Meetings

Nucleic Acid and Protein Chemistry

"Recent advances in nucleic acid and protein chemistry" was an interdisciplinary AAAS symposium organized by a committee headed by W. M. Stanley, and held on 27 and 28 December, 1965, at Berkeley, California.

G. Stark (Stanford) reviewed briefly the approaches and methods used in determining the primary structure of proteins. Methods for the selective cleavage of peptide chains were discussed, but the major section of Stark's talk concerned stepwise degradation. The work of Edman in developing an automated amino acid sequencer was discussed, and the latest modifications were presented. All extractions are carried out in a single chamber under a nitrogen atmosphere. The reaction chamber is a rotating cylindrical cup in which the reaction mixture spreads out as a thin film. Effective extractions may then be made by introducing organic solvents at the bottom, the solvent thus being allowed to spread upward across the reaction mixture and to pass out the top of the cup. Drying is carried out in the same cup simply by applying a vacuum. Additions of reagents are controlled by electronic programs, and at each cycle of the degradation, the appropriate thiazoline is collected in a fraction collector for subsequent analysis. Edman has submitted 0.5 μmole of myoglobin to 40 to 50 cycles of degradation at the rate of 16 cy/day. The final portion of Stark's talk concerned his modification of the Edman degradation. Stark converts a cross-linked copolymer of styrene and divinylbenzene to a solid phenylisothiocyanate derivative. Peptide is coupled to the polymer in 15 minutes at room temperature with a solvent of pyridine, triethylamine, and water. The polymer is washed with benzene or methylene chloride, and then the peptide is released with anhydrous trifluoroacetic acid in about 15 minutes. The eluent is submitted to amino acid determination for the subtractive analysis of the end group. This method could easily be automated, and it is important to note the speed of the reaction relative to the usual technique. The most exciting advantage of the method, however, is that contaminants normally introduced by side reactions or incomplete reactions are eliminated, since the full analysis involves a twofold selection. Stark's studies, which are still preliminary, produce impressively clean analyses. However, with an octapeptide the yields were poor owing to incomplete coupling of peptide to polymer because the solvent necessary to dissolve the peptide does not permit good penetration of the hydrophobic polymer.

Daniel Koshland (University of California, Berkeley) discussed the role of enzyme flexibility in the subunit structure of such proteins. Beginning with the kinetic evidence which led to the deduction that the template theory could not explain enzyme specificity, he summarized the physical and chemical evidence supporting flexibility as a key property of enzymes. The idea that a substrate must induce an appropriate conformational change to activate the enzyme makes it possible to understand how small molecules which are not consumed themselves can accelerate or decelerate the action of an enzyme. Moreover, such accelerations and decelerations have analogs in biological systems involving drug action and hormonal controls. Because many enzymes are composed of subunits and because cooperative effects have been observed for many years without adequate explanation, models of flexible proteins have been designed in an attempt to explain these effects. In a typical case of a protein composed of four subunits, it was assumed that each subunit could exist in two different conformations. As each subunit undergoes a conformational change induced by substrate, its interactions with its neighboring subunits may vary. Variables such as the energy of the conformational change, the extent of the attraction between neighboring subunits, and the strength of binding of the substrate or inhibitor were analyzed with the aid of a computer. From the curves so generated simple nomograms could be devised which allow a "best fit" of a theoretical model to the experimental data by measuring the substrate concentration at three points, at 90, 50, and 10 percent saturation. With such nomograms it was possible to fit the data for the oxygen binding of hemoglobin accurately over the entire concentration range. Moreover, the models give indications of the type of subunit interactions which could account for such interesting shifts as the oxygen-binding curves in tadpole hemoglobin during metamorphosis. The tadpole hemoglobin becomes saturated at much lower oxygen tensions than the frog hemoglobin, an observation which correlates with oxygen needs of the biological systems. The models can explain a variety of the data obtained from observation on enzymes under metabolic control, but may also be used to explain the action of pheromones, the small molecules responsible for communicating information between insects.

