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DETERMINATION OF ANALYTE CONCENTRATION BY RE-EQUILIBRATION

This invention relates to a process for the determination of analyte concentration in ligand binding assays for in vitro analysis of for example thyroxine (T4), insulin and thyroid stimulating hormone levels in blood.

It is known to determine analyte concentration by ligand binding assay techniques, which include competitive binding assay and sandwich assay techniques. In one form of competitive binding assay, the reactant to be measured, such as antigen A, is incubated with labelled reactant of known concentration and binder, such as antibody against antigen A, of known concentration, so that labelled and unlabelled antigen compete for binding sites on the binder. The bound antigen-antibody complex is separated from free labelled and unlabelled antigen and the signal from the bound complex bears a mathematical relationship to the unlabelled antigen A to give an estimate of the concentration thereof.

Generally, separation is achieved by immobilising the antibody on a solid phase such as plastics tube, beads, or inert particles, or by precipitation of the complexes.

In the sandwich assay technique, the reactant to be measured, such as antigen B, is added sequentially or simultaneously with another reactant of known concentration such as a labelled binder, for example labelled anti-B antibody, to excess of unlabelled binder of known concentration, to form antibody-antigen-labelled antibody sandwich complexes.

The complexes and excess antibody are separated from the free antigen and free labelled antibody, generally by immobilising the unlabelled antibody on an inert solid phase prior to the reaction so that the sandwich complexes can be physically separated, for example by decantation. The signal from the sandwich complexes bears a mathematical relationship to the unlabelled antigen B to enable an estimate of the concentration thereof to be calculated.

The competitive binding assay technique may be used for determination of thyroxine (T4) and the sandwich assay technique may be used for determination of thyroid stimulating hormone (TSH) and insulin.

In the known techniques, repeated re-exposure of the reacted antigens with the reacted antibodies is used to speed up the attainment of equilibrium between the reactants and binders, but this causes analyte exhaustion to occur. In the sandwich assay technique, an additional disadvantage is that the bound antigen tends to dissociate, with resultant blocking of the binding of the labelled antibody to the bound antigen to form the sandwich compound. Where sequential addition of reactants is practised, dissociation of the bound antigen-labelled antibody moieties from the immobilised sandwich complexes also occurs. As the concentration of antigen increases, the degree of blocking or dissociation also increases, thus rendering the assay inaccurate. The blocking or dissociation effect is known as the "hook effect".

It is an object of the present invention to increase the sensitivity of the known ligand binding assay techniques.

According to the invention, we propose an in vitro process for determination of analyte concentration in ligand binding assays, the process comprising an equilibration reaction between the analyte to be determined and a reactant immobilised on a solid phase support, wherein active sites on the support together with some immobilised reactant sites are blocked with protein before the equilibration reaction.

As applied to the competitive binding assay technique, the process according to the invention comprises blocking active sites on the support, together with some of the binding sites of the antibody, before the reaction with labelled and unlabelled antigen, and unbound labelled and unlabelled antigen is discarded after the equilibration reaction.

As applied to the sandwich assay technique, the process according to the invention comprises blocking active sites on the support, together with some of the binding sites of the unlabelled antibody, before the reaction with labelled antibody and unlabelled antigen, and unbound labelled antibody and unlabelled antigen is discarded after the equilibration reaction.

The advantage of the process according to the invention compared with the prior art is that, whereas in the prior art repeated re-equilibration causes analyte exhaustion to occur, according to the invention re-equilibration with fresh reactants may be carried out and the sensitivity of the resulting assay is enhanced with each re-equilibration by a factor of at least 3. In particular, the process according to the invention maintains the analyte concentration and, in the sandwich assay technique, decreases dissociation of

the bound antigen (where sequential addition of reactants is employed) or the antigen-labelled antibody moieties (where simultaneous addition of reactants is employed) from the immobilised sandwich complexes and thus prevents manifestation of the hook effect.

According to a preferred feature of the invention, therefore, fresh analyte to be measured is re-equilibrated with the reactant and analyte on the solid phase support as a result of the initial equilibration reaction, some of the protein blocking the reactant sites being displaced by analyte to be measured, the signal from the bound complexes being cumulated to reflect a more accurate relationship to the concentration of the analyte to be determined.

In the process according to the invention, separation of the bound complexes is carried out in the usual way and the signals from the labelled reactants measured to give an estimate of the unlabelled antigen or other reactant concentration after comparison with signals from the reactants of known concentration assayed in a similar manner.

Embodiments of the invention will now be described by way of example with reference to the accompanying drawings, in which Figures 1(a) and 1(b) illustrate diagrammatically the process according to the invention are applied to the competitive binding assay technique and

Figures 2(a) and 2(b) illustrate diagrammatically the process according to the invention as applied to the sandwich assay technique.

In the drawings, the symbols have the following meaning:-

-  Fresh unlabelled antigen
- *  Fresh labelled antigen
-  Protein
-  Unlabelled antibody
- *  Labelled antibody
- Solid phase support.

Referring firstly to Figure 1(a), the initial equilibration reaction is shown, in which fresh labelled and unlabelled antigen is reacted with antibody immobilised on a solid phase support, the remaining active sites on the support together with some of the antibody binding sites having been blocked with protein. The labelled and unlabelled antigen compete for the un-blocked binding sites and free (unbound) labelled and unlabelled antigen is discarded. In the re-equilibration reaction shown in Figure 1(b), fresh labelled and unlabelled antigen is further reacted with the immobilised antibody, some of the protein molecules being displaced by antigen so that the total amount of bound labelled and unlabelled antigen is more accurately in proportion to the amounts in the reactant mixture. Free labelled and unlabelled antigen is discarded, together with displaced protein.

Referring to Figure 2(a), which shows the initial sandwich assay equilibration reaction, fresh labelled

antibody and unlabelled antigen are reacted with unlabelled antibody immobilised on a solid phase support, the remaining active sites on the support together with some of the antibody binding sites having been blocked with protein. Unlabelled antibody-antigen-labelled antibody sandwich complexes are formed at the unblocked unlabelled antibody sites and free (unbound) labelled antibody and unlabelled antigen are discarded. In the re-equilibration reaction shown in Figure 2(b), fresh labelled antibody and unlabelled antigen are further reacted with the immobilised antibody, some of the protein molecules being displaced to allow the formation of additional sandwich complexes. Free labelled antibody and unlabelled antigen are discarded, together with displaced protein.



1. An in vitro process for determination of analyte concentration in ligand binding assays, the process comprising an equilibration reaction between the analyte to be determined and a reactant immobilised on a solid phase support, wherein active sites on the support together with some immobilised reactant sites are blocked with protein before the equilibration reaction.

2. A process according to Claim 1 comprising blocking active sites on the support, together with some of the binding sites of the antibody, before the reaction with labelled and unlabelled antigen, and discarding unbound labelled and unlabelled antigen after the equilibration reaction.

3. A process according to Claim 1, comprising blocking active sites on the support, together with some of the binding sites of the unlabelled antibody, before the reaction with labelled antibody and unlabelled antigen, and discarding unbound labelled antibody and unlabelled antigen after the equilibration reaction.

4. A process according to any preceding claim, in which fresh analyte to be measured is re-equilibrated with the reactant and analyte on the solid phase support as a result of the initial equilibration reaction, some of the protein blocking the reactant sites being displaced by analyte to be measured, the signal from the bound complexes being cumulated to reflect a more accurate relationship to the concentration of the analyte to be determined.



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5. A process as hereinbefore described with reference to and as illustrated in the accompanying drawings.
