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Antibody-Selective Membrane Electrodes

Abstract. *Direct antibody-sensing membrane electrodes have been developed by immobilizing ion-carrier immunogen conjugates in a liquid membrane matrix. The resulting potentiometric probes measure specific antibodies with high selectivity over nonspecific antibodies in the physiological* pH *range. The electrode response is shown to arise from the selective interaction of the antibody with the membrane-bound immunogen.*

Efforts to develop potentiometric electrodes have not been successful in producing practical sensors for antibodies (*1*). We now describe new electrode probes in which conjugated ion carriers in a liquid membrane configuration are used to produce selective sensors for direct antibody measurements. The resulting electrodes achieve a reproducible response to the antibody to dinitrophenol (DNP) and to the antibody to bovine serum albumin (BSA), respectively, with a high degree of selectivity over other nonspecific antibodies in dilute serum samples.

The electrodes (Fig. 1) were prepared by incorporating membrane disks (4 mm in diameter and 0.2 mm thick) in commercially available (Orion Research 92 Series) electrode bodies. Membranes were formed by immobilizing the ion-carrier conjugates in polymer films. Polyvinyl chloride was chosen as the matrix for the DNP ion-carrier conjugate and triacetylcellulose was used to form the membrane for the BSA ion-carrier conjugate. The triacetylcellulose was chosen for the latter conjugate because of compatibility with the membrane constituents.

We chose dibenzo-18-crown-6 (*2*) as the ion carrier because its electrode properties have been well documented (*3*). The carrier was activated by nitration (*4*) and then reduced to the arylamine by tin (metal) and hydrochloric acid. The model hapten, DNP, was covalently coupled to the carrier by the Sanger reaction (*5*), whereas the BSA was coupled to the diazotized carrier.

Potentiometric measurements at 30°C were compared to a single-junction reference electrode (Orion 90-01) in tris-HCl buffer (pH 7.5) and phosphate buffer (pH 7.2) for DNP and BSA, respectively. Because dibenzo-18-crown-6 is a cation carrier, all solutions were prepared to contain fixed amounts of either K^+ or Na^+, with the ionic strength adjusted to 0.154*M* with $CaCl_2$. This ensures that the observed potentiometric effects are the result of the immunochemical reactions and are not caused by changes in ion activities, pH, or ionic strength.

The results of potentiometric measurements carried out with the electrode for detecting antibody to DNP (anti-DNP electrode) are shown in Fig. 2. The electrode responds selectively to samples containing the antibody to the hapten conjugated to the ion carrier. The antibody to BSA is measured as a blank because the antiserum to DNP was produced by means of a DNP-BSA conjugate.

The potential response of the electrode for antibody to BSA is shown in Fig. 3. To prepare an appropriate blank sample, we treated the antiserum to BSA with immobilized BSA to remove the antibody to BSA (*6*). The electrode response to this "absorbed" antiserum shows a significant decrease in the magnitude of the potential changes.

As to selectivity, both the antiserum to DNP and the antiserum to BSA were in media of whole immune serums (Miles Laboratories) containing the full spectrum of serum proteins. Measurements were also carried out with membranes containing only the unconjugated ion

