Surface Reactive Peptides and Polymers

A symposium sponsored by the Division of Industrial and Engineering Chemistry of the American Chemical Society, ACS 197th NATIONAL MEETING

Dallas
April 12-13, 1989

Surface Reactive Peptides and Polymers

A symposium sponsored by the Division of Industrial and Engineering Chemistry of the American Chemical Society ACS

ORGANIZERS: C. Steven Sikes and A.P. Wheeler
The Mineralization Center
University of South Alabama
Mobile, Alabama 36688
(205) 460-6331

LOCATION:
Meetings: Dallas Convention Center

Host Hotel for Participants: Sheraton Dallas Hotel

DATE: April 12 and 13, 1989

COSPONSORS: The Coastal Research and Development Institute of the University of South Alabama
Mississippi-Alabama Sea Grant Consortium
South Carolina Sea Grant

The purpose of the symposium is to provide a forum within a major scientific society for researchers to discuss recent results concerning novel peptides and related materials having surface-reactive properties that may lead to useful applications. The 28 invited papers that comprise the symposium encompass topics that range from regulation of crystallization to regulation of growth cells through interaction of a peptide or other material with a solid surface. For example, on one extreme, there is a presentation of experiences with somatotropin, the growth substance scheduled for production in quantities of hundreds of millions of grams per year. On the other hand, there are discussions of amino-acid sequence information and activity measurements relating to proteins from biominerals that are powerful regulators of mineral formation. These proteins are currently available only in microgram to milligram amounts, but in turn may be commercialized in some form on a large scale.

As for the previous symposium, "Regulation of Mineralization", held at the ACS meeting in September of 1987, presenters come from academia and industry, which provides the potential for a further broadening of perspective. As before, a proceedings of the symposium is scheduled for publication following the meeting. Gratis copies of "Chemical Aspects of Regulation of Mineralization", the proceedings of the previous symposium, are available from the organizers.

There will be a reception in the Sheraton Dallas Hotel on Wednesday evening from 7 to 10 p.m. for more extended informal discussions between speakers, guests, and visitors. The Sheraton Hotel is located at the corner of Olive and Live Oak Streets, about 4½ blocks from the site of the symposium at the Dallas Convention Center.

PROGRAM

Wednesday, April 12, 1989

A.P. Wheeler, Presiding

7:55 - Opening Remarks. A.P. Wheeler.

8:00 - Phosphoproteins as Mediators of Biomineralization. Arthur Veis, Boris Sabsay, Chou Bing Wu.

8:30 - Peptides Enriched in Aspartate and Phosphoserine as Inhibitors of Calcium Carbonate and Phosphate Crystallization. C. Steven Sikes, M.L. Yeung.

9:00 - $CaCO_3$ Crystal-binding Properties of Polyanionic Proteins and Peptides. A.P. Wheeler, K.C. Low.

9:30 - Structural Relationship of Amelogenin Proteins to their Regulatory Function of Enamel Mineralization. T. Aoba, E.C. Moreno.

10:00- Coffee Break

10:30 - Factors Contributing to Dental Calculus Formation and Prevention. D.J. White, W.D. Bowman, A.C. Lanzalaco.

11:00 - Phosphorylated and Non-phosphorylated Carboxylic Acids: Influence of Group Substitution on Inhibition of Calcium Salt Crystallization. John D. Sallis, M. Brown, N.M. Parker.

11:30 - Inhibition of Ice Crystal Growth by Fish Antifreezes. James A. Raymond, Arthur L. DeVries.

Lunch

M. A. Crenshaw, Presiding.

1:30 - Mineral Induction by Immobilized Polyanionic Matrix Proteins. M.A. Crenshaw.

2:00 - Protein-crystal Interactions in Biomineralization. L. Addadi, A. Berman, J. Moradian-Oldak, S. Weiner.

2:30 - Crystal Engineering of Inorganic Materials at Organised Organic Surfaces. S. Mann, B.R. Heywood, S. Rajam.

3:00 - Role of Membranes in De Novo Biologic Calcification. Barbara D. Boyan.

3:30 - Coffee Break

4:00 - Purification and Characterization of a Shell Matrix Phosphoprotein from the American Oyster. K.W. Rusenko, J.E. Donachy, A.P. Wheeler.

4:30 - Organic Matrix of Calcium Concretions Isolated from the Gills of Freshwater Mussels. H. Silverman, J.M. Myers.

5:00 - Protein Polymers in Foraminiferal Shells. Lisa L. Robbins.

