Enzymatic Synthesis of C-11 Formaldehyde: Concise Communication

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We present an enzymatic synthesis of C-11 formaldehyde from C-11 methanol, with immobilized alcohol oxidase and catalase: a rapid, simple procedure, with a high and reproducible yield. Carbon-11 methanol is oxidized to C-11 formaldehyde by passage over a column on which the enzymes alcohol oxidase and catalase are immobilized. The catalase increases reaction velocity by recycling the oxygen, and prevents destruction of the alcohol oxidase by eliminating the excess of hydrogen peroxide. The yield of the enzyme-catalyzed oxidation was 80–95%. A specific activity of 400–450 mCi/μmole was obtained at EOB + 20 min. Various immobilization techniques and the optimal reaction conditions of the immobilized enzymes are investigated.

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Carbon-11 formaldehyde is a useful agent for the labeling of complex molecules of biomedical interest by reductive alkylation reactions (*1–3*). It is usually prepared by catalytic oxidation of C-11 methanol on metal catalysts at high temperature (*4–6*). The yield is strongly dependent on the temperature and the surface activity of the catalyst.

As an alternative, we present an enzyme-catalyzed oxidation of C-11 methanol, with immobilized alcohol oxidase and catalase. It is a rapid, easy procedure with a high and reproducible yield. The principle of the method is outlined in Fig. 1.

MATERIALS AND METHODS

Setup of the C-11 formaldehyde production (Fig. 2). Nitrogen gas (7 bar) is irradiated with 18-MeV protons at 15 μA for 20 min. The target gas containing $^{11}CO_2$ is pumped to a hot cell where it is trapped at a flow of 1 l/min in 0.5 ml of a 0.5 *M* solution of $LiAlH_4$ in tetrahydrofurane, cooled to −80°C. The trapping of $^{11}CO_2$ takes about 10 min. Within 1.5 min the tetrahydrofurane is evaporated to dryness by heating to 160°C. Hydrolysis of the methanolate is achieved by injection of 0.5 ml 6 *N* HCl. By heating to 160°C, the C-11 methanol is transferred by a N_2 flow of 100 ml/min to 0.5 ml phosphate buffer (0.1 *M*, pH 7.5, ambient temperature). To maintain the pH of this buffer solution, the C-11 methanol is first sent through 300 mg soda lime to trap the excess HCl. The soda lime is heated to 180°C to avoid condensation of the methanol. In 5 min, the activity is almost quantitatively trapped in the buffer solution. The ventilation outlet of the buffer solution was controlled by a wash bottle.

During a few seconds, air is bubbled through the buffer solution to introduce the substrate oxygen. The solution is sucked into a sample loop of 0.7 ml (Pump 1, Fig. 2). With a flow of 1.5 ml/min, a peristaltic pump (Pump 2, Fig. 2) transfers the loop contents to the enzyme column containing the enzymes alcohol oxidase and catalase, immobilized on porous glass beads. In 2 min, 3 ml of buffer solution are collected, containing the C-11 formaldehyde. The entire procedure takes about 20 min.

Preparation of the enzyme reactor. The enzymes are immobilized by covalent linkage to glutaraldehyde-activated controlled-pore glass (*7*) (Fig. 3).

One gram of controlled-pore glass (CPG 500/80–120 Mesh*) is rinsed in 1 *N* HCl and hydrated by boiling in

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Production of ^{11}C-isotope: $^{14}N\ (p,\alpha)\ ^{11}C \xrightarrow{\text{traces } O_2} {}^{11}CO_2$

Reduction: $4\ {}^{11}CO_2 + 3\ LiAlH_4 \longrightarrow (^{11}CH_3O)_4AlLi + 2\ AlLiO_2$

Hydrolysis: $(^{11}CH_3O)_4AlLi + 4\ H_2O \xrightarrow{HCl} Al(OH)_3 + LiOH + 4\ {}^{11}CH_3OH$

Enzymatic Oxidation: $^{11}CH_3OH + O_2 \xrightarrow[\text{(E.C.1.1.3.13)}]{\text{Immobilized Alcohol oxidase}} {}^{11}CH_2O + H_2O_2$

Recycling of O_2: $H_2O_2 \xrightarrow[\text{(E.C.1.11.1.6)}]{\text{Immobilized Catalase}} H_2O + 1/2\ O_2$

FIG. 1. Scheme for synthesis of C-11 formaldehyde.

