crystalline; but it is free from crystalline isotactic polypropylene.

The nature of the catalytic complexes, which are stereospecific in the polymerization of propylene to syndiotactic polymer, will be discussed in another paper.

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THE LABELLING OF PHOSPHOMONOESTER END GROUPS IN AMINO ACID ACCEPTOR RIBONUCLEIC ACIDS AND ITS USE IN THE DETERMINATION OF NUCLEOTIDE SEQUENCES

Sir:

To date the sequence shown in partial representation I[1] is known to be common for the presumed twenty different amino acid-acceptor ribonucleic acids.[2] While some progress has been made[3] in the determination of sequences in the vicinity of the CpCpA terminus, nothing is known about the sequences at the opposite terminus except that by alkaline hydrolysis guanosine-2′(3′),5′-diphosphate (pGp) has been identified.[4] In this communication we report on the specific conversion of the terminal phosphomonester groups in the amino acid acceptor ribonucleic acids to the C^{14}-labelled phosphoroanilidates (I→II) and on the information which we have already obtained concerning nucleotide sequences nearest to that end.

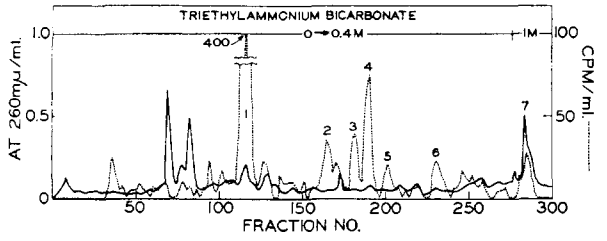
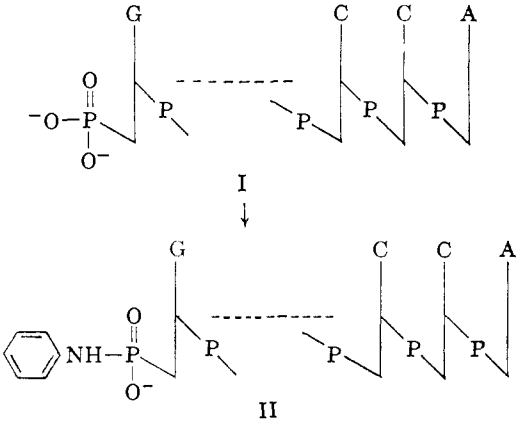


Fig. 1.—Chromatography of labelled oligonucleotides (approximately 270 optical density units at 260 mμ) on a DEAE-cellulose (carbonate) column (1 cm. × 50 cm.): elution was with a linear gradient 0 → 0.4 *M* triethylammonium bicarbonate (*p*H 7.5) with 2 l. in each vessel; rate of flow was 0.5 ml./min., ten ml. fractions being collected; one ml. of each tube was plated and radioactivity measured in a thin end window gas flow counter.



Labelling experiments were carried out with either yeast amino acid acceptor ribonucleic acid itself or with the oligonucleotide mixture[5] obtained by pancreatic ribonuclease digestion of the ribonucleic acid followed by removal of mono- and dinucleotides[5] by column chromatography. Since the results were similar, only the experiments on oligonucleotide mixture are herein summarized. Approximately 500 optical density (260 mμ) units of the mixture as triethylamine salt were treated in a mixture of water (0.3 ml.), dimethylformamide (0.6 ml.) and *tert*-butyl alcohol (0.6 ml.) with diisopropylcarbodiimide (0.12 ml.) and aniline (0.075 ml., containing 0.1 mC. of C^{14}-label) at room temperature. The pH was maintained at 8 by means of a pH stat which continually added hydrochloric acid to neutralize the guanidine (III) formed. After one day, about 80% of the total end groups were converted to the phosphoroanilidates, the 3′-phosphate end groups being converted simultaneously to 2′,3′-cyclic phosphate groups. After work-up, the total product was digested with spleen phosphodiesterase to enrich the oligonucleotides containing the 5′-phosphoroanilidate groups.[6] The resistant oligonucleotide mixture was freed from the resulting mononucleotides by column chromatography. The recovery at this stage was about 20% of the total material subjected to the action of spleen phosphodiesterase. This oligonucleotide mixture was again digested with pancreatic ribonuclease to open the 2′,3′-cyclic phosphates and the resulting mixture gave after chromatography the elution pattern shown in Fig. 1.[7]