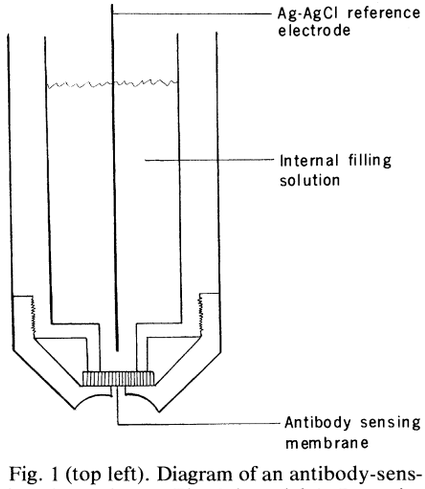
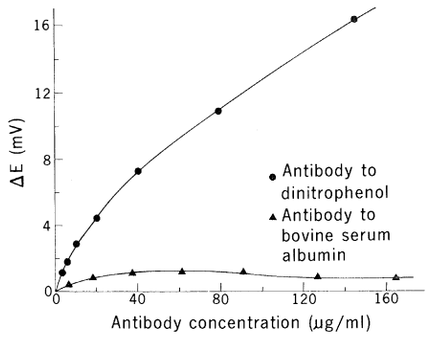
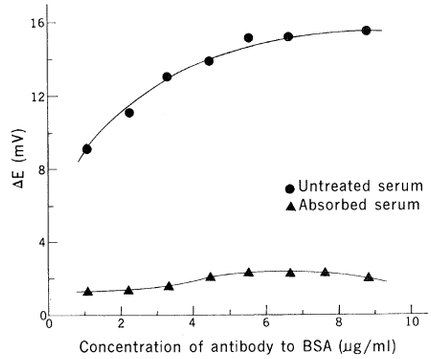


Fig. 1 (top left). Diagram of an antibody-sensing electrode. Fig. 2 (top right). Potentiometric response of the anti-DNP electrode to antibody to DNP and antibody to BSA in tris-HCl buffer (pH 7.5). ΔE is the change in potential relative to an Orion 90-01 single-junction reference electrode. Fig. 3 (bottom right). Potentiometric response of the anti-BSA electrode to antibody to BSA and the absorbed antiserum to BSA in phosphate buffer (pH 7.2). The untreated serum is whole antiserum to BSA and the absorbed serum is the immunoabsorbent-treated antiserum to BSA.





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carrier to show whether or not the potential responses were generated by simple protein absorption to the membranes. These experiments yielded electrode potentials near the baseline and indicate that the potential changes obtained with the complete immunoelectrode are the result of interactions of the conjugate with its specific antibody. Further experiments were performed with the anti-DNP electrode by adding dinitroaniline (which mimics the coupled DNP) to the test solution. The antiserum to DNP was then added, and no potential changes were observed, an indication that the reaction at the electrode is of the antigen-antibody type.

Potential readings taken for a range of concentrations of antibody to DNP from 2.8 to 145.1 μg/ml yielded a standard deviation of 4.1 percent, with individual potential readings reproducible to $\pm$ 0.2 mV. The anti-DNP electrode retained its effectiveness for at least 2 months, and the observed potential changes were fully reversible. The anti-BSA electrode measured concentrations of antibody to BSA between 1.1 and 8.8 μg/ml with similar results. The standard deviation was 4.3 percent, and potential readings were reproducible to $\pm$ 0.6 mV. Both electrodes required from 6 to 16 minutes to reach steady-state potentials after the addition of antibody samples.

Thus, such electrodes could be used to measure antibody levels in body fluids. Although we have focused our attention on a model system involving the hapten dinitrophenol and its antibody, the preliminary work with the anti-BSA electrode has shown that this principle can be extended to other antigen-antibody systems when the immunogen can be coupled with a membrane carrier.

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A Synthetic Pentapeptide with Biological Activity Characteristic of the Thymic Hormone Thymopoietin

Abstract. *The pentapeptide arginyl-lysyl-aspartyl-valyl-tyrosine, corresponding to amino acid residues 32–36 in thymopoietin, was synthesized. In vitro, this pentapeptide induced the differentiation of murine prothymocytes to thymocytes and inhibited differentiative induction of cells of the B lineage. This combination of actions is presently unique to the parent molecule thymopoietin. In vivo, the pentapeptide reduced the high numbers of autologous rosette-forming cells normally present in the spleens of athymic mice; this also is a property of thymopoietin. These results suggest that this readily synthesized pentapeptide corresponds to an active site of thymopoietin and might serve as a therapeutic substitute for thymopoietin.*

