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Bioselective Membrane Electrode Probes

Garry A. Rechnitz

One of the most rapidly expanding research areas relating to analytical measurements is the development of potentiometric membrane electrodes with selectivity for ions, dissolved gases, and biological materials. Activity in this field is so intense that new publications are appearing at a rate approaching 500 per year (*1*). Entirely aside from the appealing practical possibilities for such potentiometric membrane electrodes, it appears that new research directions have been directly stimulated by the timely infusion of concepts from physics (*2*) and biology (*3*). Some of the consequences of the latter are examined in this article.

Potentiometric membrane electrodes have their conceptual origin in the ubiquitous *p*H glass membrane electrode which, in its present form, is still the most sensitive and selective of all such electrodes. During the past 20 years, a wide variety of conventional potentiometric ion-selective membrane electrodes based on glasses (*4*), crystals (*5*), and various liquid membranes (*6–8*) has been developed and commercialized. Several recent reviews (*1*) and monographs (*9*, *10*) give comprehensive accounts of this work.

Throughout the period of ion-selective electrode development, efforts were made to extend the measurement capabilities of membrane electrodes to biological materials through the use of enzyme catalysis to convert substrates to species that could be sensed by ion-selective membrane electrodes. The resulting "enzyme electrodes" represent an increasingly practical, but now largely conventional, means of effecting bioselective measurements with membrane electrodes (*11*).

A special impetus was given to research on bioselective membrane electrodes in the early 1970's when stable and reliable potentiometric sensors for ammonia, carbon dioxide, hydrogen sulfide, and other dissolved gases became commercially available on a routine basis. Such electrodes combine the technology of ion-selective membrane electrodes with that of microporous synthetic membranes (*12*). The ammonia gas–sensing membrane electrode, for example, is a potentiometric sensor in which a hydrophobic gas-permeable membrane is superimposed on a flat *p*H-type glass membrane electrode in contact with a thin layer of ammonium chloride electrolyte solution. This arrangement gives exceptional selectivity for the measure-

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0036-8075/81/1016-0287$01.00/0

ment of ammonia in the presence of ions, biological molecules, and even other dissolved gases. It will be seen below that these gas-sensing electrodes are key elements in the development of the newer types of bioselective probes.

Tissue-Based Membrane Electrodes

The first (*13*) bioselective membrane electrode made with intact animal tissue slices utilized a rather crude arrangement (Fig. 1). This electrode required both beef liver tissue and isolated urease enzyme to mediate the conversion of the amino acid to be measured, arginine, to the electroactive product, ammonia, via the scheme:

$$\text{arginine} \xrightarrow{\text{liver tissue}} \text{ornithine} + \text{urea} \quad (1)$$

$$\text{urea} \xrightarrow{\text{urease}} CO_2 + 2NH_3 \quad (2)$$

Despite its limited utility, this early electrode effectively demonstrates the concept of using intact tissue slices as biocatalysts and, moreover, illustrates the "building-block" approach to electrode design. This approach employs a combination of biocatalytical components and membranes to give substrate selectivity and to serve as physical barriers, respectively, in a manner designed to yield overall selectivity along with electrode stability and good response characteristics.

The full power of this approach has been realized (*14*) in the design of the glutamine-selective electrode (Fig. 2). The need for an auxiliary enzyme is eliminated because the complete biocatalytic functions are being carried out by a tissue slice from the cortex portion of porcine kidney. A slice of kidney, 0.5 millimeter thick, is held at the surface of an ammonia gas–sensing membrane electrode by means of a dialysis membrane or a mesh of monofilament nylon. The dialysis membrane serves to block out high molecular weight materials, and the hydrophobic gas-permeable membrane prevents the entry of ions into the internal electrode elements. The pork kidneys are obtained from freshly killed animals but can be stored under refrigeration at 4°C for months without loss of activity.

In phosphate buffer at *p*H 7.8, the resulting electrode gives a response of approximately 50 millivolts per tenfold glutamine concentration change over the range of $6.0 \times 10^{-5}M$ to $6.7 \times 10^{-3}M$ glutamine, with a lower limit of detection of $2 \times 10^{-5}M$. The response time of the electrode is 5 to 7 minutes. The kidney tissue electrode yields negligible response to such possible interferences as urea, L-alanine, L-arginine, L-histidine, L-valine, L-serine, L-glutamic acid, L-asparagine, L-aspartic acid, D-alanine, D-aspartic acid, glycine, or creatinine.