Alkaline hydrolysis of radioactive peaks 1–7 showed that most of the radioactivity was released in compounds of the type IV (R = purine). The technique is, therefore, specific in providing a handle at the 5′-phosphomonoester ends of the polynucleotide chains and the interference from any random incorporation of label is not serious. The bulk of the radioactivity in the peak 1 is associated with the compound V which has been isolated pure and characterized. Peaks 2–6 and the peak immediately following peak 2 apparently contain mixtures of trinucleotides of the general structure VI and

(1) For the system of diagrammatic representations and abbreviations for polynucleotides see H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, New York, N. Y., 1961, Chapter 5.

(2) *Cf.* P. C. Zamecnik, "Harvey Lectures," LIV, 256 (1958–1959).

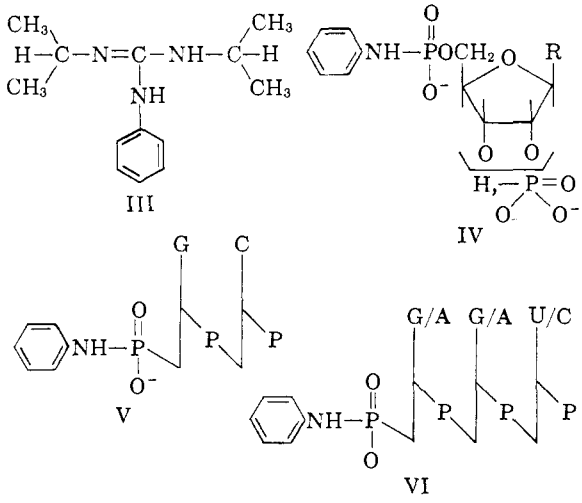
(3) U. Lagerkvist, P. Berg, M. Dieckmann and F. W. Platt, *Fed. Proc.*, **20**, 363 (1961).

(4) M. F. Singer and G. L. Cantoni, *Biochim. Biophys. Acta*, **39**, 182 (1960); W. Zillig, D. Schachtschabel and W. Krone, *Z. Physiol. Chem.*, **318**, 100 (1960).

(5) By analysis, this contained all the oligonucleotides containing pG and pA end groups that were present in the original ribonucleic acid. However, there was identified free pUp in the dinucleotide fraction discarded.

(6) Oligonucleotides released by ribonuclease from the interior of the ribonucleic acid chains would contain free 5′-hydroxyl end groups and should be degraded by the spleen phosphodiesterase. The oligonucleotides bearing 5′-phosphate or 5′-phosphoroanilidate groups would be resistant (for references, see) H. G. Khorana, in "Phosphodiesterases," "The Enzymes," Vol. V., P. D. Boyer, H. A. Lardy and K. Myrback, eds., Academic Press, New York, N. Y., 1961, p. 79).

(7) The presence of the two large peaks of nucleotide material essentially free from radioactivity shows that the digestion with spleen phosphodiesterase was not complete.



III IV V VI

the 1M fraction (peak 7) probably higher homologs. Thus after alkaline hydrolysis, the guanosine compound IV (R = guanine) has been isolated from peaks 2, 3, 4, 6 and 7. The peak immediately after 2 and probably the peaks 4 and 5 as well gave the corresponding adenosine compound IV (R = adenine). The results show that at least two of the amino acid acceptor ribonucleic acid chains end in pA groups. The most significant results of this investigation have, therefore, been (1) that while predominantly the ribonucleic acid chains end in pG groups, a few probably end in pA and one or more also in pU[5] and (2) that there is hardly any common sequence after the nucleotide containing the phosphomonoester end group.

