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CHEMISTRY OF LIVING SYSTEMS

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SUBJECT AND OBJECTIVES:

The research in this project is directed toward certain aspects of the fundamental biochemical mechanisms of heredity and gene expression, their adaptation to environmental extremes, and their possible relationships to the origin and development of life.

This general area of molecular biology is undergoing a remarkable period of expansion and discovery, and by participating in this we hope to contribute to the advancement of the field and, simultaneously, to obtain information on specific properties of terrestrial life that enable living organisms to cope with adverse conditions. The latter subject is of much current interest; discussions are planned by NASA toward organizing a national or international symposium, "Environmental Extremes and Life". Hopefully, such research can lead to an improved understanding of the possibilities for the existence of life under extraterrestrial conditions.

Terrestrial life proceeds by the "DNA-RNA-protein" pathway and hence is limited to conditions under which the DNA-RNA protein system can operate. However, these conditions have not yet been defined fully although we know that life can exist under conditions of extreme cold (Skoultchi and Morowitz, 1964), moderate heat, high salinity, and high ultraviolet flux (Dean, et al., 1966). We have started a program to investigate the mechanisms by which life resists these environmental extremes. This program will include the following:

Mechanisms of Protection of DNA Molecules

1. Enzymatic repair of damage to DNA, studied by following the replication of DNA *in vivo* and in cell-free systems.
2. The anhydrous form of DNA in spores and its resistance to ultra-violet light.
3. The protein component of the bacterial chromosome.

Thermostable Proteins

1. The amino acid sequence and mode of action of the proteolytic enzyme thermolysin.
2. The composition and structure of thermostable ribosomes.
3. The properties of heat-stable RNA polymerase and amino-acid-activating enzymes from B. stearothermophilus.

Concurrent with these studies are investigations of corresponding mesophilic systems from organisms, such as E. coli and B. subtilis.

The general studies of the Chemistry of Living Systems include the following.

Properties of RNA Polymerase

This enzyme is prepared from Azotobacter vinelandii and other bacterial sources. It is universally present in cells. It transcribes the genetic message in DNA into various forms of RNA, bringing about the first step in phenotypic expression. The enzyme uses one of two strands of DNA as a template and produces strands of complementary RNA of antiparallel sequence and of varying strand lengths that are used in the cell for translation of the genetic message. The RNA chain can be initiated with ATP, and the triphosphate group is retained at the starting point of the RNA molecule. It has been shown by this means that RNA polymerase preferentially binds to pyrimidine groups on DNA.

The Sequence of Bases in RNA

The problem of determining base sequences in the nucleic acids is fundamental to "reading the genes" directly in contrast to observing their action by indirect means. Among the difficulties are the tremendous length of the sequences and the problem of purifying the molecules. Tobacco mosaic virus RNA can be purified in comparatively large quantities; it contains about 7,000 bases and it is being studied by combinations of enzymatic degradation and the chemical tagging of end groups. The base composition of purified oligonucleotides is being analyzed by the use of the Cary 15 spectrophotometer coupled with a Datex analog-to-digital converter.

Replication of the Chromosome in B. subtilis

Recently, it has been found that an "initiator" protein is necessary for chromosome duplication and that this protein is used but once. In the absence of replication, an independent process exists for maintaining the intactness of the chromosome. A DNA-protein complex has been isolated from B. subtilis. The protein in the complex differs from histones. DNA replication is a necessary condition for the isolation of the complex. Its transforming activity is greater than that of DNA. The DNA molecule in spores has been studied with respect to ultra-violet damage, and has been found by this criterion to differ from DNA in vegetative cells. This may have bearing on the effect of environmental conditions on the replication of DNA.

