Catalyst Electrode Specific for Peroxide

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Polarographic measurement of $O_2$ and $H_2O_2$ is the basis for several enzyme-coupled electrodes (1–3). Hydrogen peroxide can be polarographically assayed by anodic oxidation (2). This method is very sensitive, but lacks specificity for peroxide in the presence of other redox-active substances that undergo anodic oxidation, and which are present in biological solutions, for example, ascorbic acid. However, if $H_2O_2$ is first catalytically decomposed to $O_2$ and $H_2O$, then the $O_2$ can be assayed polarographically using an oxygen electrode. Furthermore, specificity for just oxygen can be obtained by placing a hydrophobic membrane over the $O_2$ electrode, as originally described by Clark (2). Oxygen is the only electroactive substance in biological solutions that can cross a hydrophobic membrane and generate an electrode current.

Herein we report an inorganic catalyst electrode. This peroxide sensing device is made by covering an oxygen electrode with a membrane that catalyzes the breakdown of hydrogen peroxide to oxygen. This electrode is similar in principle to an enzyme electrode (1, 3), but has the added stability gained by using an inorganic catalyst, rather than an enzyme such as catalase.

EXPERIMENTAL

The oxygen sensor used to build the catalyst electrode was a Clark type of microcathode oxygen electrode (Radiometer, Copenhagen, Denmark, Type No. E-5046). The current output of this type of electrode is a linear function of oxygen tension (4). A membrane made of regenerated cellulose (Neflex hemodialysis membrane, 20-micron wet thickness, Union Carbide, Chicago, IL) is modified so as to carry out transmembrane catalysis of $H_2O_2$. This membrane is press-fitted over the oxygen electrode with an O-ring.

Catalyst Impregnated Membrane. Noble and transition metals form insoluble oxides and sulfides, many of which are well-known catalysts for breaking down $H_2O_2$ to $O_2$ and $H_2O$ (5). These catalysts can be precipitated within the polymer matrix of the cellulose dialysis membrane by contacting one side of the membrane with 0.3M chloride salt solution of the transition metal and contacting the other side of the membrane simultaneously with 0.1M sodium hydroxide or 0.1M sodium sulfide. A contact time of 1 minute was ordinarily used.

Initial tests for catalytic activity were made on membranes in which the following metal compounds were deposited: $Fe_3O_4$, $FeS$, $NiO$, $NiS$, $Ag_2O$, $Ag_2S$, $CuO$, $CuS$, $Zn(OH)_2$, $ZnS$, $Cr_2O_3$, $CrS_2$, $Cd(OH)_2$, $CdS$, $La(OH)_3$, $Co_2O_3$, $CoS$. All but $Zn(OH)_2$, $ZnS$, $Cd(OH)_2$, $CdS$, $La(OH)_3$, and $CoS$ showed some degree of catalytic activity; these results parallel the results of earlier (non-membrane) studies (5). (The $MnO_2$-containing membrane was prepared by contacting the membrane with 0.3M K$MnO_4$ and 0.1M NaI simultaneously for 1 minute on opposite sides of the membrane. Reduction of permanganate by iodide produced a precipitation of $MnO_2$ in the membrane. The $Co_2O_3$-containing membrane was formed by first contacting the membrane simultaneously with 0.3M Co$Cl_2$ and 0.1M NaOH to precipitate blue Co(OH)$_2$. Upon contact with $H_2O_2$, the cobalt hydroxide was oxidized to greenish-black hydrated Co$O_2$.)

Of the above listed 21 different metal compounds that were deposited in cellulose membranes, only the compounds of manganese, cobalt, and ruthenium showed high catalytic activity. Catalytic activity was measured semiquantitatively by comparing the vigor with which $O_2$ bubbles are produced on the membrane surface in a 0.2% solution of $H_2O_2$. Catalytic activity was measured quantitatively by a volumetric method detailed in a previous report (6). Only compounds of these three metals deposited in membranes were further studied for stability and washout of the catalyst.

RESULTS AND DISCUSSION

Catalyst Stability with Respect to pH and Chelation. Gamma-ray emitting isotopes of manganese, cobalt, and ruthenium were available to us through the neutron activation services of the University of Wisconsin Department of Nuclear Engineering. Catalyst washout as a function of pH was determined by observing any washout from the membrane as a function of pH in the range of 2 to 10 in the presence of $H_2O_2$ kept at a concentration of 0.2 to 0.5%. A summary of data on pH stability for the oxides of manganese, cobalt, and ruthenium is given in Table I. The oxide and sulfide of a given metal behaved identically. The oxide and sulfide of cobalt and the oxide of manganese were excellent catalysts in alkaline solution. Over a 24-hour period there was less than 3% $^{56}Mn$ washout above pH 8.0, and less than 3% $^{60}Co$ washout above pH 8.0. However, both of these metals washed out of the membrane completely within two hours at acid pH.