Thursday, April 13, 1989

C.S. Sikes, Presiding.

7:55 - Introductory Remarks. C. Steven Sikes.

8:00 - Synthesis of Bovine Somatotropin in E. coli. Gregg Bogosian, Clifton A. Baile, James F. Kane.

8:30 - Synthesis, Biological Activity and Conformation of Cyclic Growth Hormone Releasing Factor Analogs. A.M. Felix, E. Heimer, T.F. Mowles, D. Fry, V. Madison.

9:00 - A Tumor-secreted Protein Associated with Human Hypercalcemia of Malignancy: Biology and Molecular Biology. Michael Rosenblatt, Michael P. Caulfield, John E. Fisher, Noboru Horiuchi, Roberta L. McKee, Sevgi B. Rodan, Mark A. Thiede, David D. Thompson, Ruth E. Nutt, Mark E. Goldman, Thomas L. Clemens, Gideon A. Rodan.

9:30 - Potent, Nonpeptidal Cholecystokinin and Gastrin Receptor Antagonists. Roger M. Freidinger, Mark G. Bock, Robert M. DiPardo, Ben E. Evans, Kenneth E. Rittle, Daniel F. Veber, Raymond S.L. Chang, Victor J. Lotti.

10:00 - Coffee Break

10:30 - Lytic Peptides from the Skin Secretions of Xenopus laevis. Bradford W. Gibson.

11:00 - Peptide Antibiotics: Cecropins and Analogs. D. Wade, R. Bruce Merrifield, H.B. Boman.

11:30 - Synthesis of O-Phosphoserine-containing Peptides. J.W. Perich, R.B. Johns.

Lunch

J. Herbert Waite, Presiding

1:30 - Mechanistic Insights Concerning the Role of Polymers as Scale Inhibitors and Dispersants in Cooling Water Systems. Kenneth P. Fivizzani, John E. Hoots, R.W. Cloud.

2:00 - Effect of Polyelectrolytes on the Rate of Iron Oxide Growth at Heated Metal Surfaces. Claudia C. Pierce.

2:30 - Corrosion Inhibition by Thermal Polyaspartate. Brenda J. Little, C. Steven Sikes.

3:00 - Marine Metal-Chelating Proteins/Peptides. J. Herbert Waite.

3:30 - Coffee Break

4:00 - Silica Inhibition in Cooling Water Systems. Leonard Dubin.

4:30 - Macromolecular Assemblages in Controlled Biomineralisation. Carole C. Perry.

5:00 - Characteristics of the Organic Matrix-like Material Isolated from Frustules of the Freshwater Diatom Cyclotella meneghiniana. Debbie M. Swift, K.W. Rusenko, A.P. Wheeler.

Phosphoproteins as Mediators of Biomineralization

Arthur Veis, Boris Sabsay, and Chou Bing Wu. Division of Oral Biology, Northwestern University, Chicago, Illinois 60611.

Phosphoproteins may play an important role in directing the placement of the mineral crystals within and upon the organic matrix of naturally mineralized tissues such as bone and dentin. Acidic phosphoproteins may also regulate growth rate and limit the size of the formed crystals by surface adsorption. We are investigating these possibilities by examining the major phosphoprotein of dentin and some peptide analogs in the phosphorylated, dephosphorylated, and in vitro rephosphorylated states. Enzymatic methods have been used for the protein and chemical methods for the peptide analog. Neither approach provides for quantitative removal of the phosphates nor phosphorylation, but partial phosphorylation shows the profound effect of the phosphate groups on the calcium ion binding properties of the proteins and peptides. Supported by NIH Grant DE-01374 from NIDR.

Peptides Enriched in Aspartate and Phosphoserine as Inhibitors of Calcium Carbonate and Phosphate Crystallization

C. Steven Sikes and M.L. Yeung. Department of Biological Sciences, University of South Alabama, Mobile, Alabama 36688.

Peptide analogs of phosphoprotein regulators of mineralization were synthesized by solid phase methods. Serine residues were phosphorylated post-synthesis using phosphorus oxychloride, yielding 30 to 100% phosphorylation of serine, depending on the peptide. Attempts to phosphorylate peptides using diphenyl chlorophosphate were not successful, due mainly to problems associated with catalytic hydrogenolysis of the diphenyl groups attached to larger peptides. The peptides, H-(PSer-Asp)$_{10}$-OH, H-(PSer)$_{2-3}$(Asp)$_{20}$-OH, and PolyPSer-Ser (approx. 58 total residues) were all more effective inhibitors of calcium carbonate and calcium phosphate formation than their non-phosphorylated forms or equivalent polyaspartate molecules. The peptide H-(Gly-PSer-Asp)$_{10}$-OH was not an effective inhibitor. Simple polyaspartate molecules or polyaspartate molecules having a polyalanine terminus appeared to inhibit crystal nucleation and growth most effectively at a molecular size of 30 to 40 residues.

