

ENZYMATICALLY AMPLIFIED PIEZOELECTRIC SPECIFIC BINDING ASSAY

FIELD OF THE INVENTION

The present invention relates to an enzymatically amplified piezoelectric specific binding assay in which analyte suspected of being present in a liquid sample is bound either on or in the proximity of a quartz crystal microbalance by means of a capture reagent, and the bound analyte is reacted with an anti-analyte reagent/enzyme conjugate. The conjugate is reacted with a substrate specific for the enzyme to form a product that is capable of either reacting with and/or accumulating on the surface of the quartz crystal microbalance. The mass change on the surface of the quartz crystal microbalance resulting from these reactions results in a change in the resonant frequency of the quartz crystal microbalance, which can be used to determine the analyte concentration in the sample.

BACKGROUND OF THE INVENTION

The use of quartz crystal microbalances (also known as piezoelectric oscillators) in immunoassays has been described previously. These devices consist of single crystal wafers sandwiched between two electrodes. The electrodes are provided with means for connecting these devices to an external oscillator circuit that drives the quartz crystal at its resonant frequency. This frequency is dependent on the mass of the crystal, as well as the mass of any layers confined to the electrode areas of the crystal. Thus, the frequency is altered by changes in mass on the surface of the electrodes or in any layers on those electrodes. In general, the change in resonant frequency of these devices can be correlated to the amount of mass change; if the quartz crystal microbalance and any attached layers obey rigid-layer behavior, the mass change can be determined from the frequency change by the Sauerbrey relationship

$$\Delta f = - \frac{2f_0^2 \Delta m}{A \sqrt{\rho_q \mu_q}}$$

where Δf is the measured frequency shift f_0 the parent frequency of the quartz crystal, Δm the mass change, A the piezoelectrically active area, ρ_q the density of quartz (2.648 g cm^{-3}) and μ_q the shear modulus ($2.947 \times 10^{11} \text{ dynes cm}^{-2}$ for AT-cut quartz).

Shons et al. describe a piezoelectric quartz crystal microbalance which has been modified for the determination of antibody activity in solution. A quartz crystal, precoated with antigen, is exposed to antisera of varying concentration and specificity. Antisera specific for the antigen coating will form an additional protein layer on the crystal. The thickness of the antibody layer, measured by the frequency shift of the dry crystal, is proportional to the concentration of specific antibody in solution. [J. Biomed. Mater. Res., Vol 6, pp. 565-570 (1972)].

U.S. Pat. No. 4,235,983, issued to Rice on Dec. 2, 1980, discloses a method for the determination of a particular subclass of antibody. The method utilizes a piezoelectric oscillator having bound to its surface an antigen specific for the antibody to be determined. The antigen-coated oscillator is exposed to a solution containing an unknown amount of the antibody. After the antibody in the solution is attached to the antigen on the

oscillator, the oscillator is exposed to a so-called sandwiching substance which selectively binds to a specific subclass of the antibody being determined. The frequency of the oscillator is measured in the dry state before and after exposure to the sandwiching substance.

The change in frequency is related to the amount of the subclass of antibody bound to the oscillator, and the amount of the subclass of antibody in the solution can be determined by reference to a standard curve.

Roederer et al. disclose an in-situ immunoassay using piezoelectric quartz crystals, specifically, surface acoustic wave devices. Goat anti-human IgG was immobilized on the quartz crystal surface with a coupling agent. The piezoelectric crystals were then placed in an electric oscillator circuit and tested for detection of the antigen human IgG. Detection was based upon the fact that surface mass changes by adsorption are reflected as shifts in the resonant frequencies of the crystals. The authors concluded that the method suffers from both poor sensitivity and poor detection limits. The authors also concluded that the antigen to be detected must be of high molecular weight; low molecular weight analytes cannot be directly detected by this methodology. [Analytical Chemistry, Vol. 55, (1983)].

Ngeh-Ngwainbi et al. describe the use of piezoelectric quartz crystals coated with antibodies against parathion which are used for the assay of parathion in the gas phase. When the coated antibody binds with parathion by a direct reaction in the gas phase, the resulting mass change on the crystal generates a frequency shift proportional to the concentration of the pesticide. [J. Mat. Chem. Soc., Vol. 108, pp. 5444-5447 (1986)].

European patent application 0 215 669, published Mar. 25, 1987, discloses an analytical device and method for the in-situ analysis of biochemicals, microbes and cells. Again, the method is predicated on a resonant frequency change caused by a weight change on the surface of a piezoelectric crystal on which are immobilized receptor materials specific for the analyte to be detected.

Grabbe et al. describe a quartz crystal resonator, used in conjunction with cyclic voltammetry, to study the binding of human IgG and anti-IgG to a silver electrode. [G. Electroanal. Chem Vol 223, pp. 67-78 (1987)].

As discussed by Roederer et al., piezoelectric crystal-based immunoassays in which mass change is attributable only to the immunological reaction between an antigen and an antibody can, under certain circumstances, suffer from poor sensitivity and poor detection limit. Consequently there is a need in the art for a piezoelectric crystal-based specific binding assay in which the reaction between a binding agent and its ligand can be amplified to provide a more sensitive and reliable assay.

SUMMARY OF THE INVENTION

This need is met by the present invention which, in one aspect, is a process for measuring an analyte utilizing a conjugate comprising an enzyme and either an anti-analyte reagent or the analyte. The conjugate is capable of reacting with (or competing with) an analyte indirectly bound to a quartz crystal microbalance by a capture reagent that is directly bound to a surface of the quartz crystal microbalance. The quartz crystal microbalance may have at least one of its surfaces modified by any combination of chemically reactive, priming, coat-