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Table I. Catalyst Washout as a Function of pH

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>pH Range</th>
<th>Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co3O4</td>
<td>0.1Co</td>
<td>None</td>
</tr>
<tr>
<td>MnO2</td>
<td>0.1Mn</td>
<td>None</td>
</tr>
<tr>
<td>Ru3O4</td>
<td>0.54Ru</td>
<td>None</td>
</tr>
</tbody>
</table>

On the other hand, ruthenium oxide and ruthenium sulfide were excellent and stable catalysts through the entire range of pH 2 to 10. The washout of 105Ru was 2% or less over 48 hours of testing throughout this entire pH range. These washout studies were conducted in isotonic acetate and phosphate buffer solutions, and in 0.15M NaCl made pH 2 by addition of HCl.

A severe test of catalyst insolubility was conducted by treating Mn, Co, and Ru catalyst membranes at 100 °C with solutions of 0.1M EDTA at pH 4.8 and pH 10. At low pH, the Mn and Co catalysts decomposed and went into solution, while the Ru catalyst remained insoluble and membrane bound. At high pH, a high percentage of the Mn and Co catalysts also washed out while the Ru catalyst remained entrapped in the membrane.

Catalyst Inhibition. A catalyst for a peroxide electrode should not undergo poisoning or inhibition under operating conditions. For example, silver metal and silver oxide are excellent catalysts for H2O2 and can readily be deposited in membranes using technology developed in the photographic industry. However, silver catalysts are immediately poisoned by chloride ion.

To test for inhibition, Co and Ru impregnated membranes were exposed to a wide range of potential inhibitors including 0.1M sodium arsenite, 0.1M Na3PO4, 0.1M sodium pyrophosphate, 0.1M Na2CO3, 0.1M NaCN, 0.1M NaF, 1 mg/ml Heparin, and human plasma for up to 4 days. In the presence of H2O2, the vigor with which O2 bubbles form on the surface of membranes impregnated with catalyst provided an excellent semi-quantitative test of catalyst activity. Table II gives the results using membranes treated for 1 and 20 hours. At the end of the indicated interval of exposure to potential inhibitor, the catalytic activity was assayed vs. a control catalyst membrane stored in deionized water. Note that ruthenium catalyst has not shown inhibition or poisoning.

We have studied transmembrane catalysis of H2O2 using catalyst impregnated membrane as a means of transferring oxygen across a hemodialysis membrane (6-8). The breakdown of hydrogen peroxide is complete as shown by the absence of detectable hydrogen peroxide on the side of the membrane contralateral to the H2O2 solution. Titration with permanganate (9) was used as a sensitive test for the presence of H2O2. The transfer of oxygen across this type of membrane is a function of the concentration of H2O2 contacting the membrane and is one or two orders of magnitude greater than can be achieved with a partial pressure of oxygen of one atmosphere. Hydrogen peroxide concentrations greater than about 0.5% cannot be used because of blistering of the membrane caused by oxygen partial pressures in excess of forty atmospheres, as measured by an oxygen electrode pressed against the membrane. Modestly high levels of supersaturation of oxygen have been previously reported using the enzyme catalase in free solution by other investigators (10).

However, to our knowledge, the catalytic decomposition of a liquid to a dissolved gas of exceedingly high supersaturation within a polymer matrix has never been observed or studied before. The membrane obviously constrains the initiation and growth of bubbles within the cellulose polymer matrix and is undoubtedly playing a role in achieving the exceedingly high levels of O2 supersaturation.

The catalyst electrode was evaluated using a flowing stream configuration of analysis where the current output of the catalyst electrode is compared to the current output of a reference O2 electrode. A sample turntable and proportionating pump (Technicon Corporation, Chauncey, NY) were used to flow standard H2O2 solutions in series, first past the reference oxygen electrode and then past the catalyst electrode. The flow rate through the electrode cells (Radiometer, Type B-616) was 0.54 ml/min, which was rapid enough to achieve a reasonable response time. Formation of oxygen bubbles at the membrane surface was not a problem for concentrations under 0.58 M H2O2.

The difference in current outputs from these two electrodes was amplified using a high impedance differential amplifier. The data are plotted in Figure 1. In the absence of H2O2, both the catalyst electrode and reference O2 electrode responded identically to changes in partial pressure of O2, and thus the difference in current output was zero. Thus, any difference in current output represented an additional current response due to breakdown of H2O2 at the catalyst electrode. It is this difference in current output coming from the catalyst electrode that is plotted in Figure 1.