It might be expected that such animal tissue electrodes would have rather short useful lifetimes owing to deterioration of the tissue layer. This does not occur if a simple preservative such as sodium azide is added to the working buffer. In the absence of the preservative, the tissue electrode shows a loss of response and selectivity; eventually, bacterial growth on the tissue material becomes visible. However, the addition of sodium azide at a concentration of 0.02 percent completely eliminates this problem, and electrode lifetimes of at least 30 days are routinely obtained with no significant changes in the response characteristics of the electrode system during that period.

Summary. The use of intact bacterial cells or tissue slices of plant and animal origin as immobilized biocatalysts has extended the possible range of potentiometric bioselective membrane electrodes beyond that of conventional enzyme electrodes. The use of such materials as biocatalysts offers advantages in situations where isolated enzymes are not available or where multistep reaction paths are required. The resulting bioselective electrodes also offer exceptional ease of preparation, time stability, and low cost.

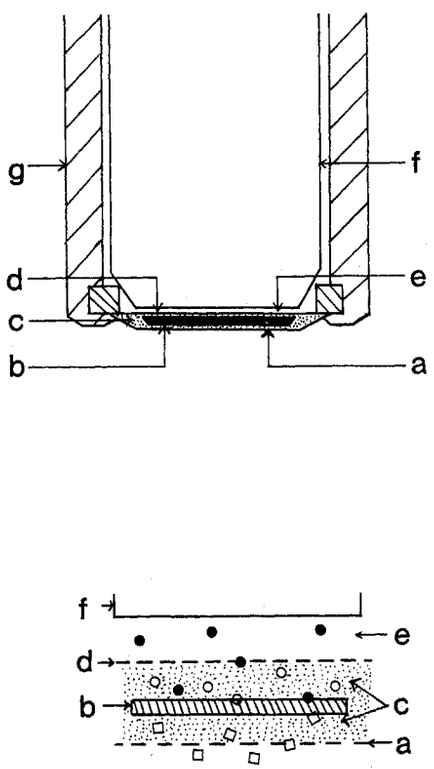

Fig. 1. Schematic of liver tissue–enzyme electrode for arginine: *a*, dialysis membrane; *b*, bovine liver tissue slice; *c*, urease enzyme suspension; *d*, gas-permeable membrane; *e*, internal electrolyte; *f*, combination *p*H electrode; *g*, plastic electrode body; □, arginine substrate; ○, urea intermediate; ●, ammonia product. (Components *d* to *g* constitute the ammonia gas–sensing electrode.)

A study (*15*) of glutamine-selective potentiometric membrane electrodes that compared the use of isolated enzyme, bacteria, mitochondria, and animal tissue as biocatalysts clearly identified the advantages of the tissue type in terms of low cost, superior mechanical and time stability, and ease of preparation. Such electrodes may become particularly attractive in situations where isolated, purified enzymes are not readily available, are unstable, too costly, or require cofactors for proper biocatalytic activity. In developing nations, moreover, fresh animal tissues are likely to be more readily available than pure enzyme preparations.

An interesting variation on the potentiometric tissue-based electrode concept has been reported by Updike and Treichel (*16*). These authors constructed an electrode with response to antidiuretic hormone (ADH) by stretching a toad bladder tissue membrane over the surface of a sodium ion–sensing glass electrode; this arrangement permits monitoring of potentials resulting from the enhanced transport of sodium ions across the toad bladder membrane in the presence of ADH, with a rough proportionality between the magnitude of the potential and the hormone concentration over a narrow range. Since ADH-enhanced sodium ion transport occurs in only one direction—from the mucosal to the serosal side of the toad bladder—the membrane can be oriented with the mucosal side touching the sodium-sensing glass electrode so that a decrease in sodium ion activity is produced at the inner sensor when ADH is present in the sample. This decrease is produced within just 10 seconds; as a result, a rapid assay for ADH might be feasible. Unfortunately, sodium transport in the toad bladder is altered by other hormones such as aldosterone, thyroxine, and angiotensin, as well as by adenosine 3′,5′-monophosphate and potassium or calcium ions. This lack of discrimination in membrane

action and the interference problems inherent to the sodium-sensing glass electrode that serves as the inner component probably mean that this particular tissue electrode cannot be made selective and thus will have limited practical usefulness.