Relationships Between RNA and Ribosomes

It has been found that ribosomes move along the mRNA strand during protein synthesis. This was demonstrated by showing that the composition of the segment of the messenger that initially attaches the ribosome differs from the segment that is attached to the ribosome after incubation with an amino-acid-incorporating system. By similar procedures evidence has been found that the direction of travel of the ribosome during protein synthesis is from the 5'-OH end of mRNA toward the 3'-OH end, i.e. from left to right. The formation and release of polypeptides in cell-free amino-acid-incorporating systems has been studied with synthetic polyribonucleotides. The results indicate that chain release is favored by 2A, 1U, or 1A 1U 1G triplets. Release of polypeptide chains did not take place unless both U and A were present in the polynucleotides. Studies with Bacillus stearothermophilus are in progress with respect to the effect of temperature on ribosomal stability, ribosomal composition, and the nature of thermostable RNA polymerase and amino-acid activating enzymes.

Metabolic Regulation in E. coli.

Thymine metabolism has been studied in E. coli B_3, a mutant that requires thymine for growth. Revertants of this strain show changes in thymine metabolism as compared with the original wild strain of E. coli B; the results indicate the existence of two pathways for the

formation of thymine compounds. It has been found that a protein is produced rapidly after infection of E. coli with T-even bacteriophages, and that the protein may function to initiate the synthesis of viral DNA.

Studies of the Primary Structure of Proteins and the Relation Between Enzyme Function and Primary Structure

Two proteins are currently under study: thermolysin, a heat-stable protease from the thermophilic organism B. thermoproteolyticus Rokko; and, in collaboration with Dr. D. I. Arnon, spinach ferredoxin. Thermolysin has been shown to attack preferentially the peptide bonds at the amino sites of leucine and isoleucine in cytochrome c, insulin and tobacco mosaic virus protein. To a somewhat lesser degree, valine and phenylalanine bonds were attacked also. Spinach ferredoxin has a composition differing markedly from those of the bacterial ferredoxins.

Optical Rotatory Dispersion, X-Ray Scattering and Birefringence of DNA and RNA

The optical rotatory dispersion curves of bacteriophages fall into three general types; the differences are thought to mirror differing conformational characteristics of the DNA inside the phage, and are suited for studies of this aspect of phage structure.

MECHANISM OF INITIATION AND TERMINATION OF PEPTIDE CHAIN SYNTHESIS - Nucleotide sequences near the 5'-end of messenger RNA

(M. Takanami. T. Okamoto, S. Goolsby, J. Levitt)

Translation of the genetic message has been shown to take place from the 5'- to 3'-end of RNA sequences; therefore, it is reasonable to assume that the 5'-end of messenger RNA contains important information for the initiation of protein synthesis. Accordingly, an attempt was made to analyze the 5'-terminal nucleotide sequence of bacteriophage f2 RNA, as described in previous progress reports.

Since the 5'-terminus of f2 RNA was found to be phosphorylated with a tri-phosphoryl group, f2 RNA was treated with alkaline phosphatase and then re-phosphorylated with ^{32}P using polynucleotide kinase. Following hydrolysis with alkali and RNases, the radioactive fragments produced were analyzed.

The results of this experiment showed considerable heterogeneity of the 5'-terminus; pApUp was the main fragment among the many radioactive fragments produced. Two interpretations are possible for such a heterogeneity: the enzymatic treatment, especially that of dephosphorylation, resulted in some degradation of molecules near the 5'-end; or, the original f2-RNA preparation contained a certain amount of damaged molecules. The latter view follows from the fact that usually the f2-phage preparation contained a considerable amount of dead (uninfectious) particles.

Ribosomal RNA has no messenger-RNA function in cell-free systems, and it is of interest to compare the 5'-terminus of ribosomal RNA with that of f2 RNA. For this purpose, ribosomal RNA was prepared with particular attention to the ratio of 16s and 23s components and separation of their individual components. Since ribosomal RNA was also found to be phosphorylated at the 5'-terminus, the 16s and 23s components were treated with alkaline phosphatase. These RNAs were phosphorylated with ^{32}P. Following hydrolysis with alkali and RNases, the resulting radioactive fragments were analyzed. In contrast to the case of f2 RNA, a clear-cut result was obtained. The 23s RNA gave pGpPupPyp as a main fragment; in contrast, the 16s RNA produced pApApApypGp predominantly. In both cases, the recovery of these fragments was about 60-70% of the phosphorylated RNAs. However, the balance of the radioactivity was found to be distributed in a number of other fragments. Therefore, we concluded that the main components of 16s and 23s ribosomal RNAs are initiated by the nucleotide sequences stated above.

