dependence in the stoichiometry of the norcamphor–Eu(FOD)$_3$ complex; this dependence may well be associated with the presence of dimeric solution species; and if the effect is at all general, it indicates that any attempts to deduce molecular conformation from Δ_B values using lanthanide shift reagents should be approached with extreme caution pending establishment of stoichiometry of the complex involved.

(9) F. J. Nicholson Scholar; H. R. MacMillan Family Fellowship, 1971–1972.
(10) Alfred P. Sloan Foundation Research Fellow, 1971–1973.

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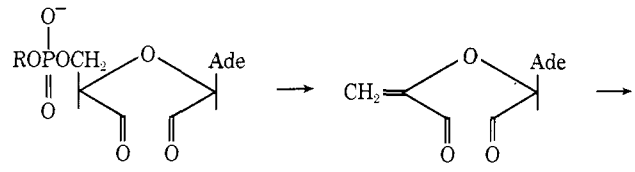
The Sequence Analysis of Polyribonucleotides by Stepwise Chemical Degradation. A Method for the Introduction of Radioactive Label into Nucleoside Fragments after Cleavage

Sir:

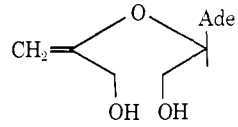
In 1953 Whitfeld and Markham[1] and Brown, *et al.*,[2] suggested that a method for the sequence analysis of polyribonucleotides might be perfected by studying the successive chemical removal of nucleotides from their 3′ terminals. The procedure would involve (i) the periodate oxidation of the terminal cis glycol group of the polynucleotide chain, (ii) the removal of the terminal nucleoside by a base-catalyzed β-elimination reaction, and (iii) the removal of the terminal phosphate group so formed by phosphatase, leaving the polynucleotide chain in a condition suitable for a second cycle of degradation. Since that time the method has been studied in many laboratories and, in all cases, primary amines have been used to catalyze the elimination reaction and, in most cases, the reaction has been carried out in the presence of the excess periodate carried over from the oxidation step. Under these conditions the product arising from the oxidized terminal nucleoside is the purine or pyrimidine base.

In earlier studies on a method for RNA sequence analysis carried out in this laboratory,[3] primary amines in the presence of excess periodate were also used for the elimination step. More recently, however, in the adaptation of these procedures to an automated solid support system[4] for the sequence analysis of fragments from large ribonucleic acids,[5] the oxidation step is physically separated from the elimination step and the latter proceeds in the absence of periodate. Under these conditions, the primary amine reacts with the oxidized glycol group and the terminal nucleoside is obtained as a morpholine derivative which is subsequently converted to the base by heat, prior to spectrophotometric analysis. The present work was directed toward increasing the sensitivity of RNA sequence analysis by developing a method by which radioactive label could be introduced into the nucleoside fragment after the elimination reaction. To this end we now show that, if the excess periodate is removed after the oxidation of an oligonucleotide and if the elimination reaction is carried out in the absence of a primary amine, a product is formed which contains all the carbon atoms of the original terminal nucleoside. The product is presumably an unsaturated dialdehyde of the type II which can be reduced with sodium borohydride to a stable derivative III, thus providing a means by which tritium can be introduced into the released fragment.



Ia, R = H
Ib, R = guanosine-3′

II



III

The β-elimination reaction has been studied with two model compounds, adenosine 5′-phosphate and guanylyl-(3′–5′)-adenosine (GpA). Adenosine 5′-phosphate (5 g, 14 mmol) was dissolved in water (50 ml) and brought to pH 8.4 with aqueous NaOH. A solution of sodium periodate (2.93 g, 14 mmol) in water (60 ml) was added and the mixture kept at 25° for 5 min. The solution was then heated at 45° for 3 hr and then cooled to 0°. Sodium borohydride (0.52 g, 14 mmol) was added and the mixture was kept at 4° for 12 hr. The solution was applied to a column (4 × 20 cm) of Dowex 1-X8 (100–200 mesh, acetate form) ion-exchange resin and the product was washed through with water. The eluate was concentrated *in vacuo* to about 10 ml and kept at 4°, yielding the white crystalline product III (2.61 g, 76%) which was recrystallized from water: mp 176–177°; uv λ_{max} 259 nm (ϵ 14,000), at pH 7; nmr (DMSO-d_6) δ 8.27 (s, 1), 8.20 (s, 1), 6.23 (t, 1), 4.27 (d, 2); mass spectrum (65 eV) m/e 251 (M^+), 194, 178, 164, 134.