In some cases, it becomes possible to improve the selectivity of a tissue-based electrode by "tuning" of the biochemical steps involved. This was recently demonstrated (*17*) by the selectivity enhancement of an adenosine-sensing electrode in which mouse small intestine mucosal tissue serves as the biocatalytic layer. In its untreated state, such tissue does not effectively discriminate between adenosine and the related phosphate nucleotides, owing to alkaline phosphatase enzyme activity in the tissue along with the desired adenosine deaminase activity. However, studies have shown (*17*) that the interfering activity can be effectively suppressed with glycerol phosphate or by an inhibitor such as L-phenylalanine to yield a final electrode system that is highly selective to the primary substrate, adenosine.

Tissue-based membrane electrodes are still in such an early stage of development that numerous practical and fundamental questions remain to be answered. What, for example, is the optimum thickness of the tissue slice to be used? Experiments in our laboratory have shown that the answer involves a compromise between maintaining mechanical integrity and holding dynamic response times of the electrodes to reasonable limits. In the case of a rabbit muscle tissue–based electrode for adenosine 5′-monophosphate (AMP), we found that a tissue thickness of 0.5 mm gives overall electrode response times of 3 minutes in the millimolar AMP concentration range; yet even this small quantity of tissue contains 50 times the effective biocatalytic activity of an equivalent volume of the purified commercially available AMP deaminase enzyme preparation.

The latest advance in the development of tissue-based electrodes is the discovery that materials of plant origin can be used as effective biocatalysts (*18*). Figure 3, for example, shows a plant tissue electrode devised from the mesocarp skin layer of the growing portion of a yellow squash, with a carbon dioxide gas–sensing probe as the inner element. The squash tissue slice serves as a biocatalyst for the breakdown of glutamic acid to yield products including carbon dioxide, whose production gives rise to a potential reading related to the concentration of glutamic acid in the sample. The possibility of using biocatalysts drawn from plant tissues may, in the future, greatly extend the possible range of tissue-based potentiometric membrane electrodes.

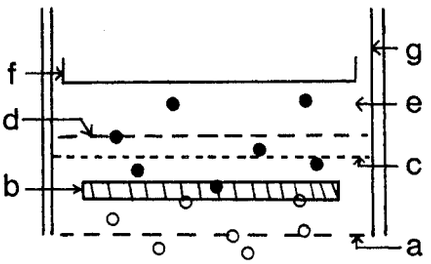

Fig. 2. Expanded view of porcine kidney tissue sensor for glutamine: *a*, nylon mesh or dialysis membrane; *b*, kidney tissue slice; *c*, dialysis membrane; *d*, gas-permeable membrane; *e*, internal electrolyte; *f*, combination *p*H electrode; *g*, plastic electrode body; ○, glutamine substrate; ●, ammonia product.

The cost of tissue electrodes is being reduced through the use of very inexpensive internal electrode elements (*19*) to a point where the possibility of completely disposable electrodes may become a reality.

Bacterial Electrodes

Although Divies (*20*) used bacteria with electrochemical detection for ethanol analysis as early as 1975, the first potentiometric membrane electrode with living bacterial cells serving as biocatalysts appears to be the arginine-selective electrode described (*21*) in 1976. An excellent review of the state of the art to 1979 was recently given by Kobos (*22*).

The experimental arrangement for potentiometric bacterial membrane electrodes is exceptionally simple. All that is necessary is to hold a layer of bacterial cells at the surface of an ion- or gas-sensing potentiometric membrane electrode selected on the basis of its response to some product of the bacterial metabolism. Although most of the successful bacterial electrodes described to date have used a selected cultured strain of bacteria, even this is not an absolute requirement, as has been demonstrated by the limited but workable sugar sensor that uses crude human dental plaque (*23*) spread on a *p*H glass electrode.