We have continued to study the special properties of thermophylic bacteria using B. stearothermophilus as a model system. The mechanism of protein synthesis in this organism has been reported to be stable at 50-60°, but the system makes "errors" at 75°. The reaction that takes place between amino acid and sRNA in this organism has been examined by studying cell-free systems prepared from it. Charging of sRNAs with amino acids by activating enzymes is uninhibited by an unidentified substance present in the extracts. The substance was not found to be present in E. coli; it was destroyed by incubation with dilute sodium hydroxide, followed by treatment with alkaline phosphatase. The

inhibitory factor appears to consist principally of organic-phosphate compounds. The substance completely inhibited the attachment of phenylalanine and valine to sRNA, but it was ineffective against proline and glutamic acid.

STUDIES OF DNA REPLICATION IN BACILLUS SUBTILIS

(H. Yoshikawa, M. Haas, B. Benjamin, E. Cook, J. Quivey)

DNA Replication by Partially-Broken Protoplasts of B. subtilis

Osmotically-broken protoplasts (OP) of B. subtilis incorporated ^{3}H-dATP into DNA in the presence of three other deoxyribonucleoside-triphosphates. The main features of this system were as follows:

1. DNA-polymerase activity was bound to a particulate fraction and was released by dialysis against 10^{-2}M EDTA.
2. OP, prepared from spores germinating in the presence of chloramphenicol, did not incorporate deoxyribonucleoside-triphosphates until they were treated with pancreatic DNase for a short time.
3. OP, prepared from spores germinating in complete medium or in thymineless medium using a thymine-requiring mutant, were active in DNA synthesis in vitro.
4. The kinetics of the activity of untreated OP were distinctly different from those of DNase-treated OP or purified enzyme system.

5-bromo-dUTP was used in place of TTP to distinguish the in vitro products from primer DNA by density difference. The in vitro products of OP, prepared from spores germinating in complete medium and thymineless medium were compared. The density of DNA with 5-bromo-dUTP incorporated was analyzed by CsCl density-gradient centrifugation before and after mechanical shear. The mean density varied with the condition of the cells from which OP was prepared. The longer the incubation under thymine-starved conditions, the heavier was the density of the in vitro product. The smallest difference of the density between 5-bromo-dUTP-incorporated DNA and primer DNA was obtained

by OP prepared from exponentially-growing cells. The maximum molecular weight of the in vitro product was 4×10^6. Part of adenine-16 marker (10 - 15%) activity was found in the heavy DNA fraction, and no other marker activities were detected.

From these results we have concluded that during thymineless germination of thymine-requiring mutant spores, initiation and unwinding of the chromosome took place, and such a partially-unwound structure was maintained in broken protoplasts and was engaged in DNA replication. Analysis of 5-bromo-deoxyuridine uptake by spores that were germinating in the absence of thymine support this conclusion.

Inhibition of Growth of B. subtilis by Amino Acids

Among twenty natural amino acids, L-serine, L-threonine and L-cysteine showed marked inhibition of the growth of B. subtilis under the following conditions:

1. when stationary cell culture was diluted in fresh medium;
2. when an amino-acid requiring mutant was starved for the required amino acid; and
3. during an early stage of germination.

In all cases synthesis of new proteins were required for the onset of DNA replication and cell growth. A careful study of DNA and protein synthesis during germination show that these three amino acids primarily inhibited protein synthesis.

We have isolated mutants to these three amino acids. A comparison will be made of the pattern of protein synthesis between wild-type and mutants during the early stage of germination.

SEQUENTIAL STUDIES OF SPINACH FERREDOXIN

(H. Matsubara, R. Sasaki, D. Ouye, J. Nakao; In collaboration with the Department of Cell Physiology)

1. Five large fragments were found in a tryptic digest of AEC-Ferredoxin. Two fragments seem to be the overlapping peptides of others. One peptide, which was identified as the carboxyl-terminal sequence, had 20 residues and the sequence of this was completely identified.