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OBSERVATION OF NH_4^+ IN THE DECOMPOSITION OF AMMONIUM ON PLATINUM

Sir:

Recent studies of catalyzed reactions in a mass spectrometer[1] have shown that this instrument is a powerful tool for the investigation of primary processes. With this instrument, direct observations may be made within a time of about one microsecond after formation of transient species such as positive and negative ions and free radicals evolving from the surface of the catalyst.

We have now utilized the techniques previously discussed[1] to investigate the decomposition of ammonia on platinum in our high pressure research mass spectrometer. *The following observations were made with the hot platinum catalyst as the only source of ionization; consequently, the charged species observed must have evolved from the hot surface of the catalyst.* Ions appearing at mass 18 and believed to be NH_4^+ were observed as displayed in Fig. 1A. In order to confirm the identity of this unknown species, D_2 was added to the system and an additional species was observed at mass 19,

(1) Charles E. Melton, *J. Chem. Phys.*, **35**, 1751 (1961).

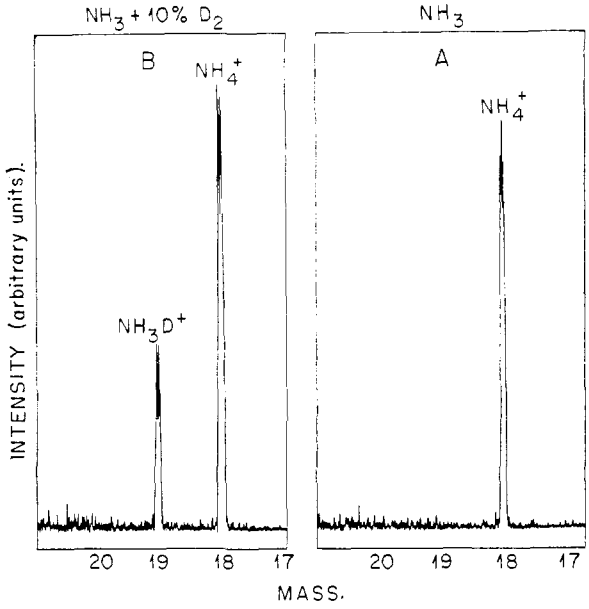

Fig. 1.—Ions released from a platinum catalyst in the catalytic decomposition of ammonia: the partial pressure of ammonia was 100 microns in both A and B and the temperature of the catalyst was 1200°. In B, 10% of deuterium was added to the system. No ionizing electrons were utilized in the production of these charged species.

NH_3D^+, as shown in Fig. 1B. Note that when D_2 was added to the system the NH_4^+ ion intensity remained essentially constant suggesting that the NH_3D^+ ion is formed on the catalytic surface by an independent reaction between NH_3 and D_2. Although the data in Figs. 1A and 1B were taken at a temperature of 1200°, the NH_4^+ ion was observed evolving from the catalyst at temperatures as low as 500°.

There appear to be two possible reactions of neutral species which would yield the NH_4^+ ion. One would be the reaction of an NH_3 molecule with another NH_3 molecule on the catalytic surface. If this were the source of NH_4^+, then the addition of D_2 to the system at constant NH_3 pressure (Fig. 1B) would not significantly change the total ion intensity. Since the data in Fig. 1B clearly show that more ions are formed when D_2 is added, we rule out this reaction. The second and much more likely source of NH_4^+ is the reaction of an ammonia molecule with a hydrogen molecule formed in the decomposition reaction (10 to 15% decomposition). Should this indeed be the reaction, then the addition of a small amount of D_2 to the system as is illustrated in Fig. 1B would not greatly change the rate of NH_3 decomposition and hence the H_2 concentration; therefore, the NH_4^+ ion intensity should not change greatly. This is in accord with the data. Furthermore, the D_2 would provide another neutral reactant to give rise to ions. As a further experimental test, we measured the NH_4^+ concentration as a function of H_2 pressure over a tenfold range with all other variables held constant. We found that the NH_4^+ ion intensity increased as a linear function of the H_2 pressure. Identical results were obtained for the concentration of NH_3D^+ when D_2 was substi-