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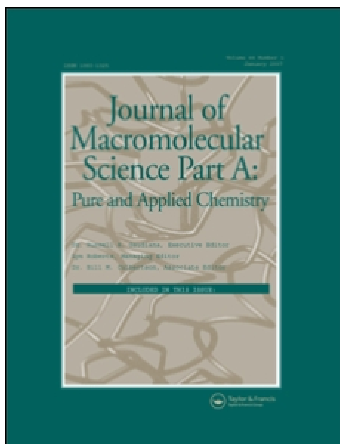

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Symposium on Polymer Grafts in Biochemistry

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SYMPOSIUM ON POLYMER GRAFTS IN BIOCHEMISTRY

PREFACE

This combined issue of the Journal of Macromolecular Science-Chemistry is the outgrowth of a symposium held at the American Chemical Society meeting in April 1974 in Los Angeles. That symposium, entitled "Polymer Grafts in Biochemistry," was sponsored by the Division of Polymer Chemistry of the ACS with Dr. Eugene P. Goldberg and me as co-chairman. Dr. Goldberg, Dr. A. H. Nishikawa, and I had participated in a research program at the Xerox Research Laboratories which attempted to merge biochemistry and polymer chemistry into a new technology. During the course of that research program it became apparent to us that a large fraction of polymer chemists were not aware of the rapid developments occurring at the interface between polymer science and biochemistry, and therefore Dr. Goldberg and I proposed this symposium to the Program Committee of the Polymer Division. Obviously the proposal was accepted and this symposium resulted.

The avowed purpose of the symposium and of these journal issues is to expose the mainstream of polymer chemists and non-biochemists as well to the significant recent advances in biochemistry resulting from the developments at the interface between polymer science and biochemistry and to indicate some exciting potential new areas where progress is likely to occur. The symposium was organized into two sections according to the two largest groupings of these recent advances: Section I, Affinity or Biospecific Chromatography; and Section II, Bioactive Materials.

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INTRODUCTION TO SECTION I-AFFINITY OR BIOSPECIFIC CHROMATOGRAPHY

The first group of polymer grafts which are active biologically is the affinity chromatography adsorbents. From a historical viewpoint the first application of affinity chromatography was in the purification of antibodies and antigens. These immunoadsorbents have been reviewed extensively [1] but only recently have these same principles been applied to the purification of other biological macromolecules and macroaggregates [2, 3]. The operating principle is quite simple. If a reversible equilibrium exists between two species A and B in solution,

$$A + B \rightleftarrows AB$$

then it is possible to covalently couple one member, A, of the equilibrium to a polymeric support in such a way that A, now called the ligand, may still participate in the equilibrium. If the equilibrium constant for association is large enough and sufficient time is allowed for equilibrium to be reached, then introduction of the polymer-bound ligand into a complex mixture containing B will result in the preferential adsorption of B to the polymer matrix. Washing of the matrix followed by dissociation of the A-B complex results in the isolation and purification of B. In actual practice the batch process described above and a column process in which the mixture is percolated through the polymer matrix are both used, with the latter being more common.

In such a process as that described above, the polymer-ligand adduct must possess unique properties, all of which offer significant challenge to the polymer chemist and biochemist. The polymer used for the support must permit rapid permeation and rapid approach to equilibrium for affinity chromatography to operate. Porath and co-workers [4, 5] and Cuatrecasas and Anfinsen [6] have defined the criteria for the ideal polymer support:

1) Insoluble
2) Mechanically resistant and rigid
3) Hydrophilic
4) Highly permeable to macromolecular substances

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5) Resistant to chemical and microbial attack
6) Free of nonspecific adsorption sites
7) Capable of chemical modification to permit covalent coupling of ligands for specific binding to the matrix.

Polyacrylamide, cellulose, porous glass, cross-linked dextran, and agarose have all been used successfully to date; however, agarose in bead form is by far the most commonly used support material. The perfect polymeric support is not yet available as recent studies demonstrating the creation of nonspecific binding centers on agarose during cyanogen bromide derivatization have shown. Cyanogen bromide activation of agarose, dextran, and cellulose is the most frequently used method of modifying the polymer so that the ligand may be attached, but bisoxiranes [7] and divinylsulfone [8] have also been used. The chemistry of coupling ligands to porous glass and to polyacrylamide is multifaceted and varied but will not be discussed in this volume.

