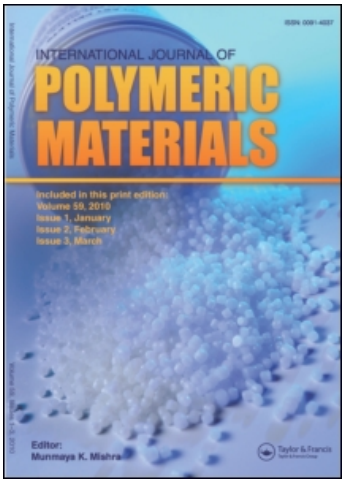

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Biological and Related Polymers

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Biological and Related Polymers

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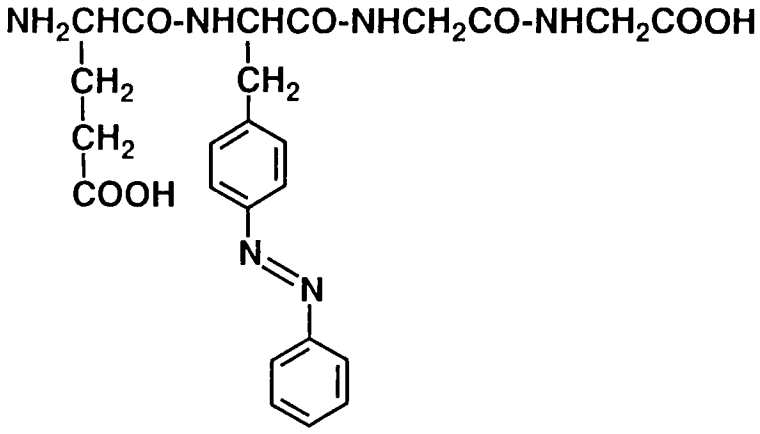
In this years meetings, 131 and 75 papers were presented at the spring and fall meetings of 1990, respectively. The numbers of papers increased remarkably as compared with 77 and 61 papers presented in 1989. This review highlights five topics related to proteins, nucleic acids, and lipids.

PHOTOCHEMICAL CONTROL OF ANTIGEN-ANTIBODY RESPONSES

An antibody can bind a particular antigen specifically. The binding is sometimes so strong that it is not possible to dissociate the antigen from the antibody. If the binding and dissociation could be controlled through some external perturbation, a wide variety of applications would be identified in the field of biotechnology.

Harada and Sisido *et al.* of Tokyo Institute of Technology (2700, E1107) succeeded in achieving this control by the use of antibodies produced in response to azobenzene as an antigenic determinant. Azobenzene shows cis-trans isomerism and the trans isomer can be converted in part to the cis isomer by UV irradiation. It is expected that the association and dissociation of antigen-antibody conjugates can be controlled by changing the structure of antigenic determinant via photoirradiation. This paper forms the first part of this investigations.

The chemical structure of the synthetic antigen is H-Glu-trans-azoAla-Gly-Gly-OH. The tetrapeptide consists of (1) a chromophore L-*p*-(phenylazo) phenylalanine



(azoAla), (2) a highly hydrophilic glutamic acid to disperse the peptide in water, and (3) a flexible diglycine spacer. This tetrapeptide hapten was then attached to a carrier protein bovine serum albumin (azo-BSA). The antigen was injected into BLAB/c mice and the immune response was boosted several times to obtain antibodies in high yields.

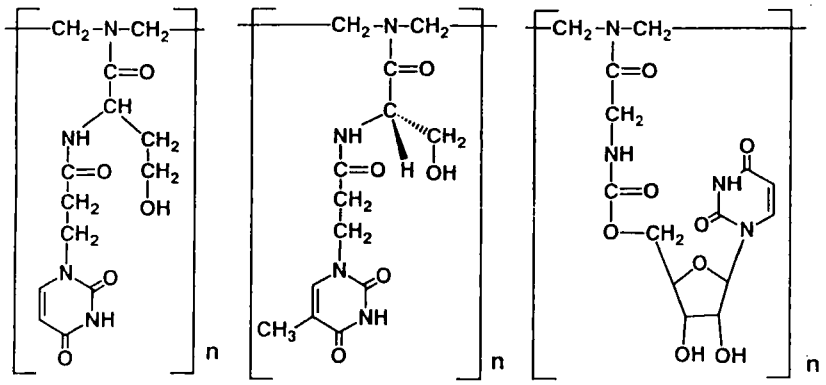
As a preliminary experiment, a modified enzyme-linked immunosorbent assay (ELISA) was carried out using the tetrapeptide-attached casein (trans-form azo-casein) as the immobilized antigen. Binding of antibodies to the immobilized casein was inhibited by the tetrapeptide containing trans-form azobenzene, but not by the tetrapeptide containing cis-form azobenzene. The polyclonal antibodies can distinguish highly specifically between the trans-form and cis-form of azobenzene.