If this sequence is compared to the amino-terminal sequence (5 or 6 residues), there is a striking similarity between them except in one position (Lys⇆Pro exchange). This suggests the repetition of similar gene units in the "protein synthesis factory" of spinach ferredoxin. The identification of another large fragment, which has the sole tryptophan residue, was partially completed (17 out of 25 residues). Also, this has a very interesting sequence in terms of repetition, although it is more complicated than the other. The identification of the sequence of the largest fragment, which has 32 or 33 residues, has not yet been approached. For these studies thermolysin was used effectively to obtain subunits of larger peptides and proved to be a valuable tool. To combine large fragments and also to get the sequence in these fragments, thermolysin digestion of oxidized ferredoxin was carried out and after fractionation on an anion exchanger, many smaller fragments were obtained. The sequential study of these is under way.

2. Recent studies suggest that the mass spectroscopic study of a peptide is very useful in establishing the sequence of peptides by using a computer system. To test the feasibility of this approach, peptides from ferredoxin with known sequences will be given to Dr. Burlingame's group at the Space Sciences Laboratory.

OPTICAL PROPERTIES OF VIRUSES

(M. F. Maestre, K. Sieux, W. Dickinson, A. Hong)

Optical rotatory dispersion (ORD) measurements of the following bacteriophages were obtained: T2, T4, T6, T2(gt) and T6(gt), T5, T7, B3, α, lambdas C, K and clear mutants, ØX-174, f2, MS2, R17 and their nucleic acids and protein envelopes or ghosts. The main results of the investigations are the following:

1. each virus shows a characteristic ORD spectrum;
2. the ORD spectra is not a simple addition of the rotational spectra of the protein envelope of the viruses plus the rotation of the nucleic acids; and
3. a perturbation or distortion of the state of the nucleic acid molecule is indicated as a possible explanation of the optical properties of the nucleic acid in the interior of the virus.

ACTION OF VARIOUS MUTAGENS ON TMV AND TMV-RNA

(B. Singer, H. Fraenkel-Conrat; In collaboration with the Department of Molecular Biology)

Hydroxylamine, which has been studied repeatedly in terms of its chemical and biological effects on viruses and nucleic acids, has been compared with two mutagens not applied previously to RNA. Methoxyamine (CH_3ONH_2) inactivated TMV-RNA slowly compared to hydroxylamine, but proved much more mutagenic than the latter at pH 6 and 9. The only chemical effect after prolonged reaction with methoxyamine was a loss of cytidylic acid, detectable only at pH 6.

Nitrosoguanidine ($CH_3N(NO)C(NHNO_2)$), which has been used widely as a mutagen for DNA-containing viruses and bacteria, inactivates TMV-RNA similarly at pH 4 and 7, with decreasing rates due to the instability of the reagent. It produces weak mutagenic effects. With extensive treatment up to 30% of the guanine could be destroyed.

Intact TMV was inactivated at similarly slow rates by all three reagents. In contrast to its ineffectiveness when acting on the RNA, nitrosoguanidine at pH 5-7 proved to be the most potent mutagen yet found for the intact virus and for the RNA isolated from the intact virus. No base changes were found in the RNA isolated from TMV treated with any of these reagents, but the fact that inactivation under practical conditions generally did not exceed 90% probably accounts for this fact.

THYMINE METABOLISM IN *E. COLI*

(H. Kammen, E. Turner, M. Strand)

1. Evidence from several laboratories has suggested that *E. coli* B_3, a strain that is defective for thymidylate synthetase, may possess an additional mutation that affects the utilization of thymine. We have identified a second mutation in this strain — these cells lack the enzyme, deoxyribose-5'-phosphate (dR-5-P) aldolase, which probably functions in the degradation of deoxyribose derivatives. This lesion may be involved in the accumulation of deoxyribose compounds and the induction of thymidine phosphorylase observed previously in this and related organisms.

We are attempting also to purify and examine a related enzyme, phosphodeoxyribomutase, which converts deoxyribose-1'-phosphate to deoxyribose-5'-phosphate. Evidence for the presence of this enzyme was described first fifteen years ago, but it has not been characterized further.