5% HNO_3. Silanization is carried out with 20 ml of a 20%-γ-aminopropyltriethoxysilane‡ solution in water, adjusted to pH 3.45 with 6 *N* HCl, by heating to 75°C for 2 hr.

Glutaraldehyde is coupled to the silanized glass by adding 25 ml 2.5% glutaraldehyde solution in 0.05 *M* phosphate buffer, pH 7. After 1 hr of reaction, the glass is exhaustively washed.

To 1 g glutaraldehyde-activated glass, 30 units of alcohol oxidase† (E.C. 1.1.3.13, from *Pichia pastoris*), and 60,000 units of catalase† (E.C. 1.11.1.6, from bovine liver) are added in 1.5 ml phosphate buffer (0.1 *M*, pH 7.0). One hour of shaking at room temperature completes the reaction. The enzyme-loaded glass beads are packed in a 0.55- by 10-cm glass column, washed thoroughly with phosphate buffer (0.1 *M*, pH 7.5) to remove any protein not covalently bound, and stored at 4°C. Under these conditions the enzymes are stable for several months. Blocking of the remaining aldehyde groups can be done with 1 *M* glycine, but blocking is not indispensable.

Before use the column is warmed to 26°C and washed with phosphate buffer (0.1 *M*, pH 7.5). After each C-11 formaldehyde production or one-half day's storage at 26°C, enzymatic activity decreases by about 5%. If properly handled there is no enzyme leakage.

RESULTS AND DISCUSSION

Yield. Table 1 shows the distribution of C-11 activity, starting from the conversion of C-11 methanol into C-11 formaldehyde. Losses of activity in $^{11}CO_2$ and C-11 methanol synthesis are not included. From the data of Table 1 and the data from radiogaschromatography, we calculate that 80% to 95% of C-11 methanol is converted into C-11 formaldehyde, yielding 200 to 250 mCi at EOB + 20 min.

Specific activity. The specific activity was 400–450 mCi/μmole (EOB + 20 min), determined as follows.

The total activity of the collection flash was determined with a calibrated gamma spectrometer equipped with a Ge(Li) detector.

The collected solution was analyzed by radiogaschromatography. The chromatographic system is described in Table 2. The result is given in Fig. 4: 93.5% of the C-11 activity is in the formaldehyde peak.

The carrier amount, determined with Nash reagent (*8*), was at most 0.65 μmole. The stock formaldehyde

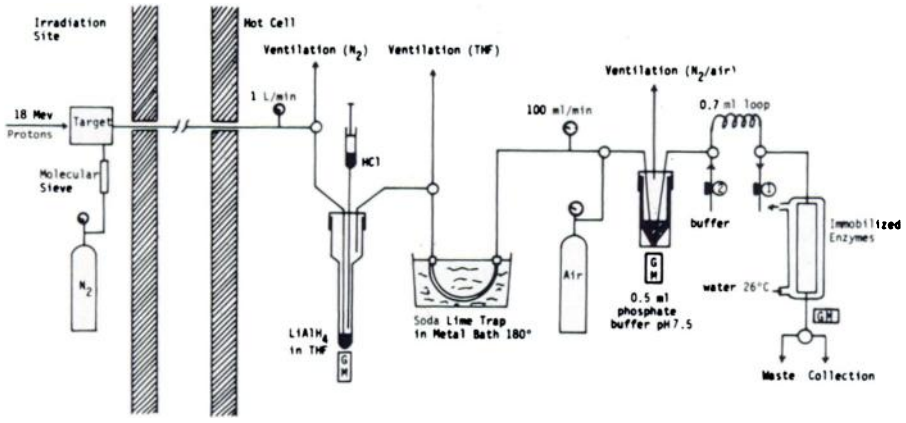

FIG. 2. Setup of C-11 formaldehyde production. ① = Pump 1. ② = Pump 2.