Monoclonal antibodies were prepared from hybridoma cells by conventional techniques. Analysis using a kit for mouse monoclonal antibody isotyping indicated that the subclass of the four isolated monoclonal antibodies was IgG1 having κ light chain.

Fluorescence of the monoclonal antibodies was quenched effectively in the presence of the trans-form tetrapeptide. The binding constant (K) was determined to be 5.4×10^7 ($Lmole^{-1}$) (25°C). Azobenzene-carrying alanine methyl ester (azoAla-OCH_3) was also an effective quencher of the fluorescence of the monoclonal antibody, and its binding constant was similar to that of the tetrapeptide. It is suggested that the monoclonal antibody recognized the trans-form azobenzene. On the other hand, other types of monoclonal antibodies had smaller binding constants and less quenching with azoAla-OCH_3. These antibodies recognized another parts of the tetrapeptide hapten and further experiments are now being carried out on monoclonal antibodies which recognize the trans azobenzene.

WATER-SOLUBLE NUCLEIC ACID ANALOGS: CONFORMATIONAL CHANGE OF DNA VIA BASE-BASE INTERACTIONS

Nucleic acid analogs have received much attention in connection with biological and pharmaceutical applications. One disadvantage of the previously reported nucleic



acid analogs is the lack of solubility in water due to the use of hydrophobic purine and pyrimidine bases.

Wada and Mochizuki *et al.* of Osaka University (2733, E1118) prepared three different types of water-soluble nucleic acid analogs[1]. Uracil, thimine, and uridine were grafted onto linear polyethyleneimine through a spacer derived from serine, homoserine, and glycine. The degree of substitution was around 90%. Hydrophilicity of the main chain as well as spacer groups made these polymers freely soluble in water.

Table I, summarizes hypochromicities between the synthetic nucleic acid analogs and synthetic or natural polynucleotides in water. A decrease of the absorbance coefficient at 260 nm is a measure of specific base-base interactions. These three synthetic analogs caused large hypochromicites of polyadenylate (poly A) which forms a complementary base pair. It took 3 days to attain equilibrium of the system with PEI-Hse-Ura, whereas the hypochromicities of PEI-Ser-Thy and PEI-Gly-Urd were attained promptly after mixing. The induced circular dichroims at 270 nm assignable to poly A was decreased by complex formation, suggesting a conformational change of poly A.

TABLE I
Hypochromicity (%) of Nucleic Acid Analogs with Polynucleotides and DNA

Analog	Poly A	Poly C	DNA
PEI-Hse-Ura	54	20	44
PEI-Ser-Thy	70	4	58
PEI-Gly-Urd	70	55	66

The hypochromicity between poly C and PEI-Ser-Thy was low. In other words, the selectivity of PEI-Ser-Thy in the base-base complement was high.

Emphasis has been placed by the research group on interactions of analogs with DNA. Not only the high hypochromicities but also drastic changes in CD spectra were reported. PEI-Hse-Ura, especially, showed a large negative circular dichroism at 270 nm: $[\theta] = -8.0 \times 10^6$ with a mixture of 1:1 composition. It is assumed that DNA takes a Z-form conformation of left-handed helix in the presence of a higher composition of the synthetic analog. These water-soluble nucleic acid analogs will be a useful tool in investigating interactions with DNA.