As can be seen from inspection of the standard deviations in Figure 1, reproducibility of identical standards was quite good. The electrode response time, to achieve 90% of steady state, was two minutes. A much shorter response time can be obtained if Teflon rather than polypropylene membrane is used to cover the oxygen electrodes. Teflon membrane is known to have a higher oxygen diffusivity than polypropylene or polyethylene membrane (11). However, polypropylene or polyethylene membrane has a more reproducible and drift free performance, and now is the most widely used hydrophobic membrane for routine oxygen polarography in the clinical laboratory.

Theoretical Considerations. Standard curves such as (b) and (c) in Figure 1 were obtained by studying different membranes treated as outlined above. For any given membrane, these calibrated curves were straight and reproducible, but never exactly superimposable from membrane to membrane. In other words, each membrane has its own calibration. The calibration curves were always lower than a theoretical calibration curve (a) obtained by calculating the oxygen partial pressure obtained assuming complete conversion of H2O2 to O2 and H2O at room temperature. This can be predicted by the fact that some of the O2 released by intramembrane catalyst will necessarily diffuse back into the sample solution. The degree to which this back diffusion of O2 occurs will depend on the density and distribution of catalyst in the membrane. Catalyst preferentially deposited on the side of the membrane proximal to the reference oxygen electrode will provide a catalyst membrane that more closely approaches the theoretical calibration curve [see (a) of Figure 1]. The technique for depositing catalyst preferentially on one side of the membrane has been described previously (6).

In summary, theoretical analysis reveals that, at steady state, the oxygen tension at various depths into the membrane will be a complex function of diffusion of H2O2 and O2 into and out of the membrane and dependent on the amount of surface area and distribution of the catalyst in the membrane.
Before milk can be microbiologically converted to cheese, the \( \text{H}_2\text{O}_2 \) must be destroyed by addition of the enzyme catalase. An arduous method for determining \( \text{H}_2\text{O}_2 \) in milk in the range of \( 3 \times 10^{-4} \) to \( 1.5 \times 10^{-2} \) percent is available. This spectrophotometric method requires deproteinization and then addition of titanium tetrachloride (13). Absorbance is measured at 415 nm. We have been able to show that the peroxide catalyst electrode has the specificity and sensitivity to measure \( \text{H}_2\text{O}_2 \) in milk. The only preliminary step is a dilution of the milk sample to decrease the concentrations of fats and proteins, which eventually foul the tubing and membrane. These complications can be avoided by first diluting the samples 1:10 in phosphate buffer solution.

Oxidase enzymes form \( \text{H}_2\text{O}_2 \) as a reaction product, and thus the peroxide electrode can be used to construct another type of enzyme electrode (3). Other applications, such as monitoring \( \text{H}_2\text{O}_2 \) in industrial processes and in the area of fuel cell technology are envisioned. Extracorporeal oxygenation by transmembrane catalysis of \( \text{H}_2\text{O}_2 \) using ruthenium impregnated hemodialysis membranes (6–8) is currently under evaluation in our laboratory.

**LITERATURE CITED**


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**Table II. Catalyst Activity after Exposure to Potential Inhibitors**

| Potential Inhibitors | Cobalt | | | | Ruthenium | | | |
|----------------------|--------|--------|--------|--------|----------------|--------|--------|
| 0.1 M EDTA | washout poor | none | good-excellent | good | | | |
| 0.1 M Arsenate | good-excellent | good | good-excellent | good-excellent | | | |
| 0.1 M Pyrophosphate | good | fair | good-excellent | good-excellent | | | |
| 0.1 M Carbonate | excellent | good | good | good | | | |
| 0.1 M CN⁻ | poor | excellent | excellent | excellent | | | |
| 0.1 M F⁻ | excellent | good-excellent | excellent | excellent | | | |
| Heparin (1 mg/ml) | excellent | good-excellent | excellent | excellent | | | |
| Human plasma | fair | poor | good | good | | | |
| H₂O control | excellent | excellent | excellent | excellent | | | |

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**Figure 1. Theoretical maximum and standard curves for catalyst electrodes**

**Calibration:** The current response going from zero oxygen tension to room air oxygen tension was assigned a value of 1.00. (a) This curve is a theoretical maximum as defined in the text. (b) and (c) are standard curves for two different catalyst membranes, both made by precipitating cobalt oxide in the membrane according to the protocol outlined in the text.