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Note

Chromatographic purification and characterization of hydrogen peroxide

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The formation of hydrogen peroxide has been observed in a wide variety of systems. Its isolation from complex reaction mixtures and subsequent characterization often is complicated by the need to use mild conditions, owing to its reactivity. We have recently developed a suitable purification method in connection with identifying H_2O_2 as a product from the photooxidation of tryptophan, and report the details below.

MATERIALS AND METHODS

Pharmacia Sephadex G-10, QAE-Sephadex, and Blue Dextran 2,000 were purchased from Sigma (St. Louis, Mo., U.S.A.). Glass-distilled water was used for all solutions. The chromatographic columns used were: (1) 2.5 × 77 cm glass column packed to a bed depth of 53 cm with 110 g of Sephadex G-10–120 (40–120 μm bead size) pre-swollen for 24 h in water; (2) Pharmacia K9/30 0.9 × 30 cm column packed to a bed depth of 15 cm with *ca.* 2 g of Q-25-120 QAE-Sephadex (30–120 μm bead size) pre-swollen for 24 h in a pH 9.1, 0.05 *M* chloride, ammonium chloride buffer prepared by diluting 5.3 g of ammonium chloride to 2.0 l with water and adjusting the pH by the addition of concentrated ammonium hydroxide; (3) Pharmacia K15/90 1.5 × 90 cm column packed to a bed depth of 88 cm with Sephadex G-10–120 pre-swollen for 24 h in 0.1 *M* ammonium sulfate. Flow detection was accomplished using an Isco UA-4 dual beam ultraviolet (UV) (254 nm) detector with 0.2-cm cells or a Waters Assoc. differential refractometer mounted on a Waters Assoc. 201 high-pressure liquid chromatograph. The pump on the chromatograph was used with the K15/90 column. Microcrystalline cellulose (Avicell, 250 μm thick) thin-layer plates were purchased from Alltech (Newark, Del., U.S.A.). Silica gel H plates were prepared from Merck HF-254/366 silica gel (10–40 μm particle size) purchased from Brinkman (Westbury, N.Y., U.S.A.). Silica gel thin-layer sheets (EK 13191) were purchased from Eastman (Rochester, N.Y., U.S.A.).

Chromatographic columns were pre-run with at least 10 bed volumes of eluent before use. The void volume of the K15/90 column was determined using Blue Dextrans; this column was then calibrated for molecular weight determinations using raffinose, sucrose, glucose, glycerol, methanol, and deuterium oxide. During all thin-layer chromatography (TLC), a glass cover was clamped over the surface. Visualization of H_2O_2 was done with *p*-N,N-dimethyldiaminobenzene and/or ammonium

thiocyanate–ferrous sulfate sprays. Quantitative H_2O_2 determinations were carried out using the titanium colorimetric reagent described by Wolfe[1].

RESULTS

In our case, H_2O_2 was to be isolated from a complex mixture, produced upon photooxidation of tryptophan. This mixture contains compounds varying in molecular weight from low (<100) to very high ("melanins"), and in acid-base properties from acidic to neutral to basic. In addition, the problem was complicated by apparent reactions between H_2O_2 and other components at temperatures in excess of 40°.

Purification procedure by column chromatography

A 100-ml sample of the crude photomixture was filtered through Whatman No. 2 paper and then reduced to a volume of 15–20 ml using a rotary-film evaporator. The resulting solution was then chromatographed using the 2.5-cm G-10 column with water as eluent. The effluent was monitored by UV absorbance (254 nm) and collected in 20-ml fractions. The fractions showing oxidizing capability by starch–iodide paper (eluent volume *ca.* 220–240 ml) were combined and carefully reduced under aspirator pressure to a volume of 5 ml. The pH of this sample was adjusted to 9.1 using 1 *M* ammonium hydroxide, and the resulting solution chromatographed on the QAE column with a pH 9.1, 0.05 *M* ammonium chloride buffer. The effluent was monitored by UV absorbance (254 nm) and fractions taken according to observed peaks. The H_2O_2 fraction eluted between 10 and 20 ml. Immediately upon collection the ammonia was removed from this fraction and the pH reduced to about 8 by removing *ca.* 10% of the volume under aspirator pressure. This starch–iodide positive fraction was further concentrated to a volume of 2–3 ml, applied to the 1.5-cm G-10 column, and eluted with 0.1 *M* ammonium sulfate at a flow-rate of 1.0 ml/min. The effluent was monitored by refractive index detection and starch–iodide assay; the purified H_2O_2 was eluted in the 112–114 ml fraction.

