M-PM-WS1-1

THE EFFECT OF STEREOCHEMISTRY UPON CARBOHYDRATE HYDRATION IN AQUEOUS SOLUTION.

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The effect of stereochemistry upon carbohydrate hydration has been studied by probing the hydration characteristics of all eight D-aldohexoses. Several techniques have been used: kinetic medium effects of D-aldohexoses, thermodynamics and molecular dynamics. In addition carbohydrate-derived surfactants have been studied. These studies are all carried out in aqueous solutions.

The fit of a carbohydrate into the tree-dimensional hydrogen-bonded network of water depends on the next-nearest neighbour oxygen distances of the carbohydrate molecule in comparison with the oxygen distances in water. The relative position of OH(2) and OH(4) determine the extent of compatibility of the carbohydrate molecule with water. The D-aldohexoses can be divided into three groups with an excellent fit (D-talose), good fit (D-glucose) and bad fit (D-galactose). This depends on whether the intramolecular next-nearest neighbour oxygen distances are equal to the nearest neighbour or next-nearest neighbour oxygen distances of water or have no fit at all. The better the compatibility of a carbohydrate with water the more it will be recognised as a hydrophobic molecule, because the hydroxy groups are camouflaged for interaction. D-talose has an enhanced apparent hydrophobicity due to the formation of an intramolecular hydrogen bond between OH(2) and OH(4).

M-PM-WS1-2

CONFORMATION AND RECOGNITION OF GLYCOLIPIDS AT THE CELL SURFACE.

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Glycolipids located in the outer leaflet of the cell membrane are involved in a number of recognition reactions e.g. with antibodies, bacterial adhesins (and toxins) and viruses. In order to improve our understanding of these interactions the favoured conformations of a number of different glycolipids have been studied using HSEA(=hard sphere exo-anomeric)-calculations and molecular mechanics (MM3). With regard to the saccharide moiety the results of these calculations were generally in good agreement with available NMR data.

For the linkage between the saccharide chain and the lipid moiety in glycosphingolipids MM3-calculations suggest the existence of three favoured conformations (1). Two of these conformations are in agreement with the saccharide-lipid linkage conformation observed in single crystal structures of monoglycosyl-ceramides (cerebroside and methylated cerebroside). Comparative calculations carried out on the corresponding diacyl- and dialkylglycerolipids indicate that the conformational preferences of the saccharide-lipid linkage are affected by structural details of the lipid moiety.

For glycolipids located in lipid bilayers the conformational space accessible to the glycolipid head group is further reduced due to restrictions imposed by the surrounding lipid layer. In the case of the Forssman glycolipid these restrictions can explain the crypticity of the Galα1-4Gal epitope with respect to interaction with the $PapG_{pap}$-adhesin of *E. coli* (2).

1) Nyholm, P.G. & Pascher, I. (1993) Biochemistry 32, 1225-1234.
2) Strömberg, N., Nyholm, P.G., Pascher, I. & Normark, S. (1991) PNAS, 88, 9340-9344.

M-PM-WS1-3

STRUCTURAL ORGANIZATION AND THERMOTROPIC PHASE BEHAVIOR OF SYNTHETIC GLYCOLIPIDS.

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Calorimetric and X-ray diffraction measurements of aqueous dispersions of a wide variety of synthetic glycosyl diacyl and dialkyl glycerols differing in their headgroup stereochemistry, anomeric configuration, glycerol chirality and hydrocarbon chain structure, question the accuracy of the present explanations of lipid structural diversity. Although these hypotheses have value as a first approximation, they are based primarily on geometric arguments rather than physico-chemical relationships. The pattern of thermotropic phase behavior observed in aqueous dispersions of these glyco-glycerolipids can be interpreted in terms of subtle changes in the relative contributions of steric interactions, hydrogen-bonding interactions and hydration in the headgroup and interfacial regions, which in turn are counter-balanced by packing constraints in the hydrocarbon chain region. Various aspects of the molecular basis of this behaviour will be discussed.

(Supported by Medical Research Council of Canada and by the Alberta Heritage Foundation for Medical Research.)

M-PM-WS1-4

THERMODYNAMICS OF CARBOHYDRATE-PROTEIN INTERACTIONS.

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M-PM-WS1-5

THE HYDROPHILIC CHARACTER OF THE POLYAMPHIPHILIC SURFACES OF OLIGOSACCHARIDES RECOGNIZED BY LECTINS AND ANTIBODIES.

