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Carbon-14 Carboxy-Labeled Polysaccharides[1]

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Procedures are given for the preparation of C^{14} carboxydextran and C^{14} carboxyinulin. These materials have C^{14}-labeled carboxyl groups in place of the reducing end groups present in the parent polysaccharides. The substances can be prepared cheaply and should find many applications as tracers in biological and chemical research.

1. Introduction

A process for labeling polysaccharides with carbon-14 was described briefly in a prior note [1] [2] and in a patent assigned to the United States Secretary of Commerce [2]. Subsequently, the labeled products have been furnished to research workers in other laboratories, but the procedures for making the compounds have not been described in detail.

The present paper reports the methods used for the production of C^{14} carboxydextran and of C^{14} carboxyinulin. These materials have already found use in the study of adsorption on blood platelets and cells, as well as for the study of kidney function [3, 4]. Other applications can be envisioned and will be realized in the future.

In the labeling process, reducing end groups present in the polysaccharide combine with C^{14}-labeled cyanide; the resulting cyanohydrin, on hydrolysis, yields a polysaccharide having a radioactive carboxyl group. The activity of the labeled product depends on the equivalent weight of the polysaccharide and on the activity of the cyanide used in the synthesis. The radioactive product differs from the parent polysaccharide in that it has a C^{14}-labeled carboxyl group in place of the reducing end group. The presence of a single carboxyl group in a large molecule has little effect on the biological behavior of the substance. Hence the carboxy-labeled polysaccharides can be used as tracers in biological and chemical research. Prior to starting the synthesis, the combining weight of the polysaccharide is determined by reaction with radioactive cyanide by the method already described [5]. The polysaccharide is then treated with C^{14}-labeled cyanide and, after removal of the excess cyanide, the product is isolated. The methods used for purification and isolation of the product depend on the properties of the material being labeled. The procedures described here can be used, with small changes, for almost any water-soluble polysaccharide.

2. Experimental Procedures

2.1. Materials

Carbon-14-labeled cyanide was prepared by the previously described method [6]. The solution used contained 0.065 mM of sodium cyanide plus 0.15 mM of sodium hydroxide per milliliter and had an activity of 1 mc/ml.

The ion-exchange resins used for release of hydrogen cyanide from the reaction mixtures were conditioned by successive treatment with 5-percent aqueous sodium hydroxide, 5-percent aqueous sodium cyanide, water, 5-percent aqueous hydrochloric acid, and water until the wash liquor was neutral and free from chloride.

The clinical dextran was supplied by the Committee on Shock, National Research Council. The sample, designated NRC–3, was part of a supply made for experimental studies by the Commercial Solvents Corporation.

The inulin used was a commercial product. Before labeling, it was recrystallized from water [7].

2.2. Determination of Cyanide-Combining Power

Approximately 5 mg of the lyophilized polysaccharide was placed in a weighed test tube which had been constricted near the middle for sealing in a flame. The sample and tube were dried at 80°C under a pressure of less than 0.1 mm, and the weight of the sample was ascertained. The analyses were set up in triplicate, with controls containing 0.02 mg of D-glucose and with blank determinations having like quantities of the reagents only.

A buffered sodium cyanide–C^{14} solution was prepared from the 0.005 N carbon-14-labeled cyanide solution by adding crystalline sodium bicarbonate equivalent to the excess sodium hydroxide. A three- to five-fold excess of the cyanide solution (approximately 0.1 ml of the solution, containing about 0.2 μc of carbon–14) and 1 drop of toluene were added to each tube. The tube was sealed and stored at room temperature. After 10 days the tube was opened, a few drops of 10 percent aqueous formic acid was added, and the solution was concentrated in a current of air. The residue was dissolved in a few drops of aqueous formic acid, and the solution was frozen and lyophilized to remove the last possible trace of hydrogen cyanide. The material in the tube was dissolved in formamide, and the radioactivity was measured with an internal, gas-flow, proportional counter [8]. The equivalent weight was obtained from the relationship:

$$\text{Equivalent weight}=\frac{(\text{cps})_G \cdot W}{(\text{cps})_W \cdot G}$$

where $(\text{cps})_G$ is the net count of the control containing G millimoles of D-glucose and $(\text{cps})_W$ is the net count for the polysaccharide sample of weight W.

[1] Part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the Division of Research, Atomic Energy Commission.

[2] Figures in brackets indicate the literature references at the end of this paper.

2.3. Carbon–14 Carboxy-Labeled Carboxydextran

Ten milliliters of a 0.065 *M* sodium cyanide-C^{14} solution was placed in a 100-ml flask containing 2.6 g of lyophilized dextran (NRC–3). The flask was sealed by a glass stopper and a ring of silicone grease. The dextran was dissolved and stored at room temperature.

After 4 days, a 10-μl sample was withdrawn for analysis, and the flask was immediately restoppered. The sample was transferred, together with washings of the pipet, to a small test tube and acidified with 3 drops of 10-percent formic acid. The mixture was evaporated to dryness in a jet of air. To insure complete removal of hydrogen cyanide, the residue was twice dissolved in 0.5 ml of water and reevaporated to dryness. Radioassay of the residue, dissolved in 1 ml of formamide, showed that approximately 0.67 mc of carbon–14 had been fixed. This was 88 percent of the amount expected (0.76 mc) from the specific activity of the cyanide (15.2 mc/mM) and from the cyanide-combining power determined by the method of section 2.2.