$CaCO_3$ Crystal-Binding Properties of Polyanionic Proteins and Peptides

A.P. Wheeler and K.C. Low. Departments of Biological Sciences, Clemson University, Clemson, South Carolina 29634 and University of South Alabama, Mobile, Alabama 36688.

Inhibition of calcitic $CaCO_3$ seed crystal growth and adsorption to the crystals by natural and synthetic polyanionic proteins and peptides labeled with C-14 were examined. In most cases one binding rate constant with a $t_{0.5}$ less than 1 minute and a second constant with a $t_{0.5}$ of hours were identified. An excellent correlation existed between inhibition of crystal growth and capacity of crystal binding by the proteins and peptides to the rapid binding site. This correlation suggests that the rapid binding occurs at discrete crystal growth sites. There was a direct relationship between percent inhibition and percent coverage of these sites, suggesting that the most effective inhibitors rapidly bound to most, if not all, of the growth sites at low doses. These inhibitors were a natural protein from calcitic oyster shell and synthetic peptides having primary structures similar to that of the protein.

Structural Relationship of Amelogenin Proteins to their Regulatory Function of Enamel Mineralization

T. Aoba and E.C. Moreno. Department of Physical Chemistry, Forsyth Dental Center, Boston, Massachusetts 02115.

We investigated the regulatory mechanism of enamel mineralization in porcine animal model. Observations in situ and in vitro suggest that the parent amelogenin (major matrix proteins secreted by the ameloblast) is selectively adsorbed onto apatite crystals, thereby inhibiting apatite crystal growth in supersaturated solutions having ionic composition similar to that of the liquid phase surrounding the enamel crystals in vivo. The adsorption affinity of amelogenin (and its inhibitory activity) is lost with cleavages of specific segments, suggesting that known enzymatic degradation of the secreted proteins plays a role in controlling kinetics of enamel mineralization. Studies using fragments of the amelogenin (and synthetic peptides) indicate that both the hydrophobic and hydrophilic sequences at the N- and C-termini, respectively, are essential for the proposed function of the amelogenin as a crystal growth regulator.

This work was supported by grants DE07623 and DE03187 from the National Institute of Dental Research.

Factors Contributing to Dental Calculus Formation and Prevention

D.J. White, W.D. Bowman and A.C. Lanzalaco. The Procter & Gamble Company, Cincinnati, Ohio 45241.

Low (LMW) and high molecular weight (HMW) salivary species have been suggested as nucleating templates or mineralization inhibitors of calculus formation. Similarly, various LMW and HMW species have been considered as topical anticalculus agents. In this study, the constant composition technique has been used to investigate the mineralization of HAP, OCP and DCPD minerals in the presence of pyrophosphate, diphosphonates, polyacrylic acid and glassy (poly) phosphate inhibitors respectively. Results show strong inhibitory activity for both LMW and HMW species, with monolayer coverage and maximal inhibitory reactivity in the concentration range from 1-100 ppm. When present at low levels in solution the HMW species tend to demonstrate greater inhibitory efficiency. However, pre-treatment of crystal phases elicits decreased relative inhibitory reactivity for some HMW species. In vivo studies corroborate reduced efficacy for some HMW agents, suggesting that the time needed for reactivity and the capacity to provide reservoir levels are limiting aspects in providing topical anticalculus benefits in situ. These factors may similarly describe the inability for natural salivary inhibitor agents to effectively reduce plaque mineralization on the teeth.

Phosphorylated and Non-Phosphorylated Carboxylic Acids: Influence of Group Substitution on Inhibition of Calcium Salt Crystallization

John D. Sallis, M. Brown and N.M. Parker. Department of Biochemistry, University of Tasmania, Hobart, Australia 7001.