2. Biochemical screening of 19 prototrophic revertants of E. coli B_3 has been completed. All of these strains are defective for the dR-5-P aldolase (less than 2% of the activity of E. coli B). These strains exhibit a continuous induction of thymidine phosphorylase during growth in the absence of thymine, similar to that found with thymine-requiring strains. The revertants fall into two chief groups. One group (8 revertants) shows a significant restoration of thymidylate synthetase (up to 25% of the activity found in E. coli B). This group exhibits more restricted utilization of exogenous thymine and more limited induction of the phosphorylase than the remaining revertants, which possess 1% or less of the normal thymidylate synthetase activity.

3. The revertant strains, which contain little or no detectable thymidylate synthetase, nevertheless grow at normal rates in the absence of thymine and contain thymidylic acid in their DNA. We have examined the possibility that the formation and deamination of 5-methyl cytosine derivatives might provide an alternative pathway for the production of thymine. The thymine requirement for growth of E. coli B_3 can be replaced by 5-methyl deoxycytidine, but only after an initial lag period. Extracts of E. coli B, B_3, and the revertants readily deaminate 5-methyl deoxycytidine, but these extracts are inactive for 5-methyl cytosine or 5-methyl dCMP. All efforts to demonstrate the enzymatic methylation of deoxycytidine have been unsuccessful thus far.

STUDIES OF THE REGULATION OF VIRAL DNA SYNTHESIS
(H. Kammen, M. Strand)

A number of electrophoretic and chromatographic procedures have been tested for the resolution of radioactively-labeled proteins formed after T_4 phage infection of E. coli. The most promising method has been that of Levinthal, et al., which employs acrylamide gel electrophoresis, and radioautography of gel slices. We hope to apply these methods for the detection of proteins involved in the regulation of viral DNA synthesis.

AZOTOBACTER VINELANDII RNA POLYMERASE — Studies on the Template Binding Site

(J. Krakow, W. Horsely, M. Karstadt, M. Fine, R. Siegel)

We have shown previously that congo red inhibits in vitro RNA synthesis by binding to the RNA polymerase and not to the DNA template. Further studies on the mechanism of the congo red inhibition showed that the dye blocked the binding of sRNA to polymerase. This indicated that the template site in the enzyme was the region to which congo red bound. The A. vinelandii polymerase is stabilized against heat denaturation by DNA. When congo red is added to the enzyme before DNA and the mixture is heated, the enzyme behaves as though DNA had not been added. This is in keeping with the contention that congo red blocks template binding. The congo red enzyme complex shows no difference in heat stability than does the free enzyme. The dye-polymerase complex can be dissociated readily by the addition of bovine serum albumin.

The ability of congo red to interfere with template binding has been used to study the affinity of polymerase for the DNA. The concentration of congo red necessary to inhibit polymerase is greater if the dye is added after the enzyme-DNA complex has formed. The dye concentration sufficient to result in a 90% inhibition of the enzyme-DNA complex gives less than a 50% inhibition of enzyme activity if added 5 minutes after RNA synthesis has begun. These results show that the binding of the enzyme at the initiator sites in the DNA is less stable than enzyme bound at other sites in DNA during the course of synthesis of RNA.

THE DETERMINATION OF BASE SEQUENCES OF TMV-RNA

(S. Mandeles, F. Fearney, M. Larsen, M. Kamio, F. Orcutt)

Sequence of Bases at the 5'-Linked End of TMV-RNA

The vicinal OH groups on the terminal ribose of TMV-RNA were oxidized with periodate to the dialdehyde derivative. This was reacted with semicarbazide-C^{14} to form the bis-semicarbazone-G^{14} to form the bis-semicarbazone-G^{14} of TMV-RNA (TMV-RNA-SC^{14}). The modified nucleic acid was partially hydrolyzed with T_1 RNase and the oligomers were separated according to chain length on a column of DEAE-Sephadex.

