Absorption-, EPR-, and EXAFS-spectroscopy [4, 5] of the freshly isolated protein are consistent with the copper bound as Cu(I) to cysteinyl residues. In contrast to the poorly resolved absorption spectrum, the protein displays circular dichroism features in the UV region attributable to highly asymmetric coordination of Cu(I). *Neurospora* copper metallothionein is further characterized by an unusual luminescence upon excitation in the UV [6]. The emission spectrum consists of a very broad band centered at 565 nm with an unusually large Stokes shift. Because the luminescence strictly depends on the integrity of the Cu(I)–thiolate complex it is attributed to transitions of the charge transfer type.

Spectroscopic titrations of *Neurospora* copper metallothionein with $HgCl_2$ and *p*-chloromercurybenzoate indicate the binding of two mercurials without loss of copper. This binding results in distinct changes in the absorption and CD spectra of the protein and in the disappearance of its luminescence. These observations lead us to suggest that the six Cu(I) ions coordinated to the seven cysteine residues are behaving as a single metal cluster, similar to those described earlier for the mammalian metallothioneins [7, 8].

From growth experiments of *Neurospora crassa* under different conditions it is suggested that copper metallothionein fulfills a multifunctional role in copper metabolism. Thus depending on the concentration of the copper present in the culture medium the protein could either serve a storage or a detoxification function. From *in vitro* reconstitution studies with *Neurospora* apotyrosinase and *Carcinus* apohemocyanin [9] it is further suggested that *Neurospora* copper metallothionein functions as a metal donor to the active site of 'type 3' copper proteins.

1 J. H. R. Kägi and M. Nordberg, (eds.) 'Metallothionein', Birkhäuser, Basel (1979).
2 K. Lerch, *Nature, 284,* 368 (1980).
3 K. Lerch, in 'Metal Ions in Biological Systems', (H. Sigel, ed.), Vol. 13, p. 299, Marcel Dekker, New York (1981).
4 M. Beltramini and K. Lerch, *Biochemistry,* in press (1983).
5 T. A. Smith, K. Lerch and K. Hodgson, unpublished results.
6 M. Beltramini and K. Lerch, *FEBS Lett., 127,* 201 (1981).
7 J. D. Otvos and I. M. Armitage, *Proc. Nat. Acad. Sci. U.S.A., 77,* 7089 (1981).
8 M. Vašák and J. H. R. Kägi, *Proc. Nat. Acad. Sci. U.S.A., 78,* 6709 (1981).
9 M. Beltramini and K. Lerch, *FEBS Lett., 142,* 219 (1982).

B5

Coordination Equilibria of Carbohydrate-type Ligands

KÁLMÁN BURGER

L. Eötvös University, Department of Inorganic and Analytical Chemistry, Budapest, Hungary

Carbohydrates are for the coordination chemist biologically active *polyfunctional ligands.* Under physiological conditions they are present in solutions containing metal ions. This alone justifies the study of their coordination equilibria. The effect of the presence of metal ions on the biological activity of carbohydrates, indicating metal complexation, has been shown in several cases, *e.g.* in calcium–heparin systems. Metal complexes of carbohydrates are of vital importance in human and veterinary therapy (*e.g.* iron(III) complexes of sugar-type ligands, *etc.*)

Investigation of the coordination equilibria of carbohydrates is made difficult, however, by the usually low stability of their complexes resulting in the appearance of competing equilibria (*e.g.* hydrolysis), by the overlap of pH-dependent and pH-independent processes, by the lack of suitable electrodes for the study of the latter, by the formation of polynuclear species due to the bridge forming ability of this type of ligand. Conformational and configurational features also influence strongly the complex formation equilibria. These are the main reasons why little research has been performed so far in this field [1, 2].

The lecture covers our recent equilibrium studies on

(a) iron(III) complexation of sugar-type ligands (lactose, galactose, lactobionic acid, gluconic acid, dextrane, *etc.*);

(b) formation of mixed ligand complexes of iron(III) in the above systems;

(c) copper(II) complexation of sugars and aminosugars;

(d) calcium(II) and zinc(II) complexation of heparin in the presence of alkali metal ions;

(e) the protonation of carbohydrate-type ligands leading to the determination of the composition and stability constants of the complexes in each system.

The experimental procedure is based on spectrophotometric, potentiometric and electrophoretic equilibrium measurements. For the separation of pH-dependent and pH-independent processes self prepared and commercial ion selective electrodes are used. The primary experimental data are processed by computer evaluation. The equilibrium studies are complemented by structural methods (*e.g.* Mössbauer spectroscopy).