Molecules with a strong negative charge are recognised inhibitors of hydroxyapatite (HA) crystallization. In particular, phosphocitrate with its charge, size and stereochemical character is very powerful. To gain insight into requirements necessary to inhibit, group substitution within a range of phosphorylated and non-phosphorylated carboxylic acids has been studied. A general strategy for the preparation of some phosphorylated compounds was to couple 1,2-phenylene phosphorochloridate to ester protected carboxylates followed by hydrogenation, base hydrolysis and ion exchange purification. For hydrocarbon chain lengthening or incorporation of a sulphate, amino or carboxyl group to the parent compound, more elaborate synthetic strategies were devised. The ability of commercial and newly synthesised products to prevent HA crystallization, and in some instances CaOx crystallization, was then compared to phosphocitrate. Data indicate that the group arrangement of the phosphocitrate molecule presents as the most ideal for preventing HA formation.

Inhibition of Ice Crystal Growth by Fish Antifreezes

James A. Raymond and Arthur L. DeVries. Institute of Marine Science, University of Alaska, Fairbanks, Alaska 99701 and Department of Physiology and Biophysics, University of Illinois, Urbana-Champaign, Illinois 61801.

Polar fishes avoid freezing by synthesizing antifreeze glycopeptides (AFGPs) or antifreeze peptides (AFPs). These antifreezes (AFs) do not affect the melting point (-1°C), but lower the freezing point (temperature of ice crystal growth) non-colligatively to -2.2° C. This thermal hysteresis results from adsorption of AF molecules to ice surface inhibiting ice crystal growth.

Studies using single ice crystals show that pure AFs bind mainly to the prism faces and completely inhibit their growth within the hysteresis gap. AFs neither bind to nor inhibit growth on the basal plane. Basal plane growth in the presence of the large AFGPs results in a surface made up of hexagonal pits 100 um deep, rotated 30° from the a-axes of the crystal. With all other AFs, basal plane growth proceeds in the c-axis direction until the single ice crystal becomes a symmetrical bipyrimidal hexagon (diamond shaped crystal).

Mineral Induction by Immobilized Polyanionic Matrix Proteins

M.A. Crenshaw. Dental Research Center, University of North Carolina, Chapel Hill, North Carolina 27599-7455.

Polyanionic proteins are thought to induce mineral formation in mineralized tissues. However, in solution, these proteins inhibit precipitation of mineral from spontaneously precipitating solutions. When we immobilized polyanionic matrix proteins by covalent attachment to insoluble substrates, they induced mineral formation from metastable solutions. The mineral induced was determined by the solution rather than by the protein. The polyanionic proteins from dentin (apatite) and molluscan shell (aragonite) induced apatite from metastable calcium phosphate solutions. Both proteins induced calcite from metastable calcium carbonate solutions. A calcium-binding capacity alone is not sufficient for a polyanionic protein to induce mineral formation because immobilized serum albumin did not induce mineral formation from either solution.

Protein-Crystal Interactions in Biomineralization

L. Addadi, A. Berman, J. Moradian-Oldak and S. Weiner. Departments of Structural Chemistry and Isotope Research, Weizmann Institute of Science, Rehovot 76100, Israel.

Acidic proteins and polysaccharides are used by organisms to control biomineralization by interacting with forming mineral. They can act as crystal nucleators or inhibitors, as well as modulators of crystal development in preferred directions. The specific type of interaction depends on structural and stereochemical rules, which we have investigated in vitro. Aspartic acid-rich, sulfated glycoproteins from mollusk shells act as nucleators of calcite from a specific crystal plane. We propose that nucleation involves cooperation between sulfates which concentrate calcium, and carboxylates which are rigidly and regularly arranged in planar beta-sheet domains. This hypothesis is supported by experiments on oriented calcite nucleation from sulfonated polystyrene films with adsorbed polyaspartate chains. Analogous acidic glycoproteins from sea urchin skeletons adsorb from solution onto specific crystal faces, and are subsequently incorporated within growing calcite crystals. The intercalation of organic material in the lattice dramatically modifies cleavage properties of the crystal. Comparison between the different systems yields information on the mechanism of biological crystal growth and for the construction of crystalline materials with controlled properties.

Crystal Engineering of Inorganic Materials at Organised Organic Surfaces

S. Mann, B.R. Heywood and S. Rajam. School of Chemistry, University of Bath, Bath BA2 7AY, United Kingdom.

The nucleation and growth of the mineral, $CaCO_3$, has been studied in inorganic and biological systems by high resolution transmission electron microscopy and electron diffraction. Recently, we have investigated the role of organised Langmuir monomolecular films on the crystallochemical specificity of $CaCO_3$ grown from supersaturated bicarbonate solutions. Negatively-charged monolayers of stearic acid induce oriented vaterite nucleation whereas only the thermodynamically stable polymorph, calcite, is formed in the absence of the organised organic surface. The influence of changes in packing density, headgroup polarity and stereochemistry will be reported.