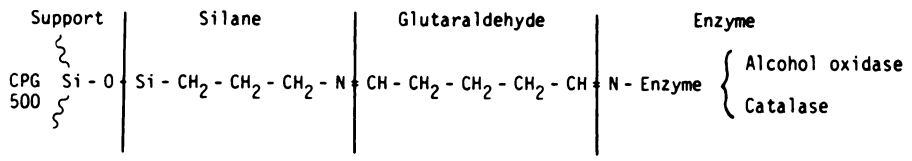

FIG. 3. Structure of immobilized alcohol oxidase and catalase.

TABLE 1. DISTRIBUTION OF C-11 ACTIVITY*

$LiAlH_4$ trap	0.5%
Soda lime trap	5.9%
Wash bottle (ventilation outlet of buffer solution)	2.6%
Enzyme reactor	3.0%
Collection flask	88.0%

* Mean value of five productions

solution was standardized by dimedone precipitation (*9*) and by iodimetric titration.

The carrier amount originates from the $^{11}CO_2$ and C-11 methanol production. The use of the enzyme process makes no significant contribution to the carrier content.

Overoxidation of formaldehyde into formic acid did not occur; it is checked by passing the C-11 formaldehyde eluate of the enzyme reactor over an AG 1X8 anion resin. Any trace of C-11 methanol and the produced C-11 formaldehyde pass through the column, whereas any trace of C-11 formic acid is retained. No activity could be detected on the anion resin, indicating that there was no C-11 formic acid present.

Comparison of immobilization techniques. Enzymes are immobilized by covalent linkage to an inert support (*7*), by means of an active group, at the end of a spacer arm, coupled to a silane. The support was controlled-pore glass in all experiments (CPG 500/80–120 Mesh or CPG 240/80–120 Mesh)*. Different silanes‡, spacer arms, and active groups were tested. To compare the different immobilization techniques, we determined the percentage of bound alcohol oxidase and the alcohol oxidase activity of the glass beads (Table 3).

TABLE 2. CHROMATOGRAPHY DATA

Column: 1/8″ Teflon tubing, i.d. 2.4 mm:
- Part 1 12.5 cm Carbosphere 60–80 Mesh*, placed in injection port,
- Part 2 200 cm Chromosorb 108/60–80 Mesh†, placed in oven

t° injection port 170°
t° oven 146°
t° thermal conductivity detector 270°
He flow = 40 ml/min
Radiochemical detection: NaI(Tl)
Injection volume 2μl

* Alltech.
† Johns Manville.

The enzymatic activity is determined by spectrophotometric measurement of formaldehyde with Nash reagent (*8*).

Incubation of soluble and immobilized enzyme is carried out with 20 μl of methanol in 2.5 ml phosphate buffer (0.05 *M*, pH 7.5) at 26°C for 10 min.

The method of choice is the glutaraldehyde coupling on γ-aminopropylcoated CPG 500 (Fig. 3). It is an easy and gentle immobilization procedure giving the highest loading with active enzyme.

Effect of coimmobilized catalase. To increase the conversion of methanol into formaldehyde, the substrate oxygen can be recycled by addition of catalase to the system (Fig. 1). When the oxygen-to-methanol ratio is not too high, addition of hydrogen peroxide to the mixture of enzymes also increases the conversion rate.

