rangle$ described by the combinations of these functions (*Figure 24*). In that case the excitement of the transition by the vibrating magnetic field parallel to the constant field, and parallel to the $Z$ axis of the molecule, is possible.

The intensity of such a transition is proportional to the square of a matrix element $\langle +|\hat{S}_z|-\rangle$, different from 0 when $E$ is different from 0. The $\Delta M_s = 0$ transition is excited. The complex $[Fe(salen)]_2OCH_2Cl_2$, which crystallized from dichloromethane, has no $X$ band spectrum. The $D = 2\,cm^{-1}$ value determined by Coggon from the magnetic susceptibility measurements of a

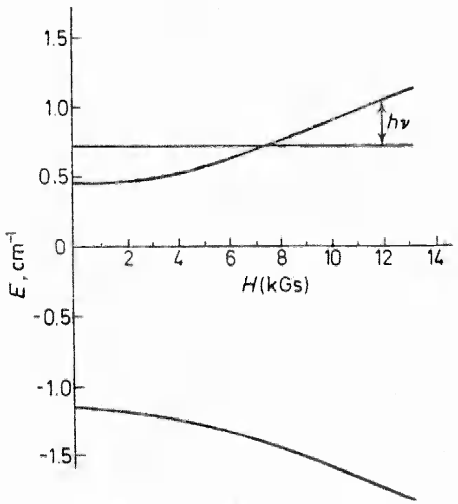



*Figure 23.* Triplet state levels at $H \| Y$.

monocrystal[39] is close to the value obtained by us for the complex crystallized from chloroform. That indicates that the $E$ parameter value for $[Fe(salen)]_2OCH_2Cl_2$ is close to 0, and this has to result from the higher symmetry of the molecule.

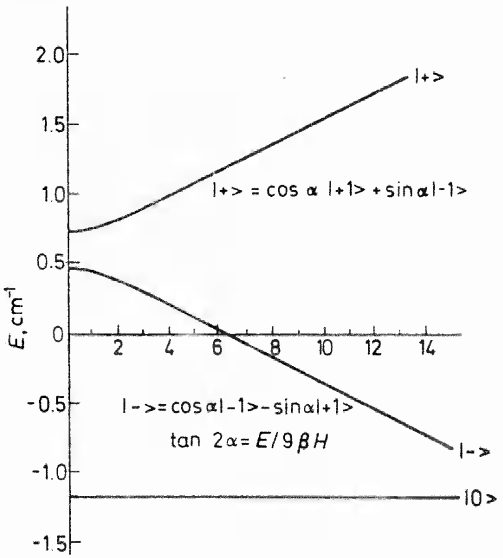



*Figure 24.* Triplet state levels at $H||Z$.

This led us to the interesting conclusion that the relatively weakly active solvent $CH_2Cl_2$ has an essential influence on complex symmetry. The determination of the influence of a solvent on the structure and magnetic properties of binuclear iron complexes can be important in considering the role of the polymer iron complexes in biological systems. That some dimers are accepted only by some fixed organism is also due to the structural and electronic properties of these compounds. Small changes in the structure of the dimeric systems could have an essential influence when the iron complexes play the role of electron carriers (sulphur polymers).

## THE ROLE OF METAL IONS IN METABOLISM

Transition metal ions, or rather transition metal complexes, affect the activity of enzymes. To obtain a better understanding of these problems we have synthesised model systems including 22 copper(II) complexes with amino acids and polypeptides, and mixed complexes with 2,2′-bipyridyl. The electronic and molecular states of these complexes were the subject of our studies. These complexes are monomers in aqueous solution and in ethylene glycol, both in fluid and frozen solution. In the solid state, the spin–spin interactions indicate the presence of dimers. The EPR spectra for all complexes in solution at room temperature are identical, with a slightly asymmetric line. The differences occur in glasses. From the EPR spectra of glasses in ethylene glycol (*Figures 25* and *26*) it follows, on the basis of calculations

