

FIGS. 2 and 3 are diagrammatic representations of electrodes employing bispecific antibody determinants.

FIG. 4 is a diagrammatic representation of a self-assembling network employing bispecific antibody determinants.

FIG. 5 is a diagrammatic representation of a multilamellar assembly useful for an assay method.

The bispecific antibody determinants of the invention are useful for a wide range of applications. Referring to FIG. 1, these applications all flow from the ability of these determinants to serve as highly specific linkers through specific sites A' and B', of any two antigenic determinants A and B capable of stimulating antibody production in animals; e.g., effective proteins, polypeptides, carbohydrates, nucleic acids, or haptens, either free or immobilized on surfaces or particles.

One application of the bispecific antibody determinants of the invention is their use as agents for bonding a desired antigenic entity to a desired surface which has a different antigenic determinant immobilized on it. For example, enzymes so immobilized on particles or membranes can be used as solid-state catalysts. Advantages of this type of immobilization over others are that antibodies can be selected which have no adverse effect on enzyme activity, and that pure enzymes can be immobilized from impure mixtures. Bispecific antibody determinants can also be used as highly specific bispecific reagents for immunoassay procedures which are used, e.g., in the diagnosis of medical disorders, or as molecular probes to study the relationships between antigenic determinants in biological systems.

An additional application of the bispecific antibody determinants is their use in electrodes. Currently-used enzyme electrodes frequently employ tissue slices as the enzyme source. For example, electrodes for measuring glutamine have been made using a conventional NH₃ electrode in combination with kidney slices as the source of glutaminase, the enzyme which breaks down glutamine to produce measurable NH₃ ions; Rechnitz (1981) Science 214, 287-291.

The present invention provides electrode apparatus for the measurement in a sample of an unknown amount of a substance which is acted on by one or more enzymes to evolve a measurable ion or compound, the ion or compound evolved being a measure of the unknown substance. The electrode apparatus includes means for measuring the measurable ion or compound, and, associated with that means, a membrane having associated therewith a plurality of molecules of each enzyme which acts on the substance to be measured and, bonded to the molecules of each enzyme, a plurality of identical, bispecific antibody determinants. Each determinant is composed of two different L-H half-molecules linked by disulfide bonds, and each half-molecule includes at least the F(ab') portion of a monoclonal IgG antibody. One said L-H half-molecule is specific for an antigenic site on the enzyme molecule to which it is bonded and the other half-molecule is specific for an antigenic determinant on the membrane to which the bispecific antibody determinant is bonded to become immobilizably associated with the membrane.

The electrode can be used to measure any substance which can be metabolized by an enzyme or combination of enzymes in a way which produces or consumes a measurable ion or compound such as NH₃, CO₂, O₂, or H⁺, provided that each enzyme can bind specifically to a site on an immobilized bispecific antibody determinant.

The reaction can be one which requires more than one enzyme. It is required in such a case that all of the required enzymes be immobilized on bispecific antibody determinants which are immobilized in the electrode.

FIGS. 2 and 3 illustrate two modes of enzyme immobilization in a two-enzyme system in which the two enzymes catalyze consecutive reactions in the conversion of a substance to an ion or compound which can be measured by the appropriate ion or compound-specific membrane electrode.

Referring to FIG. 2, membrane 2 of electrode 4 bears, on spacer arms 3 and 5, different haptens A and B, in the desired ratio, to which are immobilized different bispecific antibody determinants having, respectively, hapten-specific sites A' and B'. The second site on each bispecific antibody determinant is specific, respectively, for binding sites on enzymes C and D, which catalyze consecutive steps in the breakdown of the substance to be measured into a measurable compound or ion.

Referring to FIG. 3, membrane 6 of electrode 8 bears, on spacer 7, hapten A, to which is immobilized a bispecific antibody determinant having hapten A-specific site A' and a second site, B', which is specific for binding site B on one of the two enzymes necessary for the breakdown of the substances to be measured into a measurable compound or ion. The second bispecific antibody determinant has a site, C', specific for antigenic binding site C on the first enzyme, and a second site, D', specific for a different antigenic binding site D on the second enzyme required for the production of the measurable compound or ion. The advantage of the arrangement shown in FIG. 4 is that it assures that the two enzymes are closely linked so that the two reactions are efficiently coupled.

Enzyme electrodes made using bispecific antibody determinants possess several advantages over conventional enzyme electrodes. One advantage is their precise self-assembling property: the desired electrode assembly is generated simply by attaching the appropriate hapten or haptens to the membrane (either the electrode membrane or a separate membrane associated with the electrode) and then immersing the hapten-derived membrane into a solution containing the appropriate bispecific antibodies and enzymes. This ease of assembly also means that the electrode can be easily recharged after deterioration has occurred through prolonged use.

Another advantage of the electrodes is also a function of the specificity of the bispecific antibody determinants. Any given enzyme will possess a number of antigenic sites capable of binding to a specific site of an antibody. However, coupling at many of these sites can cause inactivation of the enzyme. In the case of bispecific monoclonal antibody determinants, this problem is avoided because the determinants are selected so that they couple with the enzyme only at a site which does not cause deactivation of the enzyme.

A further advantage is that assembly or recharging of the electrode can be done with impure enzyme mixtures because the unique specificity of the bispecific antibody determinants assures the selection of the proper enzymes from the impure mixture.

In some instances the membrane containing the immobilized enzymes can be covered with a second semi-permeable membrane to slow the deterioration of the electrode assembly, or the assembly can be stabilized by treatment with glutaraldehyde.