REVERSIBLE INTERACTIONS BETWEEN DNA AND PHENAZINE-CARRYING POLYACRYLAMIDE VIA INTERCALATION

Flat aromatic molecules sometimes induce mutation of DNA. These compounds slip in between adjacent base pairs in the DNA double helix. Consequently, they lead to the insertion or deletion of one or more base pairs. Hirai and Maeda *et al.* of Kyushu University (2730, E1120) are interested in the intercalation with DNA leading to the construct of a new type of biological conjugate[2]. This is a further study on a macromolecular assembly consisting of DNA and a synthetic vinyl polymer.

An acrylamide derivative substituted with a DNA-binding (intercalating) phenazine was radically copolymerized with acrylamide in the presence of λ phage DNA in a buffer solution. The reaction mixtures were subjected to agarose gel electrophoresis, and DNA in the gel was stained with ethidium bromide. The DNA in the mixture was found to migrate on the gel at a slower rate than the native DNA. Not only the retardation but also broadening of DNA lines was a measure of the interactions between DNA and the copolymer. The phenazine-carrying acrylamide, acrylamide comonomer, and initiator were essential for the induction of interactions to DNA.

The retardation and broadening of DNA lines were also observed even when the polymerization product was precipitated into ethanol and then the resulting polymeric precipitant was electrophoresed. When the polymeric product was treated with a strong intercalating ethidium bromide for three days, the electrophoresis profile returned to that of the native DNA. However, when ethidium bromide was removed from the solution and the resulting product was electrophoresed again, the interactions between DNA and the polymer were recovered.

These phenomena are explained as follows. DNA migrates at a rate that depends on its net charge and on its size and shape. Nonionic polyacrylamide chains were coiled to DNA to bring about an increase of the apparent size of DNA and resulted in retardation and broadening of DNA on electrophoresis. Since the interactions were reversible, the interactions are non-covalent and based on intercalation of phenazine molecule.

The research group expect that the DAN-synthetic polymer conjugates will be useful in the detection of trace amounts of DNA and also the protection of DNA against nucleases.

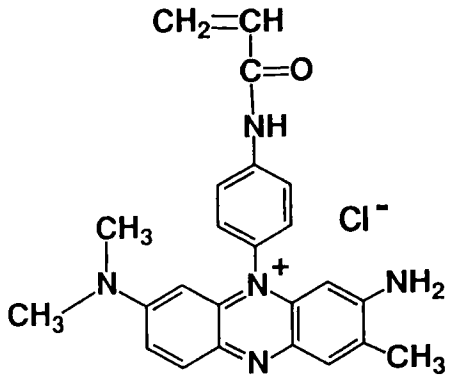
MOLECULAR ASSEMBLIES AND MOLECULAR RECOGNITION OF STEROIDAL BILE ACIDS

Steroidal acids occurring in the bile duct are known to form crystalline intermolecular compounds with a variety of organic molecules. Some of the assemblies of cholic acid and deoxycholic acid are multimolecular lattice inclusion compounds having channel type structures. In spite of the slight differences in the molecular structures, the two bile acids formed quite different crystal structures with themselves and with guest molecules.

A group of Gifu University, Sada and Miyata (2802, E1165) and Hori and Miyata *et al.* (2805, E1166), continued their investigation from the point of view of molecular recognition[3]. Cholic acid and deoxycholic acid are compared on the basis of the crystallographic data.

As illustrated in the structure, one molecule of these bile acids has (1) a head and tail array and (2) hydrophilic and hydrophobic faces. The steroidal ring skeleton is the head and the additional side chain is the tail. The hydroxyl and methyl groups protrude from the respective faces of the ring skeleton.

