Cyclic AMP Detection by Electrode Modified with 17mer Oligopeptide

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An oligopeptide (17mer) which recognizes cyclic AMP was designed and applied to electrochemical detection of cAMP. Redox peak currents on cyclic voltammograms of ferrocyanide/ferricyanide redox couple with Au electrode which was modified with the peptide showed response dependently on the cAMP concentration, while the electrode showed only slight response to ATP that is a structural analog of cAMP.

Cyclic AMP is one of the most important second messengers in intracellular signal transduction, and is produced by various extracellular stimulation like hormone molecules. Monitoring of the intracellular concentration of this messenger is an essential technique for the understanding of cellular function in cell biology, pharmacology and physiology. Immunological method using antibody against cAMP is the common way to estimate the cAMP concentration.[1] However, this method is troublesome, because it requires multi steps. We are describing here a simple electrochemical analysis of cyclic AMP using the Au electrode modified with a cAMP binding peptide of 17 amino acids.

Cyclic AMP binding peptide we used in this study was designed by using cAMP binding site sequences of cAMP-dependent protein kinase.[2] This enzyme is a major target of cAMP in intracellular space and has two distinct binding sites for cAMP. Although these two sites have different kinetics on cAMP binding,[3] there is a highly conservative region in their amino acid sequences.[4] This region, which is also conserved in many species, was reported to form some hydrogen bondings with cAMP as demonstrated by X-ray diffraction study.[5] The sequences in many cAMP-dependent protein kinases in different species were extracted and the consensus peptide, FGELALVYNTPRAATV, was designed. This was synthesized with an automatic peptide synthesizer on Fmoc chemistry and was combined with cystein at the N-terminus to immobilize the peptide on Au electrode via chemisorption, i.e., a coordination to Au with the sulfhydryl moiety in the cystein.[6] A polished Au disk electrode (1.6 mm diameter, Bioanalytical Systems) was immersed in a solution of the peptide (50 μM in 50 mM Tris-HCl, pH 7.4 containing 50 mM KCl) for 24 h at 5 ℃. The modified electrode was then washed with and stored in the same buffer at 5 ℃. Cyclic voltammetric measurements were performed in similar method as described previously[7] by using a Bioanalytical System Co. Model CV-50W potentiostat. A Pt plate (10 x 10 mm) and a standard Ag/AgCl (saturated KCl) electrode were used as counter and reference electrodes, respectively.

Cyclic voltammograms of ferrocyanide/ferricyanide redox couple with a bare Au electrode and the modified one are shown in Figure 1. The peak currents due to the reversible redox reaction of the marker ions decreased on the modified electrode comparing with the bare one. This peak current depression is explained by a decrease of local concentration of the marker ions near the electrode mainly due to the steric barrier of the peptide

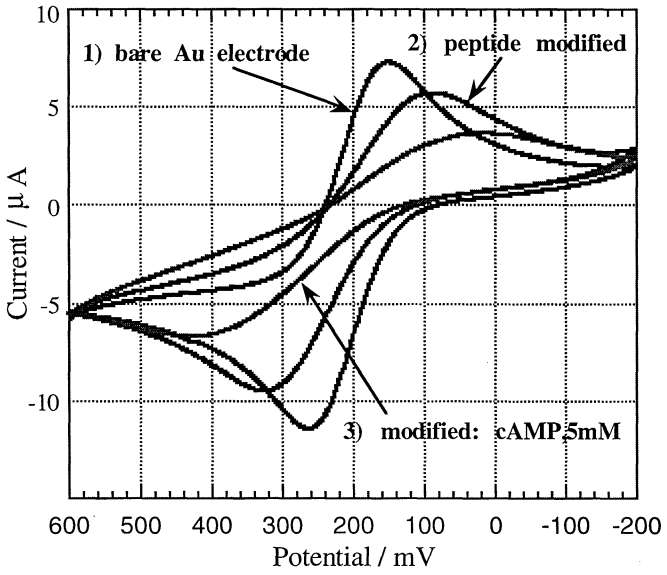

Figure 1. Cyclic voltammograms of Au electrode modified with the 17mer peptide in the absence and presence of cAMP at 25C. Scan rate, 25mVs-1; [K4[Fe(CN)6]]= [K3[Fe(CN)6]]=5mM, [KCl]=50mM. 1) bare Au electrode in the absence of cAMP. 2) Au electrode modified with the 17mer peptide in the absence of cAMP. 3) the modified electrode in the presence of cAMP (5mM).