A. Wilson (University of California, Berkeley) discussed the application of protein-structure studies to evolutionary and taxonomic problems. There are various methods for comparing the structures of enzymes that catalyze the same reaction in different species. Major consideration was given to the immunological method known as microcomplement fixation. The results discussed came mainly from the laboratories of L. Levine and N. O. Kaplan at Brandeis University, as well as of Wilson. The method is sensitive to small differences in protein structure and also appears capable of providing an approximate indication of structural resemblance between proteins from different species. Studies were conducted mainly with rabbit antiserums directed to purified, crystalline proteins that had been prepared from the domestic chicken. The proteins were H_4-lactate dehydrogenase, M_4-lactate dehydrogenase, triosephosphate dehydrogenase, aldolase, glutamic dehydrogenase, hemoglobin, and ovalbumin. Each antiserum was tested by microcomplement fixation against the homologous

chicken protein and against the corresponding protein of other species. The species chosen formed an approximate evolutionary series, ranging from the turkey, which is a close relative of the chicken, to the fishes, which are remote relatives of the chicken. The cross-reaction data led Wilson to suggest that the five enzymes and hemoglobin have changed their structure during evolution at rather similar rates but that ovalbumin is a more rapidly evolving protein. The data also suggest that enzyme evolution has proceeded more slowly in anatomically conservative fishes, such as the sturgeon group, than in fishes whose anatomy has undergone continuous, rapid change. After discussing rates of protein evolution, as indicated by the microcomplement fixation method, mention was made of the potential usefulness of enzymatic properties as taxonomic characters. Attempts to improve the classification of birds on this basis were briefly described.

A discussion of regulatory enzymes was presented by J. Gerhart (Universerity of California, Berkeley) who used aspartate transcarbamoylase as an example of this type of enzyme. Two characteristic features of the kinetics of such enzymes were illustrated. The first characteristic is a dual specificity of the enzyme. Aspartate transcarcarbamoylase specifically binds its substrates aspartate and carbamyl phosphate but does not bind their close analogs. The enzyme specifically binds a second class of metabolites, nucleotides, which do not structurally resemble the substrates and which do not participate in the enzymic reaction. This second class of compounds functions as regulators of activity and are called effectors. The second kinetic characteristic is that the binding of substrates or effectors or both is cooperative. This characteristic was discussed in terms of the model of Monod, Wyman, and Changeux which considers the enzyme in two conformational states, differing in affinity for the substrate. The equilibrium of these two states is displaced by substrate binding so that the molecular population is enriched in the high-affinity form. Effectors which act as inhibitors may then be thought of as binding preferentially to the low-affinity form. Both of these kinetic characteristics, dual specificity of binding and cooperative binding, are considered of central importance to regulatory enzymes and so were studied in terms of the structure of the enzyme. Aspartate transcarbamoylase is separated into two kinds of subunits when incubated with *p*-hydroxymercuribenzoate (*p*HMB), and the subunits can be dissociated by sedimentation or chromatography. One subunit (molecular weight 96,000) is called the catalytic subunit since it contains the catalytic activity. The other subunit (molecular weight 30,000) bears sites for binding effectors and is called the regulatory subunit since its presence was required in order to render the catalytic subunit sensitive to effectors. Apparently the native enzyme consists of two catalytic subunits combined with four regulatory subunits. When aspartate transcarbamoylase was reconstituted from subunits, aggregates closely resembling the native enzyme were formed quickly without any measurable amounts of intermediate aggregates. The conformation of the subunits seems to depend upon aggregation and upon the binding of substrates or effectors. For example, the catalytic subunit is (reversibly) inhibited as a result of aggregation. Furthermore, *p*HMB titrations of the enzyme suggested that the enzyme molecule was tautomeric between two states, one in which all the sulfhydryl groups were buried and one in which all of them were exposed. The reaction of *p*HMB with the enzyme was found to be eight to ten times faster in the presence of substrates than it was in their absence. Finally, the sedimentation velocity of the enzyme was decreased in the presence of substrate, an indication along with the other experiments just cited that the binding of the substrate produces a more open conformation of the enzyme.

