Electrochemical Behavior and Detection of Plant Hormone 6-Benzyl Adenine in Acetate Buffer at Mercury Electrode

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The electrochemical behavior of 6-benzyl adenine (6-BA) has been studied in 0.1 mol L−1 HAc–NaAc solution (pH 3.8). Cyclic voltammetry, single-sweep polarography and direct current polarography were employed to clarify the mechanism of the electrode process; the kinetic parameters of the rate-determining step were determined. Reduction of 6-BA involves two pH-dependent processes, corresponding to the overall irreversible reduction of the 1,6 and 3,2 N=C bonds. Each reduction stage consists a preprotonation of the nitrogen atom at the electroactive site and a rapid two-electron transfer. In the presence of 6-BA, the reduction potentials of some ions were shifted. Under the given conditions, 6-BA displays one irreversible reduction peak controlled by adsorption. Two linear response were observed in the range 2.0 × 10−8 – 8.0 × 10−7 mol L−1 and the range 1.0 × 10−6 – 8.0 × 10−6 mol L−1, with correlation coefficients of 0.9995 and 0.9998, respectively. The detection limit is 7.1 × 10−9 mol L−1. The method had been applied to the determination of 6-BA in bean sprout samples with satisfactory results.

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Introduction

Cytokinin is one kind of plant hormone which promotes the growth of a plant and delays the senescence of a plant. Its physiological actions has been widely studied by botanists. Of all the cytokinins, 6-benzyl adenine (6-BA) is most used for plant tissue culture, which is an important part of modern biotechnology.1 Furthermore, in recent years, many new uses of 6-BA have been found.2 However, there are few reports on its action mechanism in plants. We suggest that the action mechanism of 6-BA in plants is similar to its redox reaction on electrodes. Thus the studies of its electrochemical behavior have significant meanings.

Several studies have been made on the electrochemical behavior of 6-BA analogue. Farias et al. studied the adsorptive voltammetric behavior of adenine in the presence of guanine and some trace elements at a static mercury drop electrode by cyclic voltammetry.3,4 Jiang et al. studied the voltammetric behavior of zeatin and kinetin.5 Wang et al. studied the electrocatalytic oxidation of 6-BA by copper ions on a glassy carbon electrode.6 These results are of important theoretical and practical significance in plant physiology. However, to our knowledge, the direct electrochemical behavior of 6-BA at a mercury electrode has not been reported.

In the present work, the reduction of 6-BA in acetate buffer at mercury electrode was investigated by cyclic voltammetry (CV), single-sweep polarography and direct current polarography. The effect of the presence of 6-BA on the reduction potential of some metallic ions was studied as well. The results suggest that the reduction of 6-BA at the mercury electrode is an irreversible process and that the reduction peaks can be used for the detection of 6-BA at low levels.

Experimental

Apparatus

Electrochemical experiments were performed with a MP-2 polarographic analyzer/stripping voltameter (Shandong No.7 Telecommunicational Equipment Factory, China). This is an electrochemical instrument offering eleven standard controlled-potential techniques. Program control and data processing are conducted automatically by the microcomputer system. A conventional single-compartment cell equipped with a platinum wire counter electrode and a KCl-saturated Ag|AgCl reference electrode was employed. In cyclic voltammetry, the working electrode was a JM-01 hanging mercury drop electrode (Jiangsu Electroanalytical Instrument Plant, China). A dropping mercury electrode (DME) was used for single-sweep polarography as the working electrode. All potential values were measured and reported vs. Ag|AgCl reference electrode, and experiments were made at room temperature.

Reagents and solutions

6-BA (6-benzylaminopurine, 99%), analytically pure, was purchased from ACROS Chemical Co. The stock solution of 6-BA (1.0 mmol L−1) was prepared by dissolving an appropriate mass of 6-BA in 0.1 mol L−1 NaOH; this was kept in a refrigerator. Diluted solutions were made with an acetate buffer (0.1 mol L−1) just before using. The 0.1 mol L−1 acetate buffer was prepared with sodium acetate and acetic acid. The pH value of each solution was measured before use. All other chemicals were of analytical reagent grade. Double distilled water was used for all experiments. Pure nitrogen gas (99.99%) was used for deaeration.