The reaction mixture in the 100-ml flask was allowed to stand an additional 3 days to assure completion of the reaction. The liquid was then frozen by immersion of the flask in a liquid-nitrogen bath with gentle swirling. The stopper was removed, 5 ml of conditioned Amberlite IR–120(H) resin [3] was added and the flask, A, was connected to a receiving flask, B, as shown in figure 1. The receiver B contained excess sodium hydroxide solution (1 ml of *N* NaOH). Flasks A and B were immersed in liquid nitrogen and the system was evacuated with an efficient oil pump. Stopcocks E and C were closed, the liquid-nitrogen bath was removed from A, and the contents of the flask were thawed. After thorough mixing, the contents were again frozen in liquid nitrogen. Stopcock E was then opened and the liquid-nitrogen bath was removed from A, but not from B. As the frozen mixture became warmer, water and hydrogen cyanide were transferred to B by lyophilization. After the lyophilization was complete (5 hr), stopcock C was closed, and 10 ml of water was added to A (through funnel D) and mixed with the contents of the flask. The liquid was frozen in liquid nitrogen, the system was evacuated, and the lyophilization process was repeated. Finally, flask A was removed from the apparatus, and flask B, containing the recovered cyanide, was stoppered and set aside for future use.

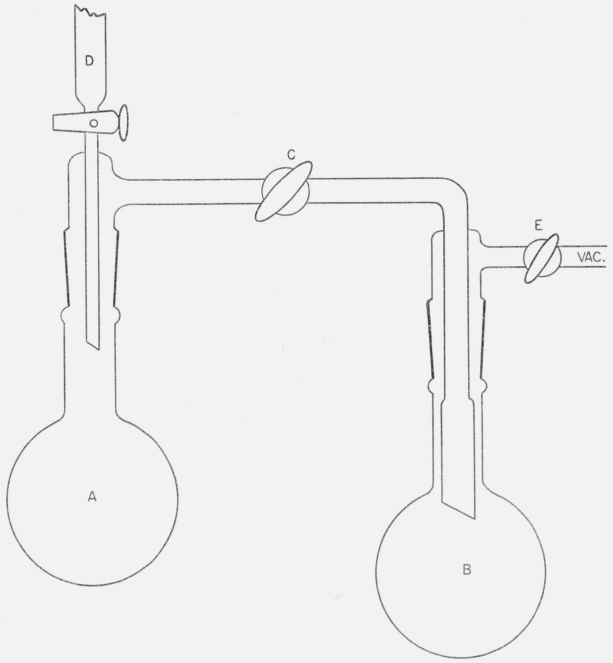

FIGURE 1. *Lyophilization apparatus for the recovery of carbon-14-labeled cyanide.*

The contents of A were passed through a column containing 10 ml of a 1:1 mixture of Amberlite IR–120(H) resin and Duolite A–4 resin.[4] The flask and resin were washed with water. The solution was acidified with a drop of 10-percent acetic acid and lyophilized. The residue was dissolved in 50 ml of water and again lyophilized. (The second lyophilization was to remove the last trace of hydrogen cyanide.) The product weighed 2.29 g and had an activity of 0.28 μc/mg.

2.4. Carbon–14 Carboxy-Labeled Carboxyinulin

Seven grams of finely powdered, lyophilized, recrystallized inulin was added slowly with constant swirling to 43 ml of an ice-cold cyanide solution in a 100-ml flask. The cyanide solution contained 0.47 mM of sodium cyanide–C^{14} (7.2 mc) and 0.3 mM of sodium hydroxide. The flask was capped with an adapter carrying a stopcock for evacuation. The contents were frozen in a liquid-nitrogen bath and the flask was evacuated. The stopcock was closed and the flask was allowed to come to room temperature. It was then heated in a water bath, with constant swirling, until the inulin had dissolved. After two days at room temperature, the inulin that had crystallized from solution was redissolved by warming. This was repeated every other day for 8 days. At this time, an analysis on a 20-μl sample showed that 5.2 mc of carbon–14 had been fixed. This amount corresponds to approximately the activity anticipated from the cyanide-combining power of inulin (previously measured) and the specific activity of the cyanide used.

The contents of the flask were frozen in liquid nitrogen and, after the addition of 5 ml of conditioned Amberlite IRC–50(H) resin, the flask was connected to a second flask (B of fig. 1) containing 1 ml of *N* NaOH. Flasks A and B were immersed in liquid nitrogen, the system was evacuated, and the hydrogen cyanide liberated in A was transferred to B by the procedure described in connection with the preparation of labeled dextran. After removal of all cyanide, the residue in A was extracted at 40° to 50° C with portions of water totaling 150 ml. The solution was filtered and passed through a column containing 5 ml of a 1:1 mixture of Amberlite IRC–50(H) resin and Duolite A–4 resin. The combined

[3] Rohm and Haas Co., Philadelphia, Pa.
[4] Chemical Process Co., Redwood City, Calif

effluent and washing were lyophilized after the addition of 3 drops of acetic acid. The residue was dissolved in 100 ml of warm water. A 20-μl aliquot was removed and counted for radioactivity in 1 ml of formamide; the remainder of the solution was lyophilized. The residue weighed 7.1 g and its radioactivity showed the presence of 5.14 mc.

Elution of the resin column with 5 ml of 5 percent HCl, and washing, resulted in the recovery of 0.16 mc of activity. The cyanide collected in flask B had an activity of 1.08 mc.

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3. References

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