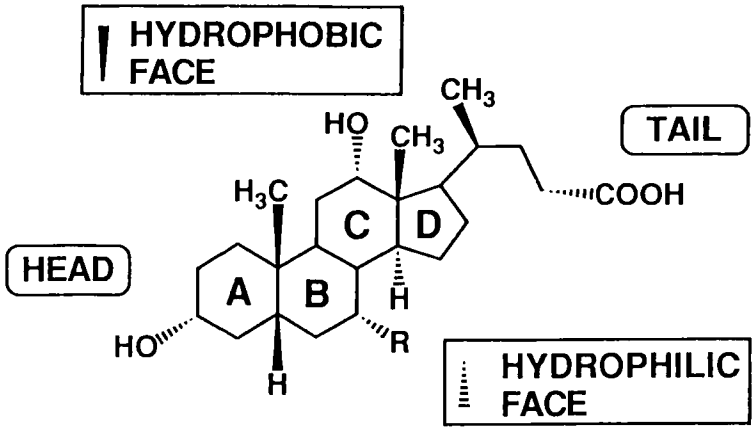
The molecules can be associated in the crystal in three dimensional arrays to form multilayered sheet structures due to the hydrophobic and hydrophobic preference.



Two different assemblies are obtained depending on the head and tail modes. Deoxycholic acid molecules were arranged in parallel (head-to-tail) fashions. In contrast, cholic acid molecules were arranged in antiparallel (head-to-head and tail-to-tail) fashions.

In deoxycholic acid, the host molecules form rigid and stable hydrophilic sheets consisting of helical networks of three kinds of hydrogen bond. Guest molecules are inserted into channels between the hydrophobic sheets. The molecular assemblies of deoxycholic acid with any organic molecules have rather similar structures.

By contrast, several kinds of crystal structures were detected from molecular assemblies between cholic acid and guest molecules. In other words, cholic acid showed guest-dependent polymorphism. Discussion is made on the modes of molecular assemblies of cholic acids on the basis of the following aspects. (1) the hydrogen-bonding modes within a hydrophilic layer in the crystals, (2) the bending of the tetracyclic steroid skeleton, and (3) flexibility of the tail chain.



R = OH, CHOLIC ACID

R = H, DEOXYCHOLIC ACID

ANALYSIS OF SELECTIVE ADSORPTIONS OF BIOACTIVE MOLECULES ONTO A LIPID MULTIBILAYER MATRIX ON A QUARTZ-CRYSTAL MICROBALANCE

A quartz-crystal microbalance (QCM) can determine the amount of adsorption of substances on an electrode from the frequency changes of the QCM. Okahata and coworkers of Tokyo Institute of Technology (2838, E1177; 2841, E1178; 2844, E1179; 2847, E1180) are investigating the adsorption behavior of various bioactive compounds onto lipid bilayer matrixes with a lipid-coated QCM. Good correlations were observed between partition coefficients and the activity of bioactive compounds such as bitter substances, odorants, anesthetics, and antibiotics.

Synthetic polyion complex between dioctadecyldimethylammonium and polystyrenesulfonate were used as the lipid of QCM in an aqueous phase. Partition coefficient were calculated from the amount of adsorption in lipid bilayers and the concentration in water. n-Nonanol was adsorbed specifically and penetrated deeply into the lipid bilayers. On the contrary, branched aliphatic C_9-alcohols or aromatic alcohols showed small partitions to the lipid matrix. This indicated that the absorption behaviour depends largely on the molecular shape of substances.

The structure of these bioactive compounds are extremely diverse and it was difficult to find molecular recognition mechanism by lipid bilayers in chemical structure levels. The first two papers of the Okahata group proposed to correlate quantitatively the adsorption behavior with molecular shape representated with "slenderness". Molecular "slenderness" (L/S) is estimated by CPK molecular models from its molecular length (L) along the longest axis and its sectional area (S). Hydrophobicity was also estimated by considering the length [L(polar)] directed along the polarity and its sectional area [S(polar)].

Plots of log P (partition coefficients) against molecular slenderness and hydrophobicity were recorded with several groups of molecules including aliphatic alcohols, aliphatic bromides, aromatic alcohols, and steroid molecules. According to multiple regression analysis, the adsorption of aliphatic C_9-alcohol into the lipid matrix were explained with both their hydrophobicity and molecular slenderness. Aliphatic C_9-bromides depended mainly on the molecular slenderness and C_9-sulfates and steroid molecules were adsorbed mainly through the hydrophobicity.

In conclusion, partition coefficients of bioactive compounds into the lipid matrix were explained with their hydrophobicity and molecular slenderness. The more hydrophobic and the more slender the molecule is, the larger partition to the lipid matrix is observed.

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