The elution was carried out in the presence of 7M urea. Each of the fractions was tested for radioactivity. It was found that fractions 1, 4, and 6 were radioactive. The relative amounts of radioactivity in each peak indicate that guanine (G) is in the 4th, 7th, and 9th positions in from the 5'-linked end of TMV-RNA. Work is continuing on the identification of the intervening bases.

Sequence of Bases at the 3'-Linked End

Polynucleotide kinase was used to catalyze the incorporation of P^{32} into the free 5'-OH at the 3'-linked end of TMV-RNA. When this P^{32}-TMV-RNA was hydrolyzed in alkali and the hydrolysate chromatographed on Dowex-1, radioactivity was found only in the position corresponding to pAp. This confirms the work of others that adenosine is the terminal base at the 3'-linked end of TMV-RNA, and also shows that ATP^{32} and polynucleotide kinase can provide the second "handle" required for our general method of base sequence determination.

FUTURE PLANS

Mutation Accompanying Transformation

Spontaneous histidine-requiring mutants differ from those isolated from indole transformants. Most of the his mutants of the indole transformants are linked to the indole marker, while spontaneous ones were not liked to the indole. We will continue to isolate these his mutants to extend this finding.

We also will study the nature of mutation, since our finding of a base-substitution type of mutation accompanying transformation differs fron the results obtained during meiosis of yeast and phage recombination.

DNA-Protein Complex and Interaction Between DNA and Protein

The chemical nature of the protein complexed with DNA in B. subtilis and its mode of binding to DNA molecules will be studied. Preliminary results with a chelating agent suggested involvement of metals in formation of the complex. The influence of the protein-DNA interaction on the biological activity of DNA will be studied by the in vitro DNA-polymerase reaction and the transforming activity of DNA.

In vitro DNA Replication

DNA synthesis by partially-broken protoplasts of B. subtilis incorporating 5-bromodeoxyuridine 5'-triphosphate, indicated that both replication and repair reaction took place in this system. Furthermore, replication is limited to only a small segment of DNA. We will continue to analyze these two reactions in vitro to study the difference in the mechanism between replication and repair synthesis. In vitro transformation will be studied by the same system.

Protein Synthesis During Early Stage in Germination

A new protein, which induces initiation of chromosome replication, is synthesized during the first step of germination of B. subtilis spores. Studies on identification and isolation of this protein will be made by using acrylamide gel fractionation techniques and by use of appropriate mutants.

Sequential Study of Spinach Ferredoxin

The sequence of spinach ferredoxin will be studied with the aid of another enzyme, such as chymotrypsin.

It is important to clarify whether methionine is truly part of ferredoxin or a contaminant. After completion of the sequence, a comparison will be made between spinach and bacterial ferredoxins.

Clostridia strains do not carry out in photosynthesis. Presumably, therefore, ferredoxins in photosynthetic organisms such as spinach, must have more complicated functions. The function-structure relationship may be approached by comparing the sequences.

The mass-spectroscopic method for sequential studies of peptides will be explored.

Enzymatic and Chemical Modification of Spinach Ferredoxin

Carboxypeptidase A cleaves only 3 residues from C-terminal of spinach ferredoxin. The activity will be examined after such cleavage. It will be necessary to determine the best conditions for this effect, because of the lability of ferredoxin under light at higher temperatures. This technique can be applied also to the reduced form of ferredoxin.

Mutation Accompanying Transformation

There are indications that the DNA in the heads of bacteriophage is packaged in an anhydrous form. We plan to investigate the mechanism by which water is removed from the head of the bacteriophage that exists in an aqueous medium. This may have a bearing on the adaptation of life to environmental extremes, such as dehydration.

Future Studies with the A. vinelandii RNA Polymerase

To gain further insight into the nature of the template binding site the enzyme will be treated with various reagents known to specifically block particular amino acid residues in proteins. The treated enzyme will be tested for its ability to synthesize RNA and also for its ability to bind ^{3}H-tRNA (which binds to the template site). The enzyme will be subjected to limited treatment with trypsin and other proteolytic enzymes to see whether the fragments are able to bind tRNA. Various procedures will be used to attempt to dissociate polymerase into its constituent subunits. These will be tested for template binding ability with the prospect of finding a template-binding subunit. This enzyme and its subunits will be studied by gel disc electrophoresis in order to resolve the various proteins thus formed.