molecules on the electrode, but not due to electrostatic barrier, because overall charge of the peptide is zero. The peak current was further dramatically suppressed in the presence of cyclic AMP in the modified electrode system. This peak current change can be explained by electrostatic interactions between marker ions and anionic charges of cAMPs which were accumulated in the peptide layer. An electrostatic repulsion between the anionic marker and anionic cAMP bound to the peptide decreased the local concentration of marker molecules near the electrode. In fact, when ruthenium trisbipyridyl ($Ru(bpy)_3$) complex which is a cationic redox marker was used, the peak current then increased depending on the increase of cAMP concentration (data not shown). Cationic redox marker should be preconcentrated onto the modified electrode to give enhanced redox currents because of the electrostatic attraction. This type of voltammetric sensing was first proposed by Sugawara, et al[8] as "ion channel sensor". Peak separation of the cyclic voltammograms also increased in Fig.1, indicating the suppression of the redox reaction of the marker. Although the reason is not clear, it may be caused by an increase of the distance between marker molecule and electrode Au surface or by a decrease of effective area, both due to the peptide modification.

Figure 2 shows the relationship between absolute values of the

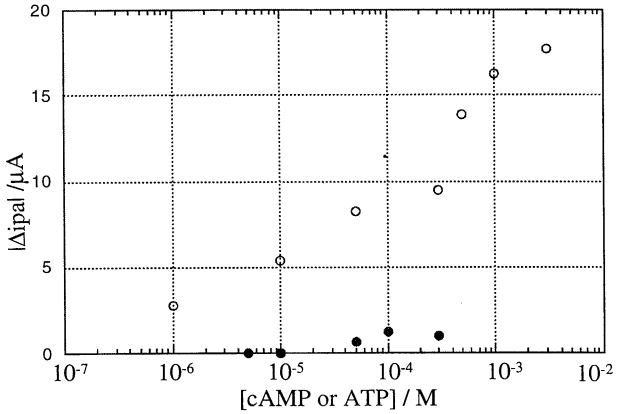

Figure 2. The adenine nucleotide-dependent changes in the anodic peak current (i_{pa}) on the Au electrode modified with the 17mer peptide. ○, cAMP; ●, ATP. Experimental conditions are the same as those in Fig.1.

diminution of the anodic peak current (Δipa) and the concentration of cAMP and ATP. The modified electrode showed good response to cAMP in the wide concentration range from μM to mM. The response can not be explained simply by nonspecific adsorption of cAMP to the peptide layer, since the response to ATP, which is a structural analog of cAMP, was very small in spite of its multi valent anionic charge (4-). If any nonspecific processes brought about the peak current change of redox markers, ATP should have caused much greater response than that for cAMP. Thus the results indicate that the present peptide sequence can recognize cAMP selectively to concentrate the cAMP anions on the electrode.

Some system have been developed for cyclic AMP detection in biolobical samples,[9, 10] although most of them were not sufficient for practical uses. Only EIA methods can be applied as usable method, while they are complicated and time-consuming . Here we reported the basic concept for the simple analysis of cAMP with the aid of a part of cAMP-dependent protein kinase as effective cAMP ligand. It was possible to analyze cAMP electrochemically using cyclic voltammetry without any preceding treatment, although further examinations would be needed prior to the practical use, e.g. control of amount of the peptide, stability on the repeated use and so on. It may also be possible to design other detecting systems of cAMP by taking advantage of the present 17mer peptide. We are now studying the fluorescent probes using this peptide.

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