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.22; N, 27.87. Found: C, 47.66; H, 5.25; N, 27.66.

A study of the rate of β elimination at 45° of the oxidation product of adenosine phosphate (Ia) was carried out in water, pH 8.4, and also in 0.4 *M* *N*,*N*,*N*′,*N*′-tetramethylglycinamide hydrochloride which had been titrated to pH 8.4 at 25° with NaOH. Samples of the reactions were withdrawn at various times, reduced with excess sodium borohydride, and chromatographed on Whatman No. 3 MM paper in the solvent system isopropyl alcohol (70 ml)–concentrated ammonia (10 ml)–water (20 ml). The amounts of the product III (R_f 0.75) and the reduced derivative of Ia (R_f 0.11) in each sample were determined spectrophotometrically after elution from the paper. Small amounts (<5%) of adenine (R_f 0.49) were also formed in these reactions. The degradation of Ia was quantitative after 3 hr in the

(1) P. R. Whitfeld and R. Markham, *Nature (London)*, **171**, 1151 (1953).
(2) D. M. Brown, M. Fried, and A. R. Todd, *Chem. Ind. (London)*, 352 (1953).
(3) H. L. Weith and P. T. Gilham, *J. Amer. Chem. Soc.*, **89**, 5473 (1967); H. L. Weith and P. T. Gilham, *Science*, **166**, 1004 (1969).
(4) H. L. Weith and P. T. Gilham, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970, Abstract BIOL 18.
(5) M. Rosenberg, G. T. Asteriadis, H. L. Weith, and P. T. Gilham, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **30**, 1101 (1971).

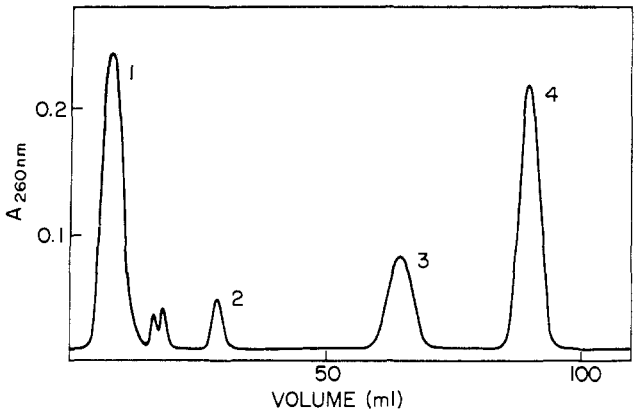


Figure 1. Elution pattern obtained from the chromatography of the reduced 30-min sample from the reaction of the dialdehyde Ib with 0.4 *M* tetramethylglycinamide at 45°. The column (0.2 × 100 cm) contained Dowex 1-X4 (−400 mesh) ion-exchange resin and the elution was carried out at 10 ml/hr with 200 ml of 20% ethanol containing a linear gradient (0.01–0.5 *M*) of ammonium chloride adjusted to pH 9 with ammonia: peak 1, compound III; peak 2, adenine; peak 3, reduced form of Ib; peak 4, guanosine 3′-phosphate.

water solution whereas complete reaction was obtained after 2 hr in the tetramethylglycinamide solution.

Initial experiments with GpA showed that its oxidized form Ib was completely degraded to guanosine 3′-phosphate and II within 1 hr at 45° in the presence of 0.4 *M* tetramethylglycinamide hydrochloride, pH 8.4. The course of this reaction was studied using a new chromatographic system[6] for the analysis of the products (Figure 1). The dinucleoside phosphate was treated with a tenfold excess of sodium periodate at 0°. The excess periodate was destroyed by the addition of excess rhamnose before the temperature was raised to 45° and the tetramethylglycinamide added. Samples were taken at various times and treated, as before, with sodium borohydride prior to analysis by column chromatography. After 1 hr the yield of guanosine 3′-phosphate was >95% and the other product was shown to be identical with III. Again, as before, a trace amount of adenine was formed early in the reaction; however, the amount of the base produced did not increase with time.