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The X-ray crystal structure of the complex of the Lewis-b tetrasaccharide with a lectin of *Griffonia simplicifolia* provided the insights required to establish a technique for the chemical mapping of the surfaces presented by oligosaccharides in forming complexes with proteins (Can. J. Chem. **70** (1992) 241). Four lectins have now been examined and, in all cases, the surfaces proved to be mosaics of short amphiphilic structural units which include a cluster of key hydroxyl groups. Since structural changes in regions of the oligosaccharide that, in the complex, come close but not in contact with the protein importantly influence the thermodynamic parameters, hydration participates in the molecular recognition. It is proposed that the hydration of a polyamphiphilic surface results in a layer of perturbed water molecules in the form of "flickering cavities" and that the association of these strongly hydrophilic surfaces at low concentrations in aqueous solution is driven by the reduction in perturbation that results on removing the polyamphiphilic surfaces from contact with the aqueous phase. The immunodominant surfaces of oligosaccharide antigenic determinants are similar in kind to those recognized by lectins.

M-PM-WS2-1

INCREASED EXPRESSION OF HEAT SHOCK GENES IN CELLS EXPOSED TO EM FIELDS. ((R. Goodman[1], A. Henderson[2] and M. Blank[3])) Columbia University Health Sciences, Departments of Pathology[1], Physiology and Cellular Biophysics[2], New York, NY 10032, and Hunter College-CUNY[3], Biological Sciences, New York, NY 10021

Specific transcripts are increased when cells are exposed to 15-120Hz EM fields (8μT-80μT). Experiments have used dipteran salivary gland cells, yeast and human HL-60 cells. Increased transcript levels were found for some oncogenes, as well as genes involved in metabolic activity, development and cell division. Recent data on protein synthetic patterns showed elevated levels of "heat shock" proteins in samples exposed to either EM fields or elevated temperature. Based on these observations, we have retested RNA from exposed and control samples, and measured increased transcript levels for the heat shock gene hsp70 in HL-60 cells and dipteran salivary gland cells, and the homologous gene SSA1 in yeast. In light of the over-expression of some heat shock genes, in the absence of elevated temperature, we conclude that EM fields appear to stimulate a natural pathway that is similar to the one used by cells in response to heat shock and other physical stresses. The response to EM fields occurs at a much lower energy density. (Supported by EPRI, DOE, ONR, and NIEHS).

M-PM-WS2-2

SPATIAL AND TEMPORAL COHERENCE AFFECTS THE RESPONSE OF BIOLOGICAL SYSTEMS TO ELECTROMAGNETIC FIELDS. ((Theodore A. Litovitz)) Physics Department, Catholic University of America, Washington, DC, 20064.

The association of biological effects with exposure to weak ELF electromagnetic fields (EMF's) remains controversial. Skepticism is due in large measure to theoretical arguments based upon signal-to-noise considerations. The signal-to-noise dilemna arises because cells, respond to exogenous EMF's 1000 times smaller than the local thermally generated noise EM fields. We propose that cells discriminate against local EM noise fields because they are *spatially incoherent*; a significant number of cell membrane receptors must be simultaneously and coherently activated to produce a bioeffect. We have already demonstrated that *temporal coherence* is necessary if bio-effects are to be observed (Litovitz, Mullins and Krause, 1991). We have hypothesized that if a spatially coherent but temporally incoherent random EM noise field were superimposed upon a coherent signal, then at some signal/noise value the observed EM induced bio-effects would be suppressed. This suppression has been observed in a number of models including, chick embryos, E. coli, human leukemia (HL60) cells, human lymphoma (Daudi) cells and breast cancer (MCF-7) cells.

M-PM-WS2-3

CA^{2+} TRANSIENTS AS A TRANSDUCTION SIGNAL FOR EXTREMELY LOW FREQUENCY ELECTRIC FIELDS

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The search for a transduction signal for low level electromagnetic fields in the extremely low frequency range has concentrated on cellular calcium fluxes and the dynamics of intracellular calcium levels. This has occurred as the energy level of these fields are so low it appears they would only be capable of perturbing a metastable system. Outside of the nervous system, tonic or phasic patterns of calcium transients are a conspicuous characteristic of many cell systems. We will review recent research addressing the sensitivity of calcium signalling to ELF fields and report on work we have undertaken on the spontaneous calcium transient activity in ensembles of growing rat osteosarcoma cells (ROS 17/2.8) during exposure to both ELF electric fields. In these cells, calcium transients lasting approximately five seconds and reaching concentration levels of 10-100 times basal (1-10 μM) can readily be recorded. Electric field exposure at 30 Hz, 500 μV/cm results in a significant decrease in transient activity occurring within 15 minutes. Full recovery of the cells occurs within 90 minutes following field removal.

