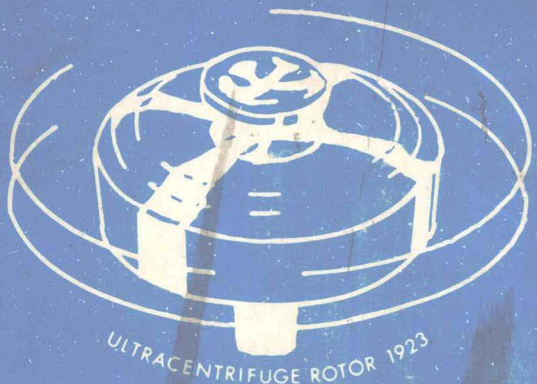
IUB

Ninth International Congress of Biochemistry

Stockholm 1-7 July 1973



ABSTRACT BOOK

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The Congress is organized by the Swedish National Committee on Biochemistry of the Royal Academy of Sciences and sponsored by the International Union of Biochemistry

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P 1 THE EVOLUTION OF ENZYMES. B.S. Hartley, M.R.C. Laboratory of Molecular Biology, Cambridge, England.

Specificity differences in serine proteinases have evolved by small changes in the binding site with negligible changes in tertiary structure. Will this hold for other enzyme families? Aminoacyl-tRNA synthetases are under immense selective pressure to conserve their specificity. They have different chain lengths, e.g. B. stearothermophilus Tyr-enzyme (2 x 40,000), E. coli Met-enzyme (2 x 90,000), yeast Leu-enzyme (1 x 120,000). They may nevertheless have diverged from a common ancestor since we find that the latter chains consist of two and three almost identical repeating sequences of 'intramers'. X-ray crystallography is in progress on each of the above enzymes. A more recent case of divergent evolution may be the enzymes responsible for episomal drug resistance. We are looking for sequence homologies between chloramphenicol transacetylase of E. coli R factors and a putative chromosomal ancestor in Proteus mirabilis.

How do such families evolve? Gene-multiplication gives one copy that could conserve the original activity and another that could rapidly mutate or recombine to produce new specificities. To test such hypotheses we need an experimental system for monitoring enzyme evolution. Klebsiella aerogenes lacks xylitol dehydrogenase (XDH). When grown on xylitol in a chemostat, the steady-state biomass is limited by the weak activity of ribitol dehydrogenase (RDH) for this substrate. Evolvants with higher XDH activity displace the ancestral organism from this ecological niche and increase the steady-state biomass. We have screened over 10^{15} organisms in this way and observed 32 such events spontaneously or after mutagenesis. In most cases the RDH content increases from 1% total soluble protein in the constitutive ancestor to over 25% in some evolvants. We have shown that this increase is frequently due to multiplication of the RDH gene. Three strains have increased XDH and decreased RDH activity. Studies are in progress to identify the sequence changes responsible.

P 2 INTERACTIONS AMONG CELLULAR MEMBRANES. George E. Palade, The Rockefeller University, New York, N.Y. U.S.A.

A wide variety of eukaryotic cells engage in intracellular transport and discharge of secretory proteins. These activities involve a series of compartments (endoplasmic reticulum, Golgi complex, secretion granules, lumen or tissue spaces) and depend on the interaction (fusion-fission) of the corresponding membranes. Morphological observations bearing on these interactions have been interpreted as indicating either: a) product transport or b) membrane transport from one compartment to the next in the series. In the second alternative, it is assumed that the membranes mentioned are biogenetically related, i.e. membrane is initially produced in the endoplasmic reticulum, transported to, and modified in, the Golgi complex to be finally inserted as plasmalemma in the cell surface. Recent advances in cell fractionation procedures, especially the isolation of satisfactory Golgi and plasmalemmal fractions from rat liver homogenates, indicate that the membranes in question are strikingly different in lipid composition, enzymatic activities, and protein composition as revealed by SDS gel electrophoresis. The differences are so marked as to exclude easily recognizable conversion. The findings lead to the conclusion that although these membranes fuse intermittently, there is no evidence of mixing of their lipid or protein components by lateral diffusion in the plane of the fused membranes or by random membrane removal from the receiving compartments. Moreover these recent findings suggest that the membrane interactions recorded in morphological observations reflect essentially product transport and that alternative procedures (assembly in situ?) may generally be involved in the biogenesis of cellular membranes.

