

many labeling substances via a polypeptide support composed of polypeptide having a nano size and low specific gravity is used. Thus, when the polypeptide support is used, it is possible to link many labeling substances while maintaining the avidities of the binding substances, unlike the case where the labeling substance is directly linked to the binding substance not via the polypeptide support. Therefore, according to the method for electrochemically detecting an analyte according to the first embodiment of the present invention, it is possible to significantly improve the detection sensitivity. The polypeptide has the structure and sequence determined for each species. Additionally, the number of a bonding site to which the labeling substance can be bound (an amino group or sulfhydryl group as a side chain of an amino acid) is determined. Therefore, according to the method for electrochemically detecting an analyte according to the first embodiment of the present invention, it is possible to detect and quantify an analyte with high reproducibility as compared with the case where a support other than polypeptide, such as a metal nanoparticle is used. Further, the polypeptide can be synthesized *in vitro*. An amino acid residue to which the labeling substance can be bound (amino acid residue having an amino group or a thiol group) can be introduced into a desired site by genetic engineering. The number of the labeling substance in the label binding substance can be increased with sufficient controllability.

**[0101]** In the method according to the first embodiment of the present invention, a photochemically or electrochemically active substance is used as the labeling substance. The photochemically active substance is detected using electrons released by excitation of the substance by light. On the other hand, the electrochemically active substance is detected using an oxidation reduction current and/or electrochemical luminescence based on the substance. Therefore, the method according to the first embodiment of the present invention can be divided broadly into the photoelectrochemical detection method (see FIGS. 6 and 8) and the oxidation reduction current/electrochemiluminescence detection method (see FIG. 9) depending on the type of detection technique of the labeling substance.

#### 1. Photoelectrochemical Detection Method

**[0102]** First, the photoelectrochemical detection method will be explained. In the photoelectrochemical detection method, the detector illustrated in FIG. 1 and the detection chip illustrated in FIG. 3 can be used, however, they are not limited thereto.

**[0103]** Hereinafter, the method will be explained taking an example of the case of using the detector illustrated in FIG. 1 and the detection chip illustrated in FIG. 3.

**[0104]** Referring to FIG. 6, in the photoelectrochemical detection method, a user injects a sample containing the analyte S through the sample inlet 30*b* of the detection chip 20 [see the process of supplying a sample of FIG. 6A]. Thus, the analyte in the sample is trapped by the trapping substance 81 on the working electrode body 61 of the upper substrate 30 constituting the detection chip 20 [see the process of trapping an analyte of FIG. 6B]. In this case, substances (contaminants F) other than the analyte S in the sample are not trapped by the trapping substance 81.

**[0105]** The trapping substance 81 can be suitably selected depending on the type of the analyte S. For example, when the analyte S is a nucleic acid, a nucleic acid probe hybridizing to the nucleic acid, an antibody to the nucleic acid, a protein

binding to the nucleic acid or the like can be used as the trapping substance 81. When the analyte S is a protein or peptide, an antibody to the protein or peptide can be used as the trapping substance 81.

**[0106]** The process of trapping an analyte by the trapping substance 81 can be performed for example, under conditions where the trapping substance 81 is bound to the analyte. The conditions where the trapping substance 81 is bound to the analyte can be suitably selected depending on the type of the analyte. For example, when the analyte is a nucleic acid and the trapping substance 81 is a nucleic acid probe to be hybridized with the nucleic acid, the process of trapping an analyte can be performed in the presence of a hybridization buffer. When the analyte is a nucleic acid, protein or peptide and the trapping substance 81 is an antibody to nucleic acid, an antibody to protein or an antibody to peptide, the process of trapping an analyte can be performed in a solution suitable for performing an antigen-antibody reaction, such as phosphate buffered saline, a HEPES buffer, a PIPES buffer or a Tris buffer. When the analyte is a ligand and the trapping substance 81 is a receptor to ligand, or when the analyte is a receptor and the trapping substance 81 is a ligand to receptor, the process of trapping an analyte can be performed in a solution suitable for binding the ligand to the receptor.

**[0107]** Then, the user injects the label binding substance 90 into the detection chip 20 from the sample inlet 30*b* to allow the label binding substance 90 to be bound to the analyte S trapped on the working electrode body 61 [see the labeling process of FIG. 6C]. In the labeling process, a complex containing the trapping substance 81, the analyte S, and the label binding substance 90 is formed on the working electrode body 61.

**[0108]** The label binding substance 90 is formed of a polypeptide support 91, a first binding substance 92 to be bound to the analyte S, a labeling substance 93, and a first linker 94. In the label binding substance 90, the first binding substance 92 to be bound to the analyte S and the first linker 94 are directly immobilized on the surface of the polypeptide support 91. The labeling substance 93 is immobilized on the support 91 via the first linker 94.

**[0109]** The polypeptide support 91 is composed of polypeptide. The diameter of the polypeptide support 91 can be suitably set depending on the type of the analyte and the labeling substance and it is usually from 3 to 100 nm.

**[0110]** The polypeptide may be any of a naturally occurring purified polypeptide, a recombinant polypeptide synthesized *in vitro*, a genetically modified artificial polypeptide, and a chemically synthesized peptide.

**[0111]** The molecular weight of the polypeptide is preferably from 1000 to 1000000 Da, more preferably from 10000 to 700000 Da, still more preferably from 50000 to 500000 Da.

**[0112]** The shape of the polypeptide may be any shape capable of being formed by the polypeptide. Examples of the shape of the polypeptide include spherical, linear, and string shapes. However, the present invention is not limited only thereto.

**[0113]** The polypeptide may contain an amino acid residue which is easily bound to the labeling substance. Examples of the amino acid residue include amino acid residues having a primary amino group at the side chain (e.g. a lysine residue, an asparagine residue, and a glutamine residue) and amino acid residues having a sulfhydryl group (e.g. a cysteine residue). Among them, a polypeptide containing many lysine residues, namely, a strongly basic protein is effective in order