Similar β-elimination reactions have been carried out on the other nucleoside 5′-phosphates and on other dinucleoside phosphates such as ApA, CpA, UpA, and ApU. In each case the patterns of reaction and products were analogous to those discussed above.

The advantage of this method of β elimination over previously developed methods is that it provides a means for selectively labeling the nucleoside fragment after it has been released from the polynucleotide chain. Using tritiated sodium borohydride it is possible to obtain the nucleoside residue labeled to a high specific activity for subsequent detection by either scintillation counting or by autoradiography. This procedure should increase the sensitivity of detection by a number of orders of magnitude over the usual spectrophotometric method.

It has also been found that both Ia and II readily form condensation products with primary amines and these products are presumed to be morpholine derivatives. In the case of the simple aliphatic amines the products formed are relatively insoluble in water, whereas reaction with glycine produces condensation products which can readily be chromatographed in aqueous systems. This observation provides another possible approach to the labeling of the nucleoside moieties from β-elimination reactions. The structural analysis and chromatographic characteristics of these condensation products will be reported in a forthcoming publication.

(6) G. T. Asteriadis and P. T. Gilham, unpublished experiments.

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Electron–Electron Double Resonance Demonstration of Cross Saturation between Trapped Electrons and Radicals in γ-Irradiated 2-Methyltetrahydrofuran Glass

Sir:

Electrons generated by γ radiation are trapped with high efficiency in a variety of aqueous and organic glasses near 77°K. 2-Methyltetrahydrofuran (MTHF) glass is one of the organic glasses in which the nature of trapped electrons has been most extensively studied.[1] After γ irradiation of MTHF, the electron paramagnetic resonance (epr) signal is found to be a superposition of a sharp single-line spectrum due to trapped electrons and a seven-line spectrum due to free radicals formed from the MTHF molecule. A recent study of recombination luminescence suggests that the latter spectrum is due to a cationic entity.[2] Paramagnetic relaxation of the trapped electron in MTHF glass has been studied by epr saturation methods by Smith and Pieroni,[3] Williams and coworkers,[4] and Kevan and coworkers.[5] These studies reveal that the spatial distribution of the trapped electrons in MTHF glass is nonuniform, but the relaxation mechanism has not been completely delineated.

Electron–electron double resonance (eldor) is a powerful new technique for studying paramagnetic relaxation processes.[6] Eldor is the change in an epr signal, as monitored by an observing microwave frequency, which is caused by a different pump microwave frequency. In the present experiment the pumping microwave frequency and the observing microwave frequency are separated by a fixed amount and the magnetic field is swept to produce an epr spectrum as shown in Figure 1. When the pumping microwave

(1) L. Kevan, *Actions Chim. Biol. Radiat.*, **15**, 81 (1971).
(2) N. Ichikawa, H. Yoshida, and K. Hayashi, *J. Nucl. Sci. Technol.*, **9**, 538 (1972).
(3) D. R. Smith and J. J. Pieroni, *Can. J. Chem.*, **43**, 876 (1965).
(4) K. Tsuji and Ff. Williams, *Trans. Faraday Soc.*, **65**, 1718 (1969); J. Lin, K. Tsuji, and Ff. Williams, *J. Amer. Chem. Soc.*, **90**, 2766 (1968).
(5) L. Kevan and D. H. Chen, *J. Chem. Phys.*, **49**, 1970 (1968); D. H. Chen and L. Kevan in "Organic Solid State Chemistry," G. Adler, Ed., Gordon and Breach, London, 1969, p 189; D. P. Lin and L. Kevan, *J. Phys. Chem.*, **76**, 636 (1972).
(6) J. S. Hyde, R. C. Sneed, and G. H. Rist, *J. Chem. Phys.*, **51**, 1404 (1969); J. S. Hyde, L. D. Kispert, R. C. Sneed, and J. C. W. Chien, *ibid.*, **48**, 3824 (1968); J. S. Hyde, J. C. W. Chien, and J. H. Freed, *ibid.*, **48**, 4211 (1968).