structure and cooperative properties of these molecules.

The change of dipole moments of DNA sites after the incorporation of certain bivalent metal ions has been established using computation methods.

The change in histone structure under the influence of metals has been demonstrated experimentally using X-ray diffraction and spectral methods. The predicted changes in chromatin structure and thermodynamic properties under the influence of metal ions have been confirmed by direct calorimetric and spectral investigations.

A general analysis of the available data is given. The program of investigations required in this field is laid out.

B3

Porphyrin Interactions with Nucleic Acids

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The interactions of tetrakis(4-N-methylpyridyl)-porphine (H_2TMpyP-4) and its copper(II), nickel(II), zinc(II), cobalt(III), iron(III), and manganese(III) derivatives with several nucleic acids have been investigated. Spectrophotometric titrations of H_2TMpyP-4 and Cu(II)TMpyP-4 with the synthetic polymer poly(dG-dC) could be analyzed by a near-neighbor exclusion model leading to $n \approx 2$ and equilibrium binding constants of 7.7×10^5 M^{-1} and 8.0×10^5 M^{-1}, respectively. The other metal derivatives (except for the nickel porphyrin) do not provide sufficiently large color changes with poly(dG-dC) to allow analysis.

In contrast, all of these porphyrins interact with poly(dA-dT) and DNA. For those porphyrins investigated the binding profiles are not adequately fit by a near-neighbor exclusion model but have bonding profiles suggesting cooperativity effects are important.

Spectral and circular dichroic experiments both suggest base specificity. With calf thymus DNA, the copper(II) and nickel(II) derivatives show primarily prominent negative CD features and large red shifts and hypochromicity of visible absorption bands in the Soret region characteristic of GC specificity (as demonstrated with the synthetic polymer); the other metal derivatives show prominent positive induced visible CD features with small red shifts and hypochromicity of the absorbance bands in the Soret region characteristic of AT specificity. Only the metal-free derivative has a conservative CD spectrum suggestive of distribution among GC and AT sites.

Stopped-flow kinetic experiments corroborate the interpretation of the static experiments. Both association and dissociation kinetics involving poly(dA-dT) are too rapid to be seen by the stopped-flow method in all the derivatives. However, the copper(II), nickel(II), and non-metallo derivatives show measurable kinetic profiles in the stopped-flow region with poly(dG-dC).

The temperature-jump method was used to investigate the reactions of H_2TMpyP-4 with DNA and poly(dG-dC). Although the former reaction is multiphasic in the former case, a single relaxation process was obtained for the latter system yielding a forward rate constant of 5.7×10^5 M^{-1} s^{-1} and 1.8 s^{-1} for the reverse rate constant.

Temperature-jump relaxation studies of mixtures of poly(dA-dT) and poly(dG-dC) indicate that the redistribution of H_2TMpyP-4 between AT and GC sites occurs via a mechanism involving dissociation rather than direct tranfer. A comparison of the relaxation times obtained for this system with those obtained for DNA strongly suggests that in the natural system the porphyrin can move from site to site without dissociation into the solvent medium.

B4

Molecular Structure and Biological Function of *Neurospora* Copper Metallothionein

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Metallothioneins are low molecular weight, cysteine-rich proteins binding unusually high amounts of zinc, cadmium and/or copper. These proteins are ubiquitous in nature and are believed to play an important role in metal metabolism. The mammalian metallothioneins have been studied in great detail from a structural point of view including amino acid sequence analysis and spectroscopic techniques [1].

The smallest and simplest metallothionein known so far is found in the cytosolic fraction of the fungus *Neurospora crassa* grown on a copper supplemented medium [2, 3]. The protein consists of a single polypeptide chain of 25 residues with a molecular weight of 2600 and a metal content of 6 g atoms per mol. It shows a striking sequence homology to the zinc- and cadmium-containing metallothioneins from vertebrates with a complete agreement in the position of the seven cysteinyl residues.

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

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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).