H. H. Fudenberg (University of California, San Francisco) discussed work which indicated that small differences in primary structure might determine the antigenicity of antibodies. Human antibodies are heterogeneous in physical, chemical, and biological properties, and they can be divided into three major families of proteins. The predominant family is γG immunoglobulin, which consists of two light and two heavy polypeptide chains. Each set of chains bears genetically determined antigens analogous to the genetically determined ABO and Rh antigens on the red cell. The antigens of γG immunoglobulin are the "Gm" factors which are present in the heavy (H) chains, and the "Inv" factors which are on the light (L) chains. Peptide mapping has demonstrated that γ-globulins of different Gm types differ in primary structure. A single peptide present in Gm(a+) H-chains is lacking in the corresponding Gm (b+, f+ and f−) structural subunits; this difference, distinguishable serologically, presumably reflects the substitution of two amino acids due to the change of two base pairs in the genetic DNA. The single peptide differences observed may be responsible for the antigenicity (serologic reactivity) of Gm(+) proteins in standard test systems, and for the immunogenicity of Gm(+) proteins in Gm(−) individuals. Such immunogenicity has been documented; anti-Gm factors have been induced by deliberate immunization of Gm(−) individuals and also by fetal-maternal Gm incompatibility; and furthermore anti-Gm factors have been found in the serums of allergic individuals treated by injections of γ-globulin. In vivo, interaction between Gm and anti-Gm factors may produce significant clinical effects, as shown in one individual who was transfused with a large amount of Gm-incompatible plasma.

P. Hanawalt (Stanford) presented direct evidence for an intracellular quality control mechanism in certain strains of bacteria. The mechanism is capable of recognizing and repairing defects in the primary genome by a process involving the excision of the damaged single strand segment of DNA and reconstruction of this segment, utilizing the information in the complementary strand as template. The experimental method involves labeling of the replicating DNA in vivo with an analog of thymine, 5-bromouracil. Density distribution analysis of the extracted DNA in CsCl density gradients in the ultracentrifuge enables a distinction to be made between normal, semiconservative replication and the nonconservative mode of repair replication. Thus molecules which have undergone limited repair will have densities between those of hybrid and nonreplicated DNA. Nonconservative DNA replication was observed after treatment of the bacteria with ultraviolet light (the primary effect being dimerization of adjacent pyrimidines in DNA) or with the bifunctional alkylating agent, nitrogen mustard (which leads to cross-linking of guanine residues). Thus, the repair mechanism can handle a variety of defects in DNA, and Hanawalt suggested that recognition may, in fact, depend on the distortion of the phosphodiester backbone rather than on

specific base defects. The discovery that thymine starvation in bacteria also leads to nonconservative replication has led Hanawalt to suggest that the transcription of the DNA into messenger RNA introduces single-strand breaks which must be repaired. He pointed out that this might be the way in which the cell overcomes the steric problems involved in transcribing the message encoded in, for example, the complex bacterial chromosome. If this were the case, the repair replication mechanism would have a function in the normal growth cycle of the cell. The existence of such repair mechanisms in bacteria raises the question of whether similar mechanisms are available in the cells of higher organisms (for example, man).

I. Tinoco, Jr. (University of California, Berkeley) described the efforts of his group to learn something about structure, particularly base sequence, of nucleic acids from a study of their optical properties. The absorption and rotation of light as a function of wavelength was measured for mononucleosides, dinucleoside phosphates, trinucleoside diphosphates, and ribonucleic acids. The optical properties of the dinucleoside phosphates were most consistent with a conformation in which the two bases are stacked, one atop the other. This is particularly true for the dinucleosides not containing uracil. Comparison of the optical rotatory dispersion of trinucleoside diphosphates and dinucleoside phosphates indicated that the conformation of the trimer is very similar to that of its constituent dimers. Thus, the optical rotatory dispersion of the trimer of sequence ABC was similar to that calculated for (AB + BC − B) but bore little resemblance to the value calculated for (A + B + C). Good agreement between the observed optical rotatory dispersion for tobacco mosaic virus RNA (TMV-RNA) at low ionic strength and that calculated from the dimers (statistically estimated) was also found. However, in the presence of 0.15*M* NaCl the optical rotatory dispersion of TMV-RNA is quite different.