Role of Membranes in De Novo Biologic Calcification

B.D. Boyan. Department of Orthopaedics, The University of Texas Health Science Center at San Antonio, Texas 78284.

Membranes and membrane components have been associated with initial formation of hydroxyapatite in bacteria and in tissues like cartilage and woven bone. Recent studies have shown that proteolipids present in the membranes may function as ion channels promoting import of calcium and phosphate ions to the initiation sites and export of protons from these sites. In addition, phosphatidylserine that is structured in the membrane phospholipid bilayer by these proteolipids, interacts with calcium and phosphate to form specific phospholipid:Ca:P_i complexes, which then support subsequent hydroxyapatite deposition. In cartilage and woven bone, these events occur in specialized extracellular organelles called matrix vesicles. The mineralization process is regulated in time and space by chondrocytes and osteoblasts, in part through changes in matrix vesicle membrane enzymes and lipid metabolism by hormones like $1,25(OH)_2D_3$.

Purification and Characterization of a Shell Matrix Phosphoprotein from the American Oyster

K.W. Rusenko, J.E. Donachy, and A.P. Wheeler. Department of Dermatology, University of North Carolina, Chapel Hill, North Carolina 27599 and Department of Biological Sciences, University of South Alabama, Mobile, Alabama 36688.

The major protein component contained in the shell matrix of the American oyster Crassostrea virginica was purified using size-exclusion and reverse phase HPLC chromatography. Nearly 90% of this acidic protein fraction is composed of aspartic acid, serine, and glycine. The majority of the serine residues were present as O-phosphoserine. A tentative carboxy-terminal sequence of four amino acids was determined and the presence of a non- polar/hydrophobic carboxy-terminal domain was implicated by carboxypeptidase digestion. The amino-terminal residue was apparently blocked by an unidentified residue. The results of mild acid hydrolysis indicated the presence of domains of polyaspartic acid and polyphosphoserine. Peptides generated by this analysis were subjected to sequencing by the Edman degradation. Polyclonal antibodies to this protein were tested for cross-reactivity with matrix proteins from other species and to various synthetic peptides with sequences proposed to exist in this purified protein.

Organic Matrix of Calcium Concretions Isolated from the Gills of Freshwater Mussels

H. Silverman and J.M. Myers. Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803.

Extracellular calcium concretions compose up to 50% of gill dry weight in some freshwater mussels. These concretions are composed of amorphous calcium phosphate associated with an organic matrix. The concretions are tightly regulated and mobilized only during brooding of embryos, serving as a source of calcium for embryo shell formation. The organic matrix of the concretions accounts for 25% of concretion weight. Analysis of this organic matrix indicates 6% by weight chloroform/methanol soluble material, and an array of proteins currently under investigation. Of note, we have identified a calmodulin-like calcium binding protein (17,000Da), and a 200,000Da band (SDS- PAGE) which covalently binds PO_4 in isolated gill preparations. While the 17,000Da protein is likely not involved in the mineralized structure following concretion formation, the 200,000Da band is likely to be an important component of the mineralized structure.

Protein Polymers in Foraminiferal Shells

Lisa L. Robbins and J.E. Donachy. Department of Geology, University of South Florida, 4202 E. Fowler Ave., Tampa, Florida 33620 and Department of Biological Sciences, University of South Alabama, Mobile, Alabama 36688.

Many of the matrix proteins associated with biomineralization of various calcitic shelled organisms appear to be analogous - often having similar compositional and chemical characteristics. However, little is still known about the specific structure, in vivo deployment, and function of the matrix proteins of most organisms. The foraminifera comprise a major group of calcium carbonate forming protozoans. Different classes of proteins in the planktonic foraminiferal shell have been analyzed for compositional as well as structural information. The interaction of these proteins appears to result in a polymeric network. Some proteins within the classes are relatively large and hydrophobic, having varying degrees of solubility, but having similar amino acid compositions. Compositional data suggest that these may be analogous to those found in elastic-like proteins. The soluble proteins may be transported to the site of deposition and then crosslinked into the matrix network. The hydrophobic proteins associate with the smaller hydrophilic proteins, and together they may act as nucleation sites. When in solution, however, the hydrophilic proteins can function to inhibit or control crystallization. The proteins may work in combination to provide a polymeric network having visco-elastic attributes of the developing organic chamber and a stimulation and/or inhibition of biomineralization as needed during chamber development and growth.