Quantitation of this scheme was carried out using an aqueous H_2O_2 solution of known concentration. The percent recovery after each chromatography step (prior concentrations being included each time) were: 90.5% after the first G-10 column, 72.6% after the QAE column, and 86.5% after the final G-10 column. This gives an overall H_2O_2 recovery of 56.8% for the procedure, including four concentration steps and three chromatographic steps.

Thin-layer chromatography of H_2O_2

Using Avicell cellulose plates, H_2O_2 was characterized by the following mobilities for the indicated solvent systems: $R_F = 0.25$ (diethyl ether) and $R_F = 0.63$ (water–diethyl ether–*n*-butanol, 1:10:10). On silica gel H_2O_2 displayed an R_F value of 0.36 using methanol–toluene (3:7).

DISCUSSION

The preparative chromatographic sequence described herein allows the separation of H_2O_2 from a wide variety of compounds, under very mild conditions. It will be noted that it is possible to concentrate these solutions, avoiding temperatures above

35°, under aspirator pressures (*ca.* 25 mm Hg) without significant loss of H_2O_2 in spite of its volatility. Another important point in this sequence is the pH of the eluent used for the QAE column: at pH values greater than 9.1, H_2O_2 decomposed rapidly on the column, as evidenced by gas evolution and loss of activity of the eluent.

The quantitation data indicate that the step which accounts for most of the H_2O_2 loss is the QAE column. This is due to decomposition of H_2O_2 when exposed to the QAE resin at pH 9.1. For separation problems that can be carried out at lower pH, such a loss will be eliminated.

The elution volume for H_2O_2 on G-10 is of interest as a characteristic value. Using the 1.5-cm G-10 column, the elution volumes for H_2O_2, Blue Dextran 2,000, and D_2O are 112–114 ml, 46 ml, and 99 ml, respectively. The K_d value[2] for H_2O_2 is therefore 1.26. This value indicates that some affinity effects, in addition to gel permeation, give rise to retardation of H_2O_2; this observation is in line with reported observations on other small, electron-rich molecules[3].

Finally, we have improved considerably on the TLC analysis of H_2O_2 previously described[4–9]. Using microcrystalline cellulose plates in place of paper, the resolution is substantially improved. Additionally, we have found that for purposes of maximum resolution, progressive multiple development (increasing degrees of solvent advancement) using diethyl ether gave best results. The solvent systems described above provide R_F values which are in a range that allows most ready differentiation from other compounds (*e.g.*, organic hydroperoxides).

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REFERENCES

1 W. C. Wolfe, *Anal. Chem.*, 34 (1962) 1328.
2 J. Janson, *J. Chromatogr.*, 28 (1967) 12.
3 D. Eaker and J. Porath, *Separ. Sci.*, 2 (1967) 507.
4 M. H. Abraham, A. G. Davies, D. R. Llewellyn and E. M. Thain, *Anal. Chim. Acta*, 17 (1957) 449.
5 A. Rieche and M. Schultz, *Angew. Chem.*, 70 (1958) 694.
6 N. A. Milas and I. Belic, *J. Amer. Chem. Soc.*, 81 (1959) 3358.
7 J. Cartlidge and C. F. H. Tipper, *Anal. Chim. Acta*, 22 (1960) 106.
8 E. Kanppe and D. Peter, *Z. Anal. Chem.*, 190 (1962) 386.
9 M. M. Buzlanova, V. F. Stepanovskaga, A. F. Nesterov and V. L. Antonovskii, *J. Anal. Chem. U.S.S.R.*, 21 (1966) 454.