| Complex | Spin Hamiltonian parameters | | | | |
|---|---|---|---|---|---|
| | $g_{\parallel}$ | $g_{\perp}$ | $g_{av}$ | $A_{\parallel} \times 10^{-4}$ cm$^{-1}$ | $B \times 10^{-4}$ cm$^{-1}$ |
| Cu[glycine]$_2$ | 2.258 | 2.069 | 2.132 | 172.9 | 13.5 |
| Cu[DL-α-alanine]$_2$. $H_2O$ | 2.255 | 2.054 | 2.121 | 175.8 | |
| Cu[β-alanine]$_2$. $H_2O$ | 2.257 | 2.050 | 2.199 | 148.8 | |
| Cu[DL-valine]$_2$ | 2.252 | 2.063 | 2.126 | 175.4 | 12.9 |
| Cu[DL-ornithine]$_2$. $2H_2O$ | 2.250 | 2.072 | 2.123 | 176.5 | 11.6 |
| Cu[glutamic acid]$_2$ | 2.244 | 2.064 | 2.123 | 176.0 | 11.27 |
| Cu[L-lysine]$_2$ $Cl_2$ | 2.246 | 2.071 | 2.114 | 184.6 | 10.6 |
| Cu[L-β-phenyl-α-alanine]$_2$ | 2.244 | 2.063 | 2.123 | 180.3 | 10.8 |
| Cu[L-tyrosine]$_2$ | 2.246 | 2.067 | 2.125 | 181.9 | 9.65 |
| Cu[GLGL]. $H_2O$ | 2.243 | 2.063 | 2.121 | 188.5 | 10.6 |
| Cu[histamine]$_2$ | 2.247 | 2.088 | 2.141 | 182.7 | 12.95 |

*Figure 25.* The Hamiltonian parameters of the copper(II) complex with amino acids and polypeptides in frozen ethylene glycol solutions.

| Complex | $\alpha^2$ | $\beta_1^2$ | $\beta^2$ | $\alpha\beta$ | $\alpha\beta_1$ | $1-\beta_1^2$ |
|---|---|---|---|---|---|---|
| Cu[glycine]$_2$ | 0.75 | 0.90 | 0.97 | 0.85 | 0.83 | 0.10 |
| | 0.75 | 0.90 | | | | |
| Cu[GLGL]. $H_2O$ | 0.82 | 0.62 | 0.96 | 0.71 | 0.88 | 0.38 |
| Cu[DL-valine]$_2$ | 0.77 | 0.69 | 0.85 | 0.73 | 0.81 | 0.31 |
| Cu[DL-ornithine]$_2$. $2H_2O$ | 20.79 | 0.73 | 0.86 | 0.76 | 0.83 | 0.27 |
| Cu[L-lysine]$_2$ $Cl_2$ | 0.81 | 0.69 | 0.88 | 0.75 | 0.85 | 0.31 |
| Cu[histamine]$_2$ | 0.79 | | | | | |
| Cu[L-tyrosine]$_2$ | 0.79 | | | | | |

*Figure 26.* Values $\alpha^2$, $\beta_1^2$, $\beta^2$, $\alpha\beta$, $\alpha\beta_1$, $1-\beta_1^2$ in frozen ethylene glycol solutions.

of the $\alpha^2$ molecular orbital coefficients, that $\sigma$-bonds are almost equally strong in all complexes. The $\beta^2$ coefficients indicate that the $\pi$-bonds are the weakest in the complexes Cu(glycine)$_2$ and Cu(glycylglycine). $H_2O$. Magnetic susceptibility measurements of the solid complex Cu(β-alanine)$_2$. $H_2O$ in the temperature range 2–300 K indicated the presence of spin–spin interactions, leading to the triplet ground state, and to the singlet state over 10 cm$^{-1}$ (*Figure 27*). At temperatures below 10 K the influence of the lattice antiferromagnetic interactions is observed. The EPR spectra at low fields confirm the presence of spin–spin interactions and the formation of dimer systems[40–44]. Similar exchange interactions were found in the following complexes: Cu(DL-valine)$_2$.$H_2O$, Cu(DL-α-amino-n-butyric acid)$_2$.$2H_2O$, Cu(L-leucine)$_2$, Cu(asparagine)$_2$, Cu(L-methionine)$_2$.$2H_2O$, Cu(DL-homocysteine)$_2$, Cu(DL-homocysteine)$_2$.$2H_2O$, Cu(DL-ornithine)$_2$, Cu(histamine)$_2$, Cu(DL-leucine)$_2$.$3H_2O$, Cu(DL-α-alanine)$_2$.$H_2O$, Cu(DL-α-alanine-DL-α-alanine).$3H_2O$.