Synthesis of Bovine Somatotropin in E. coli

Gregg Bogosian, Clifton A. Baile and James F. Kane. Monsanto Company, Chesterfield Village Parkway, Chesterfield, Missouri 63198.

The market projections for the commercial use of bovine somatotropin (BST) is in the hundreds of millions of grams per year. When one considers that this protein has to be a highly purified, high quality, biologically active protein that is produced cheaply, the technological challenges are immense. Cloning the BST gene into E. coli was the easy part. The first major task was to achieve BST expression levels that made commercialization a reality. This goal was reached by developing high cell density fermentations of the E. coli host/vector to the point that 30% of the cellular protein was the product of interest. One problem that was encountered and solved was the incorporation of norleucine, an amino acid analog of methionine, into the protein. Secondly, the product had to be purified by "bucket chemistry" techniques to allow for the low cost large scale production. One major step toward this goal was the isolation of the product in the form of inclusion bodies. The fermentation of the E. coli host/vector and the characteristics of these proteinaceous aggregates are the subjects of this presentation.

Synthesis, Biological Activity and Conformation of Cyclic Growth Hormone Releasing Factor Analogs

A.M. Felix, E. Heimer, T.F. Mowles, D. Fry and V. Madison. Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.

Growth hormone-releasing factor, GRF(1-44)-NH_2, has been shown to stimulate growth hormone release in vitro and in vivo in humans and a variety of animal species. Structure-activity studies have shown that the shortened analog, GRF(1-29)-NH_2, retains the full intrinsic activity with only slightly reduced potency. Replacement analogs of GRF(1-29)-NH_2 have resulted in peptides with increased potency which are being used for application to domestic livestock (performance enhancement). Conformational analysis (molecular dynamics calculations based on NOE-derived distance constraints) have been carried out to correlate bioactivity with conformation. These studies have led to the design and synthesis of cyclic analogs of GRF(1-29)-NH_2 which retain the bioactive conformation. Novel methods for the solid phase synthesis of these cyclic peptides were developed and will also be described.

A Tumor-Secreted Protein Associated with Human Hypercalcemia of Malignancy: Biology and Molecular Biology

Michael Rosenblatt, Michael P. Caulfield, John E. Fisher, Noboru Horiuchi*, Roberta L. McKee, Sevgi B. Rodan, Mark A. Thiede, David D. Thompson, Ruth E. Nutt, Mark E. Goldman, Thomas L. Clemens*, and Gideon A. Rodan. Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486 and *Helen Hayes Hospital, West Haverstraw, New York 10993.

Tumors can produce the paraneoplastic syndrome of humoral hypercalcemia of malignancy (HHM). Recently, a peptide from human tumors was cloned: within the N-terminal 13 residues, there is homology between the tumor peptide (hHCF) and parathyroid hormone (PTH). A synthetic fragment, hHCF-(1–34)NH_2, has been prepared and evaluated. The peptide displays multiple actions similar to PTH and can produce the components of HHM. Like PTH, it increases levels of 1,25-dihydroxyvitamin D_3 and acts on bone. The finding that hHCF-(1–34)NH_2 is more potent than PTH is of considerable interest for the future design of hormone analogs. Also, the actions of hHCF-(1–34)NH_2 are inhibited by a PTH antagonist, demonstrating that hHCF-(1–34)NH_2 occupies PTH receptors. These studies establish a new mechanism-based approach using PTH antagonists for treatment of tumor-associated hypercalcemia.

Potent, Nonpeptidal Cholecystokinin and Gastrin Receptor Antagonists

Roger M. Freidinger, Mark G. Bock, Robert M. DiPardo, Ben E. Evans, Kenneth E. Rittle, Daniel F. Veber, Raymond S.L. Chang, and Victor J. Lotti, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486.

Cholecystokinin(CCK) and gastrin are important peptide neurohormones found in the gastrointestinal tract and the central nervous system. Their actions are mediated via membrane surface receptors. This presentation will describe the design and synthesis of nonpeptide antagonists with selectivity for different CCK or gastrin receptor subtypes and receptor affinity comparable to native ligands. The structural requirements of various antagonists for optimal receptor interaction will be compared. The CCK-A and CCK-B/gastrin selective antagonists L-364,718 and L-365,260, respectively, will be highlighted.