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illustrates that the process is adsorption-controlled. More regression line of currents solution without 6-BA is obviously low in comparison with that of the acetate buffer. The dependence of cyclic voltammetry current on scan rate (v) is shown in Fig. 1c. Figure 1c indicates that the peak current is linearly proportional to the scan rate at v > 100 mV s⁻¹. The regression line of \( i_p \) vs. scan rate may be expressed by the equation: \( i_p = -0.224 + 4.72 \times 10^{-3}v \), \( r = 0.999 \), which illustrates that the process is adsorption-controlled. More evidence for the adsorptive behavior of 6-BA was demonstrated by the following experimental results. The electrocapillary curve of \( 1.0 \times 10^{-5} \) mol L⁻¹ 6-BA in acetate buffer pH 3.8 was obviously low in comparison with that of the acetate buffer solution without 6-BA.

Results and Discussion

Cyclic voltammetry

We measured the cyclic voltammogram (Fig. 1) of \( 1.0 \times 10^{-5} \) mol L⁻¹ 6-BA in 0.1 mol L⁻¹ acetate buffer. As can be seen in Fig. 1, a well-defined cathodic peak appears at −1.31 V. However, no peak is observed in the anodic branch. This indicates an irreversible redox process of 6-BA.

Further investigation was made of the transport characteristics of 6-BA at the hanging mercury drop electrode (HMDE). The dependence of cyclic voltammetry current on scan rate (v) is shown in Fig. 1c. Figure 1c indicates that the peak current is linearly proportional to the scan rate at v > 100 mV s⁻¹. The regression line of \( i_p \) vs. scan rate may be expressed by the equation: \( i_p = -0.224 + 4.72 \times 10^{-3}v \), \( r = 0.999 \), which illustrates that the process is adsorption-controlled. More evidence for the adsorptive behavior of 6-BA was demonstrated by the following experimental results. The electrocapillary curve of \( 1.0 \times 10^{-5} \) mol L⁻¹ 6-BA in acetate buffer pH 3.8 was obviously low in comparison with that of the acetate buffer solution without 6-BA.

Effect of pH on the reduction of 6-BA

The effect of pH on the peak current and peak potential of the reduction of 6-BA was investigated using single-sweep polarography. The results are shown in Fig. 2. As can be seen, the peak potential for 6-BA reduction shows a linear variation with the increase of pH and is shifted to a more negative potential with a slope of about −62 mV pH⁻¹, as illustrated in curve a of Fig. 2. Such a result indicates the participation of protons in the reduction process. Curve b in Fig. 2 shows the variation of peak current of 6-BA reduction at the DME with the increase of pH. The peak current increases from pH 2.78 to 3.80 and reaches a maximum value at pH 3.80, then decreases quickly with the increase of pH above pH 3.80. The maximum peak current probably indicates a optimum complete protonation of 6-BA. The decrease of the peak current at pH < 3.8 can be attributed to excessive hydriions gathered on the electrode. Therefore, the solution pH was selected as 3.80.

Reaction mechanism and kinetics

6-BA, zeatin and kinetin are derivatives of purine. Philip et al. reported that reduction of purine involved two pH-dependent processes corresponding to the overall irreversible reduction of the 1,6 and 3,2 N=C bonds, in which two electrons and two protons were added to each bond. The reduction mechanism of 6-BA was investigated by single-sweep polargraphy and by direct current polarography. Under the same conditions (0.1 mol L⁻¹ pH 3.8 acetate buffer), 6-BA was very similar to kinetin in the wave shape obtained by single-sweep polarography. The peak potential values were −1.32 V and −1.30 V for 6-BA and for kinetin. Purine gave two reduction peaks, at about −0.88 V and −1.18 V. For further comparisons on the polarographic behavior of the three compounds, we measured their DC polarogram data in the light of reference (Table 1). Comparing data of the half wave potential (\( E_{1/2} \)) in Table 1 with that of above the peak potential, we see that the value of the half-wave potential obtained in DC polargraphy for each compound approximates that of the peak potential obtained in single-sweep polargraphy, and the values of reduction current (the sum of two reductive current values for each compound) obtained in DC polargraphy for all the compounds are close to each other on the whole. The reasons probably include similarity of the molecule structure; identity of experimental conditions; and lack of effect on the current by diffusion coefficients. Based on this, we infer that the number of electrons added for 6-BA was equal to that of purine and kinetin, i.e., n = 4.