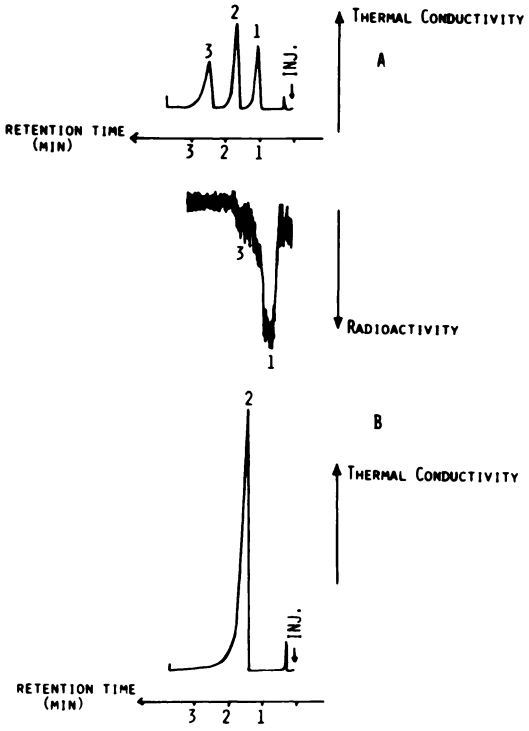

FIG. 4. Radiogaschromatography. Peak 1 = formaldehyde, Peak 2 = water, Peak 3 = methanol. A = test mixture; B = C-11 formaldehyde solution. Peak 1 represents 93.5% of the activity, Peak 3, 6.5%.

TABLE 3. COMPARISON OF IMMOBILIZATION TECHNIQUES

Glass	Silane	Spacer arm	Active group	Coupling	% Bound	% Active
CPG 240	A1100*	Glutaraldehyde	Aldehyde	Shiff base	100	9
CPG 500	A1120†	Glutaraldehyde	Aldehyde	Shiff base	100	5
CPG 500	A 187‡	—	Aldehyde	Shiff base	40	15
CPG 500	—	—	Cyanogen bromide	Carbamate	15	15
CPG 500	A1100*	Arylamine	Diazonium	Azo	80	0.5
CPG 500	A1100*	Glutaraldehyde	Aldehyde	Shiff base	100	30
CPG 500	A1100*	—	Thiocyanate	Thiourea	90	9
CPG 500	A1100*	Succinate	Acid Chloride	Amide	60	15
CPG 500	A1100*	Succinate	Active Ester	Amide	6	2

* $(C_2H_5O)_3$-Si-CH_2-CH_2-CH_2-NH_2

† $(CH_3O)_3$-Si-CH_2-CH_2-CH_2-NH-CH_2-CH_2-NH_2

‡ $(CH_3O)_3$-Si-CH_2-CH_2-CH_2-O-CH_2-CH-CH_2 (O)

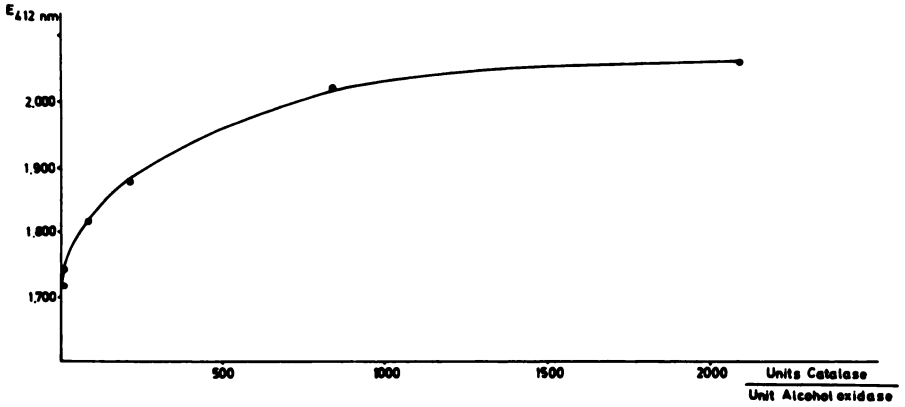

FIG. 5. Effect of coimmobilized catalase on activity of alcohol oxidase. Ordinate gives extinction by Nash reaction.