A more typical arrangement is that shown in Fig. 4 for the highly selective glutamine electrode (*24*). Bacterial cells of the pure strain *Sarcina flava* (American Type Culture Collection No. 147) are held at the surface of an ammonia gas–sensing membrane electrode by means of a dialysis membrane. Although the volume of cells held at the electrode surface is only 10 to 15 microliters, even this quantity corresponds to 10^8 to 10^9 living bacteria. The bacterial cell layer is obtained quite readily by culturing the appropriate strain under sterile conditions, harvesting and washing the cells, compacting the cells from suspension by gentle centrifugation, and spreading the cells manually on the surface of the inner potentiometric electrode. The *Sarcina flava* strain is sufficiently selective in its biocatalytic activity that this electrode can be used for the measurement of L-glutamine in the presence of the other essential amino acids, even in such complex samples as human blood serum. Provided that care is taken to prevent

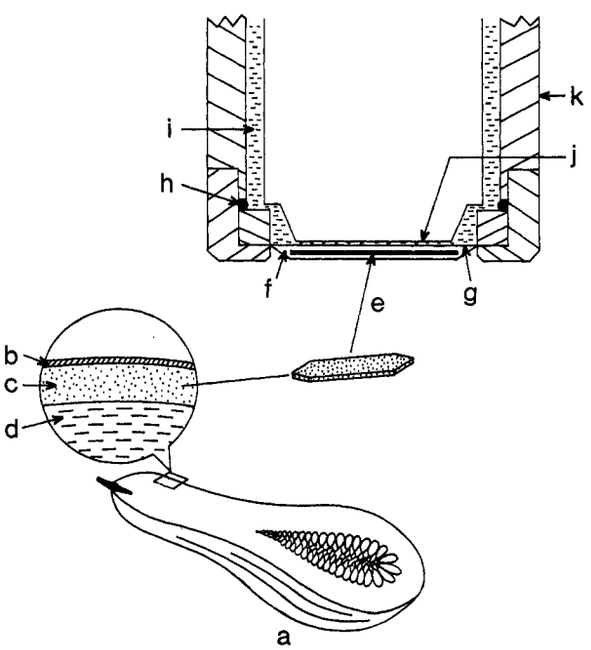

Fig. 3. Pictorial diagram of a plant tissue–based membrane electrode for glutamic acid; *a*, yellow squash plant; *b*, pericarp skin layer; *c*, mesocarp skin layer; *d*, endocarp skin layer; *e*, squash tissue slice from mesocarp layer; *f*, immobilizing matrix; *g*, gas-permeable membrane; *h*, spacer; *i*, internal electrolyte; *j*, combination *p*H electrode; *k*, plastic electrode body. (Components *g* to *k* constitute the carbon dioxide gas–sensing electrode.)

contamination, useful electrode lifetimes of 20 days or more are easily attained with no significant changes in response characteristics. Walters *et al.* (*25*) recently improved the physical design of bacterial electrodes by substituting a sterilizing filter membrane for the dialysis membrane in their preparation of a L-histidine–sensing electrode based on the strain *Pseudomonas* sp. (American Type Culture Collection No. 112996).

A distinctive feature of bacterial membrane electrodes is the possibility that their lifetimes may be extended through regeneration, that is, through the regrowth of fresh cells on the electrode surface. This effect has been demonstrated with an electrode for L-aspartate (*26*), based on the strain *Bacterium cadaveris* at an ammonia gas–sensing membrane electrode, and the L-cysteine (*27*) electrode, which uses *Proteus morganii* in conjunction with a hydrogen sulfide gas–sensing membrane electrode. In both cases, the biocatalytic activity can be regenerated by placing the spent electrode back into the nutrient growth medium used for the culturing of the respective bacterial strain. Apparently, fresh cells are grown in situ at the electrode surface so that the initial biocatalytic activity can be largely restored and the electrode lifetime extended; this method becomes self-limiting after a few regrowth cycles owing to the buildup of cellular debris at the electrode surface.