L-364,718 L-365,260

Lytic Peptides from the Skin Secretions of Xenopus laevis

Bradford W. Gibson. Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-0446.

Several novel classes of peptides have been isolated from the skin secretions of the South African frog, xenopus laevis. These peptides are between 21-27 amino acids in length and are expected to form alpha-helices in an anisotropic environment based on their amphipathic structures. Indeed, many of these peptides have been shown to possess a broad range of anti-microbial activities and may serve this function in vivo. The precursors to all these peptides have now been identified and they include the precursors to several known prohormones (xenopsin and caerulein), a novel neurohormone-like peptide called 'levitide', as well as two precursors that apparently encode only these cytolytic peptides (preproPGS and preproPGLa).

Peptide Antibiotics: Cecropins and Analogs

D. Wade[1], R.Bruce Merrifield[1], and H.G. Bowman[2]. [1]The Rockefeller University, New York, New York 10021-6399 and [2]University of Stockholm, Sweden.

The immune system of insects is composed of both cellular and cell-free components. Bacterial infection of the giant silkworm moth (Hyalophora cecropia) and other insects induces the synthesis of a family of peptides, the cecropins, which are strongly basic and exhibit potent antibacterial activity. The antibacterial spectrum of cecropins is broad and includes both Gram- positive and Gram-negative bacteria, whereas most eucaryotic cell types tested are resistant.

The amphipathic structure and mode of action of several cecropins have been examined with the aid of a series of synthetic analogs. Conformational analyses and the design of analogs were based upon Chou and Fasman predictive methods. Peptides were synthesized by solid-phase techniques, and purified by reverse phase high performance liquid chromatography. Secondary structures were studied by circular dichroism measurements. Studies with planar lipid bilayer membranes suggest that the antibacterial activity of cecropins is due to formation of large pores in bacterial cell membranes, and that the insensitivity of eucaryotic cells may be due to the presence of cholesterol in the plasma membrane.

Synthesis of O-Phosphoserine-containing Peptides

J.W. Perich and R.B. Johns. Department of Organic Chemistry, University of Melbourne, Parkville, 3052, Victoria, Australia.

Over the past seven years, our work has been aimed at the development of an efficient synthetic procedure for the synthesis of complex O-phosphoseryl-containing peptides. This presentation describes the synthesis of several casein-related phosphopeptides by (a) the use of novel phosphorotriester and phosphite-triester phosphorylation procedures for the synthesis of various Boc-Ser(PO_3R_2)-OH derivatives, (b) their use in peptide synthesis and (c) the efficient deprotection of Ser(PO_3R_2)-peptides.

Recently we have diversified in our studies and have examined the application of 'phosphite-triester' phosphorylation procedure to solid-phase systems (e.g., resin-bound peptides, etc.) and the biochemical properties of PSer-containing peptides.

Mechanistic Insights Concerning the Role of Polymers as Scale Inhibitors and Dispersants in Cooling Water Systems

Kenneth P. Fivizzani, John E. Hoots, and R.W. Cloud. Nalco Chemical Company, One Nalco Center, Naperville, Illinois 60566.

Water soluble polymers serve an important function in the treatment of industrial cooling water systems. Classical terms used to describe polymer activity are based on observations of macroscopic properties. A "scale inhibitor" is any material that limits formation of scaling salts, while the term "dispersant" describes the ability of a chemical to prevent particulate settling for an extended time period. Markedly similar performance behavior for scale inhibitors and dispersants has been observed in benchtop activity tests utilizing a variety of scaling ions. In these tests, the precipitation process involves initial formation of microparticles and subsequent agglomeration to produce large particulates. Effective polymers limit the agglomeration of smaller particles. Particle size distribution studies and scanning electron microscopy (SEM) have characterized the particulates formed in benchtop activity tests and provided experimental support for the proposed mechanism which encompasses both inhibition and dispersion.

Effect of Polyelectrolytes on the Rate of Iron Oxide Growth at Heated Metal Surfaces

Claudia C. Pierce. Nalco Chemical Company, One Nalco Center, Naperville, Illinois 60566.

A kinetic method is described for the study of iron oxide growth at heated metal surfaces. Several physical parameters (heat flux, surface roughness and specific surface area) affecting the rate of iron oxide deposition will be described. The influence of polyelectrolytes used as iron dispersing agents on the rate of iron oxide growth will be presented and discussed.