Thymopoietin is a thymic hormone, and the complete sequence of the 49 amino acids of this single polypeptide chain is known (*1*). Investigation of the active site or sites of this molecule has practical as well as theoretical value. For clinical use, the yield of thymopoietin from natural sources would be insufficient (*2*), it might be prohibitively antigenic, and synthesis of the entire molecule, although this has been achieved (*3*), would be impractical on a sufficiently large scale. A tridecapeptide of thymopoietin, consisting of amino acids 24 to 41 (thymopoietin$_{24-41}$), has been synthesized (*4*) and shown to share biological properties of the native molecule (*4*). We report here the synthesis of a pentapeptide that corresponds to amino acids 32 to 36 of thymopoietin, thymopoietin$_{32-36}$, (TP5), and show that it also has biological properties characteristic of thymopoietin.

The pentapeptide arginyl-lysyl-aspartyl-valyl-tyrosine was synthesized both by solid-phase methods and by classical solution synthesis. The products from both types of synthesis, purified by molecular sieving, ion exchange, and partition chromatography, were indistinguishable, and were greater than 99 percent pure according to thin-layer chromatography in five solvent systems and on high-pressure liquid chromatography. In vitro activity of TP5 was studied in induction assays of T cell and B cell differentiation (*5*), and also in vivo for effects on autologous rosette-forming cells (ARFC) (*6*) in the spleens of athymic *nu/nu* mice.

Induction assays in vitro have been of great value in elucidating the nature and mechanisms of some of the differentiative processes whereby lymphocytes progress from immunoincompetent precursors to immunocompetent cells (*5*, *7*, *8*). This progression is marked by selective expression of cell surface com-

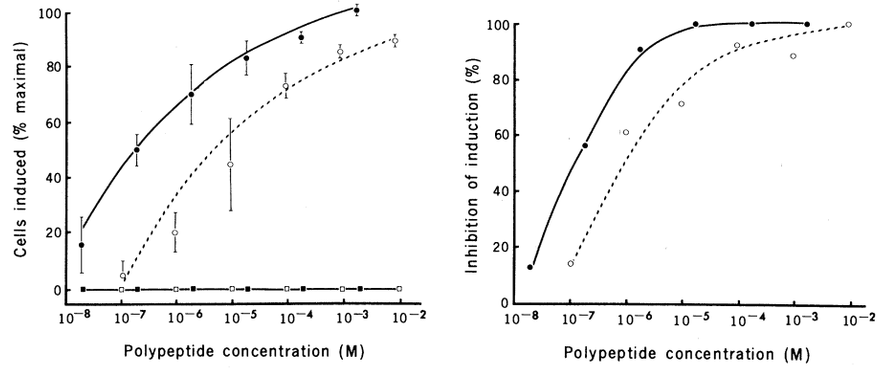

Fig. 1 (left). Selective induction of Thy-1$^+$ cells (● – ○) but not of CR$^+$ B cells (■ – □) by thymopoietin (● – ■) and TP5 (○ – □). Conditions and technique of induction of fractionated spleen cells from *nu/nu* mice were according to Scheid *et al.* (*5*). In concentrations of 10^{-6} *M* to 10^{-3}*M*, TP5 induced Thy-1$^+$ differentiation corresponding to thymopoietin over the concentration range 1.8×10^{-8}*M* to 1.8×10^{-5}*M*. To allow for variation in the proportion of inducible and induced cells in different assays, the data shown (mean $\pm$ standard error, three assays) were standardized for each assay as percent of maximal induction with optimal concentration of inducer (range, 15 to 25 percent) (*5*). Fig. 2 (right). Dose response data showing similar inhibition by thymopoietin (●) and TP5 (○) in the PC$^+$ B cell induction assay on Peyer's patch lymphocytes from *Corynebacterium parvum*–primed HSF/SN mice. Carbamyl choline (10^{-5}*M*) was used as the inducer for these experiments [induction technique of Scheid *et al.* (*5*)]. The data are standardized as percent of maximal inhibition which is complete at the higher concentrations. One of four representative experiments is shown.

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