Bacterial electrodes need not be restricted to sensing biochemical substances. Kobos *et al.* (*28*), for example, have developed a bacterial electrode for the determination of nitrate ion. This electrode uses bacteria of the strain *Azotobacter vinelandii* in conjunction with an ammonia gas–sensing internal electrode. Nitrate is reduced to ammonia in the two-step sequence

$$NO_3^- + NADH \xrightarrow[\text{reductase}]{\text{nitrate}} NO_2^- + NAD^+ + H_2O \quad (3)$$

$$NO_2^- + 3NADH \xrightarrow[\text{reductase}]{\text{nitrite}} NH_3 + 3NAD^+ + 2H_2O \quad (4)$$

where both enzymes are contained in the bacterial cells. Nitrate concentrations as low as $10^{-5}M$ can be determined with a precision of 3 to 4 percent.

This electrode illustrates another favorable aspect of bacterial electrodes; in addition to the two enzymes, the cofactor NADH (nicotinamide adenine dinucleotide, reduced) and a means for its regeneration are all contained within the bacterial cells in an optimized environment. Thus, it is not necessary to find a method for the joint immobilization of these several materials, and this represents a desirable simplification in the preparation of bioselective membrane electrodes.

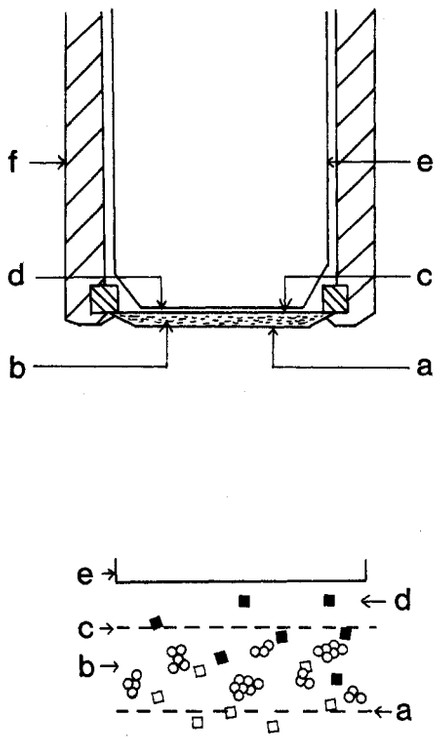

Fig. 4. Cross-sectional view of bacterial electrode for glutamine: *a*, dialysis membrane; *b*, bacterial layer; *c*, gas-permeable membrane; *d*, internal electrolyte; *e*, combination *p*H electrode; *f*, plastic electrode body; □, glutamine substrate; ■, ammonia product.

Such a consideration becomes especially important when complex multistep biocatalytic pathways are necessary to convert the substrate to be determined into a form that can be sensed by the internal electrode element. Two approaches have been taken in such cases. First, "hybrid" electrodes have been constructed in which a bacterial strain and a separate enzyme catalyst are jointly immobilized at the electrode surface as, for example, in the nicotinamide adenine dinucleotide (NAD) (*29*) electrode system. In this case, the enzyme NAD^+ nucleosidase was used with whole cells of *Escherichia coli* to provide the sequence

$$NAD^+ + H_2O \xrightarrow{NAD^+ \text{ nucleosidase}} \text{nicotinamide} + \text{adenosine diphosphate–ribose} \quad (5)$$

$$\text{nicotinamide} + H_2O \xrightarrow[\text{deaminase}]{\text{nicotinamide}} \text{nicotinic acid} + NH_3 \quad (6)$$

where the ammonia gas produced is sensed by the internal membrane electrode element.

A much more elegant solution to the multistep sequence problem is to utilize bacterial strains that already contain all the necessary enzymes and cofactors to carry out the entire complex reaction sequence. An exceptionally clear example of this approach was recently reported by Kobos and Pyon (*30*), who developed a bacterial sensor for nitrilotriacetic acid. In this case, the bacterial cells of the *Pseudomonas* strain must carry out a four-step reaction sequence to convert the nitrilotriacetic acid to ammonia. The greatest biocatalytic activity was obtained with bacterial cells harvested early in the exponential growth phase; as a result, good sensitivity and response slope could be achieved, although several significant interferences may limit the practical usefulness of this sensor.