In their EPR spectra the bands ($H_{min}$) corresponding to the $\Delta_{M_S} = \pm 2$ transition are present at $g = 4$. That indicates that in all complexes the dimeric systems are present, in which the triplet and singlet states are formed due to the spin–spin interactions (*Figure 28*). In the remaining complexes, Cu(glycine)$_2$, Cu(glycylglycine).$H_2O$, Cu(L-β-phenyl-α-alanine)$_2$, the spin–spin interactions do not occur, since the $H_{min}$ lines have not been found. It

should be noted that the complexes Cu(DL-α-alanine)$_2$,$H_2O$, Cu(β-alanine)$_2$. $H_2O$ and Cu(DL-α-alanine)(2,2′-bipyridyl).2$H_2O$ present very broad $H_{min}$ bands. The interactions are more complicated, and broadening of the lines is caused by the dipole interaction.

Our studies have allowed us to systematize the electronic structures of copper(II) complexes, to determine the influence of changing the character

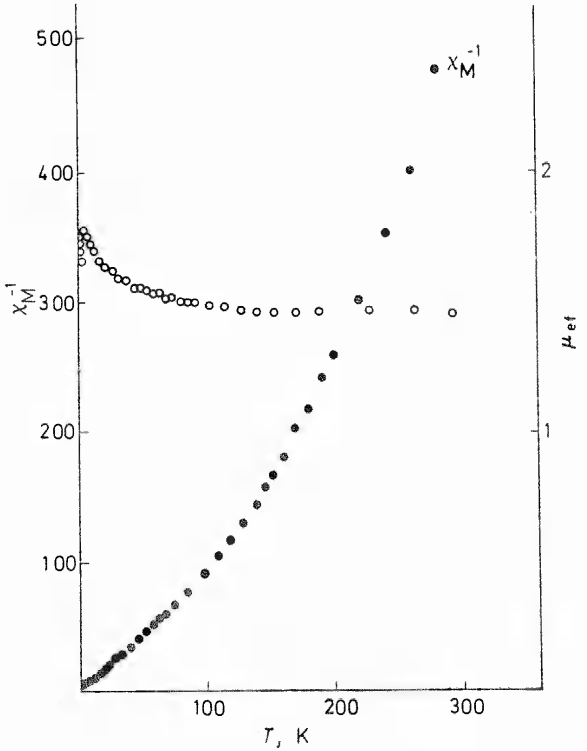



*Figure 27.* The inverse magnetic susceptibility and magnetic moment of [Cu(β-alanine)$_2$. $H_2O$]$_2$ in the temperature range 2–300 K.

of the ligand by increasing the chain and by changing the nature of the peptide bond. Studies of simple and mixed complexes of various angles have become very important in recent years, as models of metal–protein and metal–enzyme bonds; and since they can be regarded as models for metallo–enzyme–substrate complexes.

The action of the enzyme after the approach of the peptide depends upon the shifting of a negative charge in the active centre, cleavage of the peptide bond, with liberation of an amine and an acid, and upon return of the charge to the initial position. Consideration of the electronic structure of the

compounds under investigation, in particular the spin–spin interactions, and the different $\pi$-bond strengths in complexes with equal $\sigma$-bonds, suggests that copper complexes differ in their effect on the active centre of the enzyme. They can either facilitate the migration charge, or they can act as inhibitors by combining sufficiently strongly with the active centre of the enzyme to inhibit any movement of a charge.

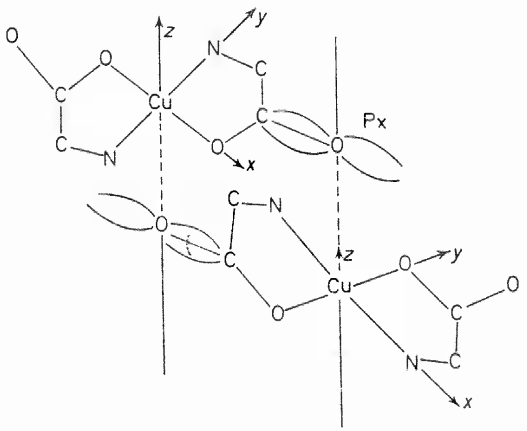



*Figure 28.* Schematic representation of the structure of $[Cu(DL\text{-}\alpha\text{-alanine})_2 . H_2O]_2$.