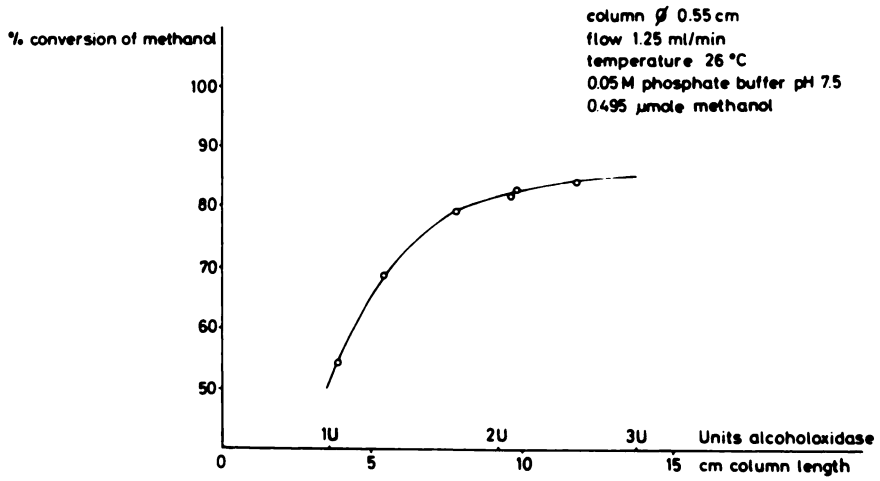

FIG. 6. Conversion of methanol into formaldehyde, plotted against amount of enzyme in column.

However, we observed that by addition of hydrogen peroxide to a buffered solution of alcohol oxidase without catalase, the enzymatic activity decreases irreversibly from day to day and more rapidly than the same enzyme solution without hydrogen peroxide. To obtain an enzyme column with high stability, addition of hydrogen peroxide in the buffered C-11 methanol solution must be avoided. Therefore an enzyme column of alcohol

oxidase with a sufficient excess of catalase was preferred, so that not oxygen but the substrate methanol limits the conversion rate.

One unit of alcohol oxidase is immobilized with variable amounts of catalase (0.5–2000 units, Fig. 5). For routine preparation of an enzyme reactor, the alcohol oxidase is immobilized with a 2000-fold excess of catalase.

Optimization of enzyme reactor. The amount of immobilized enzyme needed for high-yield conversion of methanol into formaldehyde is determined for 0.5 μmole of methanol, which is about the carrier amount present in the preparation.

The percentage of methanol conversion into formaldehyde is determined with different packed bed volumes in the enzyme column (Fig. 6). A column, containing 2.5 units of alcohol oxidase, that gives a 80–95% conversion of methanol into formaldehyde, is used for routine production.

FOOTNOTES

* Corning Glass Works.
† Sigma Chemical Company.
‡ Union Carbide Benelux.

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REFERENCES

1. STRAATMAN MG, WELCH MJ: A general method for labeling proteins with ^{11}C. *J Nucl Med* 165:425–428, 1975
2. MAZIERE M, BERGER G, COMAR D: Optimization of ^{11}C-labelling of radiopharmaceuticals: ^{11}C-clomipramine as an example. *J Label Compd Radiopharm* 132:196–197, 1977
3. BERGER G, MAZIERE M, MARAZANO C, et al: Carbon 11 labeling of the psychoactive drug O-methyl-bufotenine and its distribution in the animal organism. *Eur J Nucl Med* 3: 101–104, 1978
4. CHRISTMAN D, CRAWFORD J, FRIEDKIN M, et al: Detection of DNA synthesis in intact organisms with positron-emitting [methyl-^{11}C]thymidine. *Proc Natl Acad Sci USA* 69(4):988–992, 1972
5. MARAZANO C, MAZIERE M, BERGER G, et al: Synthesis of methyl iodide-^{11}C and formaldehyde-^{11}C. *Int J Appl Radiat Isot* 28:49–52, 1977
6. BERGER G, MAZIERE M, SASTRE J, et al: Carrier free ^{11}C-formaldehyde: An approach. *J Label Compd Radiopharm* 17(1):59–71, 1980
7. WEETALL HH: Covalent coupling methods for inorganic support materials. In *Immobilized Enzymes. Methods in Enzymology*, Vol 44, 1976, pp 134–148
8. NASH T: The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem J* 55:416–421, 1953
9. HORNING EC, HORNING MG: Methone derivatives of aldehydes. *J Org Chem* 11:95–99, 1946