Future Prospects

Recent work in Suzuki's laboratory on the use of immobilized whole cells in conjunction with a *p*H electrode in a flow system (*31, 32*), could easily lead to new bioselective membrane electrodes if the cells were held directly at the electrode surface. Specifically, it was shown that cephalosporin (*31*) could be determined with immobilized *Citrobacter freundii* cells and nicotinic acid (*32*) with immobilized *Lactobacillus arabinosus*, on the basis of the *p*H changes produced.

Work in progress in our laboratory suggests that yet a new class of bioelective membrane probes may be possible through the use of anaerobic bacteria. Intact cells of the type *Clostridium acidiurici* immobilized at a potentiometric ammonia gas sensor yield (*33*) an electrode for L-serine with good response characteristics. Although these bacterial cells must be grown under anaerobic conditions, the final sensor can be effectively employed in nondeaerated samples. It may also be possible to prepare electrodes for high-temperature measurement by using thermophilic bacteria.

Both practical and fundamental studies need to be carried out on bacterial and tissue-based potentiometric electrodes. There is as yet no general theoretical formulation for the steady-state and time-dependent behavior of these electrodes in terms of geometric and kinetic parameters. A systematic investigation of the various possible immobilization procedures and their effect on electrode response is urgently needed. Finally, considerably greater effort is required to investigate means—through blocking or inhibition of undesired metabolic pathways—of improving the selec-

tivity of whole cell bioselective membrane electrodes.

At present, significant research on bacterial and tissue-based potentiometric membrane electrodes is being carried out in just three or four laboratories around the world. This situation is likely to change, however, as improved techniques are found to improve the characteristics of such electrodes and to demonstrate their practical utility for bioanalytical measurements. In view of the great number and range of biological materials that might be used in conjunction with ion- or gas-sensing membrane electrodes to make potentiometric sensors, future research is almost certain to result in some exceptionally attractive new measurement devices. The advantages of simplicity and low cost that can be realized with such electrodes are already fully apparent.

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Recent Developments in Nuclear Magnetic Resonance Spectroscopy

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From modest but promising beginnings in the 1940's and 1950's, nuclear magnetic resonance (NMR) spectroscopy has developed into an important research tool (*1*). Early in its history, NMR spectroscopy was adapted from sole use by physicists, who had first discovered it, to the realm of chemists, who saw the potential of the so-called chemical shift phenomenon as a structural probe. This first useful parameter has now been supplemented by many other experimentally accessible quantities. In this article we outline some of these new developments in NMR spectroscopy.

In a review of this scope it is not possible to cover all developments in an area. We have attempted to provide a brief overview of the advances in technology during the past several years together with a discussion of some of the many new applications. We have stressed applications in chemistry and biology, as these are the areas in which most of the new uses of NMR have occurred. Of course, there are also many applications in physics, and the future holds promise for the further expansion of NMR spectroscopy into other disciplines. For example, routine medical diagnosis through NMR seems possible in the foreseeable future. Geological applications are also under development, largely as a result of advances in the technology associated with NMR of solid samples. We hope that this review will demonstrate some of the strengths of NMR to scientists not familiar with the technique and perhaps stimulate some further new applications.

Instrumentation

In broad terms, most new applications of NMR in recent years have derived from parallel improvements in instrumentation and methods. The instrumental improvements may be categorized as follows:

1) The development and use of spectrometers operating at higher magnetic fields, in some cases with large and versatile probe (sample) geometries.

2) The development of multinuclear spectrometers.

3) Improved spectrometer design for Fourier transform techniques and higher sensitivity for NMR with protons and other nuclei.

4) Advances in computer capabilities.

Magnetic field strength. One of the primary instrumental improvements has been the use of stronger magnetic fields. In the early 1960's it was rare for an NMR spectrometer to be other than a 60-megahertz instrument, capable of observing only sensitive nuclei such as protons. Commercial spectrometers of the day were based on iron permanent or electromagnets with fields of about 1.4 tesla (14 kilogauss). In the middle 1960's electromagnets with fields of 2.3 tesla (equivalent to a resonant frequency of 100 MHz for protons) became available, and they are still in extensive use. This field represents about the limiting strength of a conventional NMR magnet. However, since the early 1970's there has been increasing use of superconducting solenoid–based systems, which are capable of much higher magnetic fields.

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0036-8075/81/1016-0291$01.00/0