We have also studied the complexes of vitamin $B_{12}$ with proteins and metal ions. The $B_{12}$-cyanocobalmin, which plays the role of catalyst in the synthesis of nucleic acids, is necessary for the proper function of nerve systems. It forms complexes with proteins; that with mucoprotein, for example, is essential for this sorption on vitamin.

To elucidate the role of the metal ion in the sorption of vitamin $B_{12}$, we have synthesized the vitamin $B_{12}$ complexes with mucoprotein and with glutathione. The formation of the complexes was confirmed by their infrared spectra. The complexes were saturated in solution with molybdenum, copper, iron and cobalt ions. The EPR and infrared spectra indicate further coordination of the vitamin $B_{12}$ complexes with metal ions[45–53]. Some results of our studies on complexes of constituents of nucleic acid with metals should also be mentioned. We have obtained a number of copper(II) complexes with adenine[54] and iron(III) complexes with guanine and glutathione[55,56]. We have found that dimers and trimers are easily formed. The EPR studies allowed us to determine the electronic structure of the dimers and the electronic density change of the bridge bonding the copper(II) ion after deprotonation of the purine ring at high pH (>7) (*Figure 29*). On

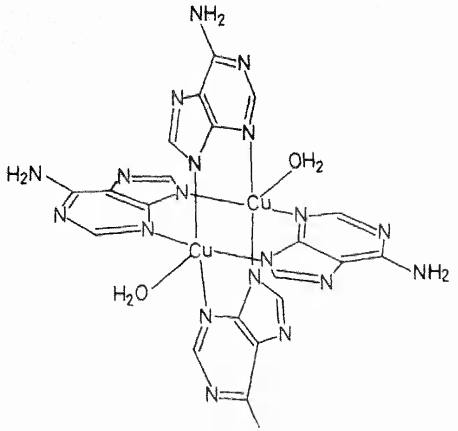



*Figure 29*. Binuclear structure of the complex $Cu_2(adenine)_4 \cdot 4H_2O$.

deprotonation of adenine and guanine, the $\pi$-bond system changes with delocalization of the electronic density via the N—C—N bridge connecting the ions, and a change in the electron density on the metal atoms (*Figure 30*).

The strong antiferromagnetism in those dimers is caused by the fact that the $\pi$-orbitals of the purine rings are readily accessible to the copper(II) or iron(III) electrons. Small changes in the bond system cause a change of electron delocalization. The copper(II) trimer with chloride bridges exhibits small exchange interaction because of the small delocalization of the chloride

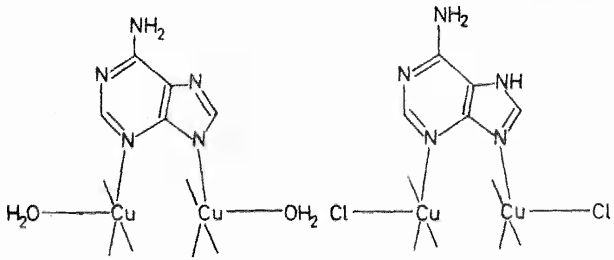



*Figure 30*. Bonding arrangements in the binuclear clusters of the complex $Cu_2(adenine)_4 \cdot 4H_2O$.

ions. The EPR spectrum exhibits a broadened line because of dipole interactions. The lack of the appropriate $\pi$-orbitals in the bridging chlorine atoms causes the decay of the strong antiferromagnetic interactions. These changes are essential in abnormal growth processes in a cell and in mutation. These studies will perhaps contribute to a better understanding of the processes controlling living organisms.

Major contributions to the studies described were made by my co-workers A. Vogt, P. Sobota, J. Jezierska, A. Jezierski, H. Kozłowski, A. Ożarowski and A. Antonow.

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