The results reflect the complexity of each system, the simultaneous presence of several species in them, the sensitivity of the complex formation equilibria on internal (structure of ligand) and external (matrix) factors. The equilibrium constants have been used for the calculation of the concentration distribution of species of different compositions.

1 S. J.Angyal, *Chem. Soc. Rev., 9,* 415 (1980) and references therein.
2 A. E. Martell and R. M.Smith, 'Critical Stability Constants', Vol. 3, Plenum Press, New York (1977).

B6

A New Model of Coenzyme B_{12}?

I. YA. LEVITIN* and M. E. VOL'PIN

Institute of Organo-Element Compounds, Academy of Sciences of the U.S.S.R., Moscow B-334, U.S.S.R.

A survey of vitamin B_{12}-dependent rearrangements suggests that they involve homolytic splitting of the Co–C bond in 5′-deoxyadenosylcobalamin. Numerous attempts were made to simulate these processes using 5′-deoxyadenosylcobalamin in nonenzymatic systems as well as its most popular models, organocobaloximes. Nevertheless, striking features of the homolysis step, namely mild conditions, reversibility and controllability, have been neither imitated nor properly explained so far. In this connection, certain results of our studies with a new type of organocobalt chelate [1] may be of interest.

The cationic complexes in question involve the trivalent metal bound to an alkyl group, a chelating diamine and a mixed tridentate ligand derived from a Schiff base constituted by the same diamine and an *o*-hydroxycarbonyl compound at a 1:1 ratio. Conditions of their formation and its mechanism are considered; the spatial and electronic structure of the complexes is also studied. Some of the reactions related to modelling vitamin B_{12} (*e.g.* photolysis, reduction and oxidation) are discussed, with emphasis being put on the unusual behaviour of the complexes under the influence of acids [2].

Decomposing readily in acidic media, the alkylcobalt chelates under consideration give all the products of disproportionation and coupling of the alkyl groups (RH, R_{-H} and R_2), the yields of the RH alkanes being substantially higher than those of alkenes. Experiments with an isotope label (D_2O) revealed that the excess of the former was due to the abstraction of hydrogen atoms from chelating ligands by alkyl free radicals rather than to protolysis of the Co–C bond. These findings suggest homolytic cleavage of the organocobalt complexes under the action of protons.

The formation of alkyl free radicals in the course of decomposition was directly proved by the spin-trapping technique. Spin adducts of the radicals (*viz.* Me, Et and c-C_6H_{11}) with Bu^tNO and $PhCH{=}N(O)Bu^t$ were identified by ESR spectroscopy in phosphate buffer solutions. Furthermore, kinetic measurements with the latter trap at various pHs indicated that protons are involved in steps leading to the formation of alkyl free radicals. The intermediacy of protonated complexes still holding the Co–C bond was established by means of spin-trapping, NMR and spectrophotometric techniques.

The ability of the complexes in question to generate alkyl free radicals under mild conditions and at a conveniently regulated (pH-controlled) rate was used to imitate vitamin B_{12}-dependent dehydration of α-glycols. Some positive results give support to the speculation that protonation-deprotonation or related polar interactions may control the dissociation of the Co–C bond of 5′-deoxycobalamin in enzymatic systems, thus triggering the biological dehydratation of glycols as well as other vitamin B_{12}-dependent rearrangements.

The potential use of the complexes as sources of free radicals in living organisms is also discussed.

1 I. Levitin, A. Sigan, E. Kazarina, G. Alexandrov, Yu. Struchkov and M. Vol'pin, *J. Chem. Soc. Chem. Comm.,* 441 (1981).
2 I. Ya. Levitin, A. L.Sigan, R. M. Bodnar, R. G. Gasanov and M. E. Vol'pin, *Inorg. Chim. Acta, 76,* L169 (1983).

B7

Iron–Carbon Bond Formation During Substrate Activation by Hemoproteins

D. MANSUY

Laboratoire de Chimie de l'Ecole Normale Supérieure, 24, rue Lhomond, 75231 Paris Cédex 05, France

Recent results point to the existence of an important organometallic chemistry of certain hemoproteins, with the formation of iron–carbon bonds during substrate activation. Evidence for such iron–carbon bond formation comes both from spectroscopic studies on the hemoproteins themselves and from model studies on iron-porphyrins. These iron–carbon bonds are formed either upon reduction or oxidation of several substrates [1].

Reduction of benzylhalides, $ArCH_2X$, by microsomal cytochrome P450 leads to σ-alkyl complexes of this cytochrome involving a $Fe(III)–CH_2Ar$ bond. Reduction of halothane, $CF_3CHClBr$, leads also to a σ-alkyl cytochrome $P450–Fe(III)–CHClCF_3$