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Electrochemical Detection of Polyamines Using Immobilized Enzyme as Post-Column Reactor in High Performance Liquid Chromatography

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Novel detection method of biological polyamines was developed. Polyamine oxidase immobilized column worked as a post-column reactor, in which hydrogen peroxide was produced. Then, hydrogen peroxide was electrochemically detected on a platinum electrode placed in down stream. The detection limit at the sub-pico mole level was obtained.

Naturally occuring polyamines(putrescine, spermidine and spermine) are ubiquitously found in prokaryotic and eukaryotic cells and involved in cell proliferation. Since the levels of urinary polyamines were found to be elevated in cancer bearing patients,[1] much attentions have been taken as a tumor marker.[2]

Many high performance liquid chromatographic methods have been reported for analysis of polyamines so far. Polyamines have no chromophore in their molecular skeletons. Hence, pre- or post-column derivatizations have been usually the way for the detection. These include the derivatizations by benzoyl chloride[3] for UV absorption, dabsyl chloride[4] for visible absorption and dansyl chloride[5] or o-phthalaldehyde[6] for fluorescence detection. These derivatizations are tedious and/or time consuming. The selectivity of these detection methods is not necessarily sufficient, even by fluorescence detections because many other amine compounds are also derivatized with these labeling reagents. Therefore, a conventional method with higher sensitivity and selectivity is still being pursued.

Immobilized polyamine oxidase column was used as a post-column reactor in this study. The immobilized enzyme reacted with polyamines to produce hydrogen peroxide, which was eventually detected by electrochemical oxidation on a platinum electrode. Polyamine oxidase was purified from soybean seedling according to the previous work.[7] The enzyme was immobilized on aminopropyl derivatized glass beads with controlled pore size by using coupling reaction with glutaraldehyde.

The separation of polyamines was performed on polymer-based octadecyl bonded column(Biophase-III, BAS Co., Tokyo) by ion-paired reversed phase isocratic elution. Chromatograms obtained with authentic mixture of polyamines were shown in Fig.1. Figure 1(a) was obtained with the enzyme column removed off, while Fig.1(b) was obtained with the injection of the same sample as Fig.1(a) but with the enzyme column installed. It is obviously noticed from comparison of (a) and (b) that the enzyme column functioned effectively. The detection limits were 0.3, 0.6, and 4 pmol at S/N=5 for putrescine, spermidine and spermine, respectively with linear ranges of three orders of magnitude.

The reproducibility in short-term operation was examined by the replicate injection of authentic mixture. Coefficient of variance in the peak response was 2.6, 2.9, and 5.8% (n=10) for putrescine(10 pmol injected), spermidine(20 pmol) and spermine(40 pmol), respectively. The loss of the enzyme activity was not noticeable after continuous usage for four months at least. Most of amino acids had no response except with tyrosine. Only tyrosine was detected by electrochemical oxidation, but it was not due to enzymatic conversion. Moreover, tyrosine was eluted before putrescine. Therefore, amino acids practically have no interference with the present method. The method proposed here needs neither tedious derivatization nor complicated gradient elution, allowing simple and rapid analysis of polyamines. Applications to the determination of polyamines in biological samples such as rat brain or human urine are now underway.

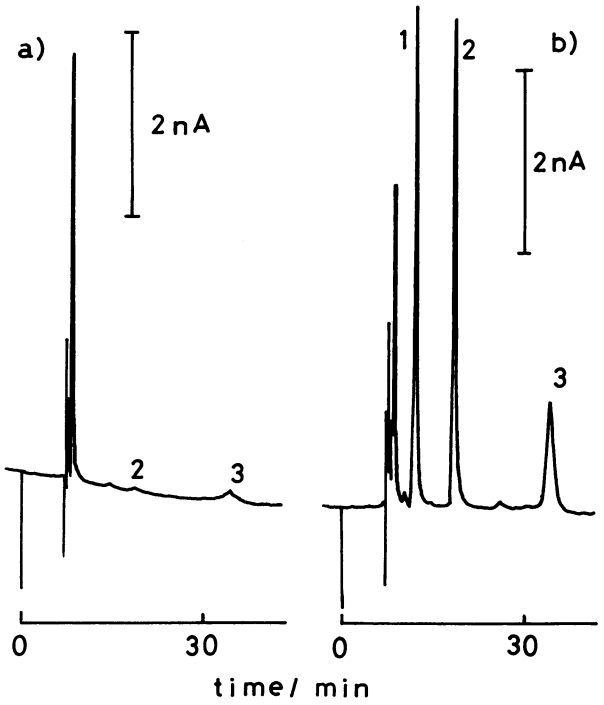

Fig.1. Chromatograms of authentic mixture of polyamines.
Eluent: 0.2 M phosphate buffer(pH 7.7) + 0.05% sodium octanesulfonate, 0.40 ml/min
Applied potential: +500 mV vs. Ag/AgCl
a) without enzyme column, b) with enzyme column, 1=putrescine(10 pmol), 2=spermidine(30 pmol), 3=spermine(40 pmol)

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