This work is supported by the Electric Power Research Institute Grant #2965-21, and grant #ES06287 from the National Institute of Environmental Health Sciences, NIH.

M-PM-WS2-4

Na,K-ATPASE FUNCTION IN 60Hz ELECTROMAGNETIC FIELDS. ((M. Blank)) Department of Physiology and Cellular Biophysics, Columbia University, New York, NY 10032

The Na,K-ATPase is a model membrane protein for studying interactions with electromagnetic fields. These studies have also elucidated active transport mechanisms. Decreased ATP-splitting by low frequency electric fields appears due to increased binding of activating ions on the Na,K-ATPase surface (Blank, FASEB J 6:2434-2438, 1992). Activation by ions also accounts for the frequency dependence (Blank, J Electrochem Soc 134:1112-1117, 1987), as well as the difference between optimal frequencies for ion influx and efflux observed by Tsong et al (Bioelectrochem Bioenerg 21:319-331, 1989). Using an Electric Research & Management (Pittsburg) exposure system, we measured effects of low frequency magnetic fields between 0-300Hz and 0-300mG, and found increases in enzyme activity of 5 to 15%, with optima around 50mG and 60Hz. Inhibition by electric fields and stimulation by magnetic fields probably arise from different charge movements in the enzyme. Electric fields increase ion binding at the surface, and magnetic fields increase charge movements within the protein that coordinate the surfaces. The frequency dependence of electric (Blank & Soo, Bioelectromag 13:329-333, 1992) and magnetic field effects is similar, suggesting that the two charge movements are part of a closely coupled rate limiting process. (We thank EPRI for their support.)

IMMUNE FUNCTION

M-Pos1

THE INFLUENCE OF A FREE THIOL IN THE CDR REGION ON THE ASSEMBLY AND STABILITY OF HUMANIZED IgG4 ANTIBODIES.

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The assembly of an immunoglobulin G (IgG) molecule requires the proper formation of both intrachain and interchain disulfide bonds. In the case of IgG4 antibodies, it has been observed that the formation of inter-heavy chain disulfide bonds is incomplete, resulting in a heterogeneous population of whole (H_2L_2) and half (HL) antibody molecules. The CDR regions of the murine antibodies OKT®3 and OKT®4a have been used to generate humanized antibodies of the G1 and G4 isotype. The amino acid sequence of OKT®3 shows that there is a cysteine residue in the third loop of the heavy chain CDR region. Based on the structure of known antibodies, this cysteine is expected to exist as a free thiol in the native molecule. In this study, we present the biophysical characterization and comparison of a number of related humanized OKT®3 molecules containing mutations of the cysteine in the CDR loop, as well as residues in the hinge region and the CH2 domain. Quantitation of the disulfide bonds and free thiol groups indicates that in the native state, the free thiol is not solvent exposed, and the remaining cysteine residues are involved in disulfide bonds. The antibodies migrate as a single peak on gel filtration HPLC, and have comparable stabilities to guanidine denaturation. However, on non-reducing SDS polyacrylamide gels, these antibodies show HL populations ranging from 0 to 50%. The implications of these results on the assembly of antibody molecules will be discussed.

M-Pos2

A KNOWLEDGE-BASED APPROACH TO MODELING THREE-DIMENSIONAL STRUCTURES OF IMMUNOGLOBULIN FRAGMENTS.

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Immunoglobulin (Ig) amino acid sequences are highly conserved and often have sequence homology ranging from 70-95%. Antigen binding fragments (Fab) of more than 25 antibodies have been crystallized and display a high degree of structural similarity. Based on this observation, several homology modeling approaches have been recently developed for the prediction of Fab structures prior to their experimental determination. We have extracted features from existing Ig sequences and known structures to build an automated AntiBody structure GENeration (ABGEN) method for obtaining structural models of antibody fragments. The features we have used include invariant and strictly conserved residues, structural motifs of known Fab, canonical features of hypervariable loops, torsional constraints for residue replacements and key intra-residue interactions. In addition, molecular mechanics and dynamics methods have been used to further refine the ABGEN structures. We have explored the validity of our approach using a five-fold cross validation using the existing Ig fragment structures. Using ABGEN we generated the Fab structure of an anti-sweetener antibody prior to crystallographic refinement and the model structure has an RMSD of less than 2 Å when compared with the crystal structure. Supported by NIH/NIGMS GM46535.