Tinoco concluded that the TMV-RNA at low salt concentration is approximately a single-strand helix of stacked bases. In the presence of salt, the TMV-RNA can fold to form loops held together by hydrogen bonds and other intramolecular forces. It was found that the optical rotatory dispersion, but not the absorption spectrum, is sensitive to sequence. The six pairs of sequence isomers in the dinucleoside phosphates of the four bases (adenine, cytosine, guanine, uracil) show distinct optical rotatory dispersion curves. Thus this method can be used to identify sequence isomers for dimers, trimers, and some tetramers without degradation. When the number of isomers is small, it should be possible to determine the amount of each component present in the mixture without separating the components. Because of the small amount of material required in this technique (10 to 20 μg, all recoverable) this appears to be a method of considerable merit.

D. Hogness (Stanford) in his discussion of the structure and function of the DNA from bacteriophage λ emphasized two particular aspects. The first was the direct measure of the position of various genes on this linear molecule which is about 15 μ long. The technique was to break the DNA into fragments by exposure to hydrodynamic shear gradients, to fractionate the fragments into different classes, and to determine what genes each class contains by a transformation-like assay devised by Kaisar and Hogness. The first fragments investigated, resulting from single, center-directed breaks of the whole molecules were loosely termed "halves," which in turn were fractionated into "left halves" and "right halves," so called because the genes they contain are located on the left and right halves of the genetic map of vegetative λ. Recent experiments have allowed Hogness and associates to specify the position of some genes within each half. The technique, as applied to the positioning of five genes in the right half, involved further fragmentation of the molecule, fractionation according to size by zone sedimentation, and measurement of the fractions for five different pairs of genes in which *R*, the gene at the right end of the map, is a member of each pair. In this way the relative size of the smallest fragment containing both genes of a given pair could be determined. The order of sizes is (*N* to *R*) > (*O* to *R*) > (*P* to *R*) > (*Q* to *R*) > (*R*), where *N*, *O*, *P*, *Q*, and *R* are genes in the right half. Since only those fragments which contain one of the ends of the whole molecules are active, the data indicated that the order of the genes in the right half of λ DNA is—*N-O-P-Q-R*-end, which is the order of the genes on the genetic map of vegetative λ. By estimating the sedimentation coefficient of these fragments and computing their sizes from these estimates, the number of base pairs separating each gene from the end was estimated, thus giving the position of each gene in the λ DNA. The order of the three genes of the galactose operon located on the left half of the λdg DNA (λdg is a variant of λ) was also determined by further fragmentation of left halves. The second aspect of λ DNA considered was the relative orientation of the genes. Orientation in this context means the direction of transcription along the DNA molecule, either from left to right or from right to left.

The question was asked whether this direction is different for different genes and, if so, what are the governing rules. The general method used was to determine which of the two strands of λ DNA is used as template during transcription of a given gene. One strand should be used if transcription is from left to right, and the other strand used if the direction is opposite. Results were described for one gene, *N*, the expression of which is necessary for normal replication of λ DNA. The specific technique was to separate and isolate the intact strands from both wild-type λ and an N^- mutant of λ. The four types of isolated single strands were used to form, separately, the two possible heteroduplex molecules in which one strand is wild-type and one strand is mutant. In these molecules there is a mismatch of bases at the site of the mutation. Under conditions where neither replication of these heteroduplex molecules nor repair of the mismatch of bases in the strand lacking the *N* function can take place, one would expect only one of the two heteroduplexes to be active, namely, that one which contains the wild-type base sequence in the strand which serves as template during transcription of the *N* gene. This was observed experimentally, and the template strand for gene *N* was thus determined. In these experiments repair processes were prevented by adding to the system an excess of ultraviolet irradiated foreign DNA which presumably bound all available repair enzymes.

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