P 3 ELECTRON TRANSFER AND ENERGY CONSERVATION. E.C. Slater, Laboratory of Biochemistry, University of Amsterdam, The Netherlands.

EPR measurements at very low temperatures (5-35°K) have revealed the presence of new iron-sulphur centres and unidentified non-haem iron centres in the proteins of high molecular weight (electron-transfer complexes) that constitute the mitochondrial respiratory chain. Spectrophotometric, potentiometric and EPR techniques have identified at least two and maybe four species of cytochrome b. A total of at least 20 different electron acceptors have been identified in mitochondrial preparations. Reconstitution, immunological and genetic evidence strongly favours a role of the mitochondrial ATPase (coupling factor-1) in the synthesis of ATP linked with electron transport. Like the electron-transfer complexes, the ATPase is an oligomeric protein containing 5 different polypeptide chains. A small protein (OSCP) is required for the binding of the ATPase to the mitochondrial membrane. Heart and liver mitochondria contain 1 molecule of ATPase, 1 molecule of QH_2-cytochrome c reductase (containing 1 antimycin-binding site) and 1 oligomycin-binding site (on the membrane in the region of the attachment of the ATPase), for every two molecules of cytochrome c oxidase. The ATPase is inhibited by a polypeptide (ATPase inhibitor) that is bound to the ATPase when electron transport is inhibited or uncoupled from phosphorylation. Coupled electron transport induces dissociation of the inhibitor, with concomitant increase in the fluorescence yield of aurovertin bound to the ATPase. Co-operative binding of antimycin to the electron-transfer chain and of aurovertin to the ATPase in mitochondrial preparations reveal an interaction between the binding sites. It is proposed that these interactions and those between polypeptide chains within single molecules of the electron-transfer complexes and the ATPase are involved in oxidative phosphorylation.

P 4 MOLECULAR PROBES FOR A VIRAL ETIOLOGY OF HUMAN CANCER. S. Spiegelman. Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

The central purpose will be to describe the use of the techniques of molecular biology to determine how much of the knowledge gained from animal viral oncology is applicable to the etiology and pathogenesis of human cancer. Evidence will be presented for the existence in human neoplasias of RNA molecules related in sequence to those found in RNA tumor viruses known to cause corresponding cancers in experimental animals. The diseases studied include human breast cancer, leukemias, lymphomas, and sarcomas. Further experiments demonstrated that the RNA molecules identified in these human neoplasias are viral in size and are associated in a particle with "reverse transcriptase," an enzyme found uniquely in the RNA tumor viruses.

Finally, we will describe experiments with human neoplastic cells that test the validity of the "virogene" theory, which proposes that every cell contains in its DNA a segment coding for a tumor virus. The data obtained suggest that this concept is not applicable to human leukemia.

1Sa 1 STABLE SEPHAROSE DERIVATIVES FOR AFFINITY CHROMATOGRAPHY OF PEPTIDES AND PROTEINS.

Meir Wilchek, Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel.

Ligands coupled monovalently to cyanogen bromide activated Sepharose suffer from a small but constant leakage. This phenomenon could limit the use of affinity chromatography for the isolation of very small amounts of proteins and would make the interaction between Sepharose bound hormones and intact cells questionable. Stable and high capacity agarose derivatives were prepared by coupling poly lysine or poly glutamic acid hydrazide to cyanogen bromide activated Sepharose. The high stability results from the multipoint attachment of these polymers to the solid matrix. The poly lysyl Sepharose was used for further ligand substitutions. The efficiency of the derivatives prepared from poly lysyl Sepharose was determined by the specific adsorption of different proteins. The poly glutamic acid hydrazide was also used for the covalent binding of periodate oxidized nucleotides. Affinity chromatography can also be used for the isolation of small ligands. Anti-DNP-antibody columns are being used for the specific isolation of peptides containing cysteine, histidine, methionine, tyrosine, tryptophan or glutamic acid to which a dinitrophenyl group has been covalently attached by various methods. Antibodies to other haptens such as arsanylic acid can also be used efficiently. The advantages of using the DNP group are (a) it has a high molar extinction and therefore is monitored easily and (b) anti-DNP-antibodies can be prepared easily. Of particular interest are modifications of methionine residues with α-bromoacetyl-ε-DNP lysine for the purpose of obtaining overlapping peptides to establish the positions of cyanogen bromide cleavage.