Corrosion Inhibition by Thermal Polyaspartate

Brenda J. Little[1] and C. Steven Sikes[2]. [1]Naval Ocean Research and Development Activity, NSTL, Mississippi 39529-5004 and [2]Department of Biological Sciences, University of South Alabama. Mobile, Alabama 36688.

Polyaspartic acid, prepared by thermal polymerization of L-aspartic acid at 190°C for 24 hours, was evaluated as a regulator of crystallization and as a corrosion inhibitor of mild steel exposed to a salt solution. The bulk product was formulated on a 1:1 weight basis with Na_2CO_3 and hydrolyzed at 55°C for 30 minutes to convert aspartimide to aspartate residues, resulting in a pale yellow aqueous stock solution of 1% polyaspartate, pH 9.0. This material gave a single, well-defined peak on gel permeation chromatography with an estimated molecular weight of 5000 daltons. Its UV spectrum and activity as a regulator of crystallization were very similar to those of polyaspartic acid molecules prepared by solid phase methods. A 0.01% solution of polyaspartic acid appeared to inhibit corrosion by forming a surface film that acted as a physical barrier to restrict diffusion, retarding both anodic and cathodic reactions.

Marine Metal-Chelating Proteins/Peptides

J. Herbert Waite. College of Marine Studies, University of Delaware, Lewes, Delaware 19958.

The marine bioadhesives of barnacles, oysters, mussels, reef-building polychaetes, etc., have long intrigued chemical engineers. In recent years, the structure and chemistry of a number of marine adhesive proteins have been elucidated. Although these are very diverse in their primary structures, they share basic isoelectric points and have high ($>10^{30}$) affinities for a variety of metals including Al, Fe, V, Ti, Zn, Cu, etc. This affinity is, at least in part, due to stable organometallic complexes formed between the metal and 1,2-benzenediol-functional groups represented by residues of 3,4- dihydroxyphenyl-L-alanine (DOPA) in the protein. Stable complexes are formed both with metal salts in solution and with insoluble metal oxides. Curiously, despite the large size of some of the adhesives (>100 kD), spectroscopic evidence suggests that they can form mono-, bis- and tris- complexes with Fe (III), for example. This reflects considerable flexibility in the peptide backbone. Future studies will attempt to characterize the mechanism of metal binding.

Silica Inhibition in Cooling Water Systems

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Amorphous silica (SiO_2) deposition is a significant problem in recirculating cooling water systems due to the low solubility of silica. Because silica deposits are difficult to remove, avoidance of the problem by minimizing cycles of water concentration is the common practice. In water short areas, this is a significant problem. A discussion of the chemistry of silica as it relates to cooling water systems will be presented. A brief description of the operation of a cooling tower will be included. Experimental laboratory work on the evaluation of chemical treatments as amorphorous silica inhibitors and the identification of active materials will be presented. Some process simulation and field data on the efficacy of an active silica inhibitor will be presented. A possible mechanism by which the inhibitor operates will be discussed.

Macromolecular Assemblages in Controlled Biomineralisation

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This lecture will address the relevance of membrane structures and organic matrices on the path of controlled mineralisation of amorphous (SiO_2) and crystalline ($SrSO_4$, $BaSO_4$) materials in biological organisms.

Characteristics of the Organic Matrix-Like Material Isolated from Frustules of the Freshwater Diatom Cyclotella Meneghiniana

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Frustules from the freshwater diatom Cyclotella meneghiniana were isolated by differential centrifugation. After cleaning, they were exposed to 1 M HF, 3 M NH_4F (pH 5) at 4°C to dissolve the silica. Soluble and insoluble materials were separated by centrifugation. The soluble fraction extracted from the frustule contained both protein and carbohydrate, but little or no phosphate. Amino acid analyses, indicated that glutamate, serine, and glycine comprised 62 mole % of the soluble protein. The protein in the insoluble material consisted largely of hydrophobic amino acids. Neither soluble or insoluble materials had any significant effect on silica polymerization in vitro assays developed to date. However, the soluble material inhibited $CaCO_3$ crystallization in a dose dependent fashion indicating the existence of components with structural similarity to matrix isolated from calcified biomineral. The insoluble material neither stimulated nor inhibited $CaCO_3$ crystal growth.

MASGP-89-018

This work is a result of research sponsored in part by NOAA, National Sea Grant College Program, U.S. Department of Commerce under Grant #NA89AA-D-SG016. The Mississippi-Alabama Sea Grant Consortium and the University of South Alabama are authorized to produce and distribute reprints for information and governmental purposes notwithstanding any copyright notation that may appear hereon.