As stated above, the ligand (A) must be attached to the polymeric support in such a fashion that it is able to participate unhindered in the equilibrium with B. This problem takes on two aspects: (1) difunctionality and (2) distance from the polymer support. It is obvious that in addition to whatever structural feature the ligand possesses which permits it to participate in the equilibrium with B, it must also possess a functional group which enables the ligand to be coupled to the polymer support. It is also important that these two functional properties must not interact to any great extent. The design and synthesis of ligand molecules which can be permanently coupled to the support and which are fully active vis-a-vis species B and the equilibrium are critical features of any affinity chromatography system. Cuatrecasas et al. [9] demonstrated the importance of placing a spacer or extension arm between the ligand and the polymer support in the affinity purification of some enzymes. Distances of 10 to 12 A were shown to be required for satisfactory binding of these enzymes. These spacer arms are mixed blessings and in some cases

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actually operate to the detriment of the separation as may be seen in some of the following articles. From all of these considerations it can readily be seen that the design and synthesis of these affinity chromatography matrices is a challenging problem to the chemist and is in need of new and improved ideas and materials.

Some brief comments concerning the papers in the first section follow.

Bioaffinity Chromatography-Methodology and Application by Jerker Porath begins with a short history of affinity chromatography and then turns to the chemistry of the support matrix, especially the agar and agar derivatives which Professor Porath has been so instrumental in developing to the current level of great utility. The chemistry of such promising new derivitization methods which eliminate some of the disadvantages of the cyanogen bromide method is also discussed.

Design Parameters in Affinity Chromatography by Nishikawa, Bailon, and Ramel addresses affinity chromatography from a theoretical point of view and then tests that theory with well-designed experiments. Numerous efforts have been made to develop a theory of affinity chromatography but no one has tested his theory with direct experiment as have these authors. This is a difficult subject since all theoretical approaches require the assumption of reversible equilibrium, and the abundance of non-specific binding effects always complicates experimental testing of the theory. Nishikawa et al. have provided here a useful framework for the analysis and design of new affinity chromatography systems.

General Ligand Affinity Chromatography in Enzyme Purification by Lee and Kaplan discusses the use of ligand which is general to a class of enzymes in the isolation and purification of that class of enzymes from a complex mixture and the separation of the enzymes within that class from each other. This work represents the beginning of an attempt by numerous workers to design and

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synthesize a set of general ligand affinity matrices which could potentially be used to purify all enzymes. The success of such a strategy would eliminate the need to devise a new ligand-polymer combination for each new enzyme separation desired.

Application of Immunoadsorbents for Isolation of Placental Alkaline Phosphatase, Carboxypeptidase G-1, and Serum Hepatitis Antigen by Charm and Wong concerns the use of antibody-polymer combinations (immunoadsorbents) for the purification of biologically active materials. Previously, the dissociative conditions for removing the biologically active material from the immunoadsorbent frequently resulted in the destruction of its activity, but in this work suitable dissociating conditions have been worked out to retain activity for two enzymes. The system for purification of hepatitis B associated antigen from human serum represents a potential large-scale, economically useful, affinity chromatography process if the antigen is the ineffective agent for hepatitis B. Evidence at this date is that it is not, however.

Affinity Chromatography and Characterization of an Acetylcholine Receptor from Torpedo Californica by Vandlen, Schmidt, and Raftery demonstrates the diversity of uses to which affinity chromatography has been put. The purification of the membrane receptor for acetylcholine also illustrates two problems not considered in enzyme affinity chromatography. First, the ligand is a charged species, and substitution of the ligand onto the support matrix renders it an ion-exchange resin. Second, the equilibrium constants for this class of ligands are sufficiently large that the substitution level of the ligand in the matrix becomes a sensitive parameter, requiring optimization of the substitution level in the matrix.

Hydrophobic Effects in Adsorptive Protein Immobilization by Hofstee presents a systematic and thorough study of the effects of hydrophobic forces in affinity chromatography and related techniques. These effects can represent undesireable side effects in affinity chromatography or they may be used to advantage when

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understood and desired. This study points out that the affinity chromatography world is not an ideal world and that the practitioner must be concerned with a multitude of factors:

In summary, these articles cover the diversity of current affinity chromatography applications and also deal with recent problems and progress in the technique. Major problems which require further effort or new approaches are:

1) Better supports and coupling chemistries to minimize or eliminate nonspecific binding
2) Better dissociation methods for immunosorbents in particular so as to retain biological activity
3) Further development and especially extensive experimental testing of theoretical concepts
4) More rigorous testing of affinity methods prior to publication to determine if they are indeed affinity and not nonspecific separations

This is a new and burgeoning field, one rich in success and with even greater promise. Continued development should enable all chemists to reap the rewards of this powerful concept.

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