Session Lectures

B1

Reversed Micelles to Mimic Active Site of Metalloenzymes

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Reversed micelle is an aggregate of surfactant formed in an aprotic solvent that usually contains a small amount of water. The interior core of the reversed micelle, *i.e.* the micellar interface and the inner aqueous phase, provides a unique reaction field which mimics the structure and function of the enzyme active site. We would like to show that metal ions in reversed micelles behave in a way similar to those found in metalloenzymes.

The reversed micelle of CTACl in $CHCl_3$ solubilizes a considerable amount of halide or nitrate salts of cobalt(II), nickel(II) and copper(II) that are otherwise insoluble in simple apolar media. The cobalt and nickel ions undergo a structural transformation between tetrahedral and octahedral configurations in the cationic reversed micelle [1]. At low water concentrations in the micelle, the tetrahedral complex is favored. This is easily converted to the octahedral species by lowering the temperature (thermochromism) or simply by the addition of water (solvochromism). Cupric halides undergo a more complex structural transformation in cationic reversed micelles [2]. At low water concentrations a polymeric cupric complex bridged by halide ions is produced, which transforms into monomeric species with an increase in water concentration. The complex is converted eventually into an octahedral species at high water concentrations.

The halide bridge of the polymeric copper mentioned above is readily broken by either reduction to the cuprous ion with 2-mercaptoethanol or by the addition of such common ligands as imidazole and azide ion. In the latter case, the resulting imidazole-copper complex showed an electronic absorption spectrum with λ_{max} at 810 nm. This spectrum resembles that of copper carbonic anhydrase, though the λ_{max} is red shifted from the latter by ~80 nm.

The hydrolysis of norleucine p-nitrophenyl ester was accelerated drastically by copper ion in 0.10 *M* AOT reversed micelles in CCl_4 [3]. The effect of copper was maximal at low water concentration (~0.15 *M*) in the micelle, and a further increase in water concentration diminished the rate acceleration by the metal sharply. The intramolecular cyclization of the pyridoxal Schiff base of histidine is also affected by metal ions in reversed micelles [4]. Of interest is the fact that even the magnesium ion was effective in prohibiting cyclization in the micelle, while it is totally ineffective in non-micellar solutions. All these phenomena of metal ions may be explained by their 'naked' character in reversed micelles, especially at low water concentrations therein.

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3 J. Sunamoto, H. Kondo and K. Akimaru, *Chem. Lett.,* 821 (1978).
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B2

The Role of Metals in the Initiation and the Development of Malignant Transformation

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The concentration of metal ions in DNA molecules of a malignant growth is higher than in the norm. The genetic consequences appearing in the DNA molecule as a result of mutations of certain type and of the penetration of metal ions into its structure are considered.

The present state of investigation of metal influence upon the structural and thermodynamic properties of DNA, histones and chromatin in the norm and in the malignant transformation is considered. Metals endogen-bound with DNA leave the latter susceptible to the influence of X-rays and certain antitumor preparations. The change in the physical properties (electronic density distribution, thermostability) of DNA, histones and their complexes in the case of cancer are interpreted from the view-point of the influence of endogen-bound metal ions on the

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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 $7.7 \times 10^5\ M^{-1}$ and $8.0 \times 10^5\ M^{-1}$, 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 $5.7 \times 10^5\ M^{-1}\ s^{-1}$ 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.

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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.