1Sa 2 ASPECTS ON BIOSPECIFIC ADSORPTION AND AFFINITY CHROMATOGRAPHY.

Jerker Porath, Inst. of Biochem., Uppsala, Sweden.

A discussion of the physico-chemical background for biospecific adsorption and affinity chromatography will be given. In particular, the association constants for the formation of adsorption complexes and the corresponding complexes in free solution will be compared as illustrated with lectin-carbohydrate interaction.
The potentialities and limitations of the specific adsorption methods will be discussed and possible improvements will be suggested.

1Sa 3 STUDIES OF PROTEIN INTERACTION BY SCANNING GEL CHROMATOGRAPHY.

Gary K. Ackers. Department of Biochemistry, University of Virginia, Charlottesville, Va. 22901. U.S.A.

Scanning gel chromatography is a useful technique for the study of interacting systems of macromolecules, including: (1) Interactions between protein subunits (2) Interactions between macromolecules and small ligand species (binding reactions). The gel partitioning and transport behavior of such systems permits determination of interaction parameters (stoichiometries and equilibrium constants) as well as molecular size parameters of the interacting species. Two types of experiment are generally useful: (1) Nonequilibrium transport experiments in which the migration properties of solute zones are analyzed. (2) Equilibrium saturation experiments in which a gel column is completely saturated with solution containing solute species of interest and scanned. This approach is especially useful for determination of binding ratios between macromolecular components and small ligand molecules in solution. The method is based on direct optical scanning of gel columns saturated with a solution containing both ligand and macromolecule.

A further application of scanning gel chromatography is the use of substrate-saturated columns to study migration of enzyme systems (active enzyme technique). Information regarding molecular size of the catalytically active form is readily obtained with small quantities of material. This work has been supported by USPHS Grant GM-14493.

1Sa 4 CHROMATOGRAPHY OF PROTEINS AND NUCLEIC ACIDS ON HYDROXYAPATITE COLUMNS.

Giorgio Bernardi
Laboratoire de Génétique Moléculaire,
Institut de Biologie Moléculaire, Paris 5°, France.

Some recent developments in the field will be presented.

1Sa 5 HYDROPHOBIC INTERACTION CHROMATOGRAPHY OF PROTEINS. Stellan Hjertén. Institute of Biochemistry, University of Uppsala, Uppsala, Sweden.
As different proteins often exhibit different degree of hydrophobicity it should in principle be possible to fractionate proteins by chromatography on columns of a hydrophobic nature. Different bed materials have therefore been examined and utilized for the separation of both "common" water-soluble proteins and hydrophobic membrane proteins. The chromatograms can be developed by increasing concentrations of organic solvents (for instance propanol) or detergents (for instance Tween 20 or sodium dodecyl sulfate).

1Sb 1 SEPARATION AND CHARACTERIZATION OF BIOLOGICAL STRUCTURES WITH THE APPLICATION OF FREE FLOW ELECTROPHORESIS. K. Hannig. Max-Planck-Institut für Biochemie, 8033 Martinsried bei München, Germany.
Transformation and differentiation processes are known to be accompanied by alterations of the cell membrane concerning the surface charge. The main surface constituents of different biological structures as cells, cell organelles or membranes possess different electrical charges. Consequently, variations of electrophoretic mobilities directly reflect the differences described. These facts have encouraged us to adapt our preparative electrophoretic separation technique "Continuous Free Flow Electrophoresis" to problems envisaged with complex biological structures. As important results, the time of exposure of the sample to the electrical field has been reduced to 3-5 min at a temperature of 5° C whereas the capacity of the apparatus increased to provide material sufficient for further analysis. It has been also shown that morphological structure and biological functions were fully maintained after the separation. Since our technique bases on a parameter other than used in classical separation methods it may be added to these methods in many cases or otherwise enable new approaches to certain biological or biochemical problems. As an example, a survey of possible applications of the technique on the separation of distinct immunocompetent cell populations (T- and B-lymphocytes), cell organelles and membrane components (mitochondrial membrane systems, plasma membranes) will be presented.