Determination of Base Sequences in Nucleic Acids

Our research plan covers three closely-related areas: basic sequence experiments, mapping experiments, and formation of subunits.

Basic sequence experiments. We have been implementing a method that does not depend on the determination of overlapping sequences or on step-wise degradation. Briefly, the process involves the chemical modification of one end of the molecule, followed by partial enzymatic degradation and separation according to chain legnth of the modified polymers. A second reagent or "handle" is attached to the exposed end of the individual modified fractions; these double-handled fractions are degraded enzymatically to completion, and the sequence of the oligomer attached to the second handle is determined. In this manner, the original nucleotide sequence can be reassembled in terms of sequential oligomeric fragments. The features of this general method include: construction of long sequences from shorter fragments, unequivocal

positioning of fragments, independence from quantitative procedures, determination of sequences from both ends simultaneously, applicability to DNA and RNA. The work in progress indicates that a suitable handle for the 3'-OH end of TMV-RNA is C^{14}-semicarbazide. Similarly, for the 5'-OH end, P^{32}, incorporated with the aid of polynucleotide kinase, fits the requirement for the second handle.

Mapping experiments. Based on certain chromatographic and base composition data, there are strong indications that TMV-RNA contains sequences of 15 or more bases in which guanine is excluded. This means that hydrolyses of TMV-RNA with T_1-RNase will produce one or more long oligomers of unique sequence — that is, these oligomers occur only once in the whole molecule. These long unique oligomers can be used in mapping in that their location along the TMV-RNA can be determined approximately. The rationale for these experiments depends on the observation that sodium dodecylsulfate (SDS) removes the protein coat from TMV-RNA starting only at the 5'-linked end 3'-OH end of the molecule. At any time before the completion of the stripping, one should ordinarily observe a distribution of TMV particles ranging from completely stripped to completely covered, depending on the time and temperature of exposure to SDS. These particles can be sedimented, after removal of the exposed nucleic acid "tails" by ribonuclease, to reveal this distribution, and fractionated. If the original particles were uniformly labeled with P^{32}, each of the fractions will be labeled with P^{32}. Each fraction can be diluted with non-isotopic, intact TMV, the RNA isolated, hydrolyzed with T_1 RNAse, chromatographed and the unique long oligomer isolated and examined for radioactivity. If the long oligomer naturally occurs at the end of the molecule last affected by the SDS, it should appear as a radioactive peak in the RNA from almost all of the sedimented fractions. If, however, it occurs near the point where SDS stripping begins, then it should be radioactive only from fractions containing the longest virus particles. In this fashion, the position of a unique, long oligomer in TMV-RNA can be determined, depending only on the precision of fractionation.

Formation of sub-units. The rationale for work in this area is as follows: oligomers of known sequence and containing at least one cytosine are allowed to complex with complementary sequences in TMV-RNA at low temperatures. The TMV-RNA oligomer complex is reacted with a reagent that is specific for guanine, such as trinitrobenzene sulfonic acid (TNBS), thus modifying the guanine residues not participating in the complex with the oligomer. The oligomer is melted off and the TNBS modified TMV-RNA is cleaved with T_1 RNAse. Presumably, T_1 RNAse will act only on those guanine sites that were protected from reaction with TNBS by the presence of the complexing oligomer. In this way, an oligomer such as CpCpCpCpCp would be expected to complex with its complement of five Gs, and since runs of five Gs are comparatively rare, the TMV-RNA could be cleaved into a small number of subunits.

Studies of Ribosomal Structure

It has been found that the units of ribosomes may be broken down into a form that may be reconstituted. This step makes it possible to study the active subfractions of ribosomes with respect o to protein composition, etc. These studies may cast light on how transfer RNA molecules become attached to ribosomes.

The evolution of enzymes will be studied by examining the subunits of p-hydroxybenzoate oxidase from bacteria. We wish to test a theory that new enzymes may originate from alterations in single subunits of 6 existing enzymes.

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