In the light of the reduction mechanisms of purine,5 zeatin and kinetin,6 we proposed the reduction process of 6-BA as shown in reaction (1):

In order to confirm that 6-BA is reduced in the form of protonation, we added tetra-n-butylammonium iodide and sodium dodecylsulfonate in the solution containing 6-BA.
respectively. The former shifted the peak potential from –1.31 V to –1.35 V, while the latter did not affect it. According to the literature,5 this indicates that 6-BA is reduced in the form of positive ions (i.e., protonation).

For a totally irreversible and reactant adsorption controlled process, the relationship between the $E_p$ and $v$ (scan rate) can be represented by the following Eq. (1):10

$$E_p = E'_p + \frac{RT}{n F} \ln \frac{k_a}{k_d} - \frac{RT}{n F} \ln v$$

(1)

Here $k_i$ is the standard rate constant of electrochemical reaction. The symbol $n_i$ is the electro-transfer number of rate-determining step for electrochemical reaction. The symbol $E'_p$ is the formal potential; it can be evaluated by outwardly extending the plot of $E_p$ vs. $v$ obtained in low scan rate until the $E_p$ axis.11,12 On the basis of this, we obtained the $E_p$ vs. $v$ plot (Fig. 3) in 1–1000 mV s$^{-1}$. The $E'_p$ obtained from Fig. 3 is −1.22 V. At lower pH, a split of the reduction peak for 6-BA was observed (Fig. 4). Also, the split of the reduction peak was observed at high scan rate (3 V/s). The effect of the pH and the scan rate on the two-step reactions might be the main cause for the peak split. The split verified once again the two-step mechanism of 6-BA reduction.

**Calibration curves and statistical parameters**

The calibration graph for the determination of 6-BA was constructed under optimum conditions. It consists of two segments with different slopes. The linear regression equations of the two segments are as follows

$$i_p \mu A^{-1} = 1.725 \times 10^9 c \text{ mol L}^{-1} + 0.5902$$

(2)

and

$$i_p \mu A^{-1} = 4.697 \times 10^9 c \text{ mol L}^{-1} + 0.1586$$

(3)

with correlation coefficients of 0.9982 ($n = 7$) in the range of $1.0 \times 10^{-6} - 8.0 \times 10^{-4} \text{ mol L}^{-1}$ and 0.9995 ($n = 7$) in the range of $1.0 \times 10^{-5} - 5.0 \times 10^{-5} \text{ mol L}^{-1}$.
Effect of 6-BA on metal ions

Some metal ions, for example, Zn$^{2+}$ and Cu$^{2+}$, are bound up with growth of plants. Rare earth ions enable agricultural output to increase. Therefore, the effects of 6-BA on electrochemical reduction of these metal ions were investigated by cyclic voltammetry.

Figure 5 shows the effects of 6-BA on the redox of Cu$^{2+}$. As shown in Fig. 5, 2.0 × 10^{-4} mol L^{-1} Cu$^{2+}$ gave a pair of well-defined oxidation-reduction peaks at about +0.075 V($E_{pa}$) and -0.010 V($E_{pc}$) (Fig. 5A). Another pair of peaks appeared at about -0.050 V($E_{pa}$) and -0.110 V($E_{pc}$) when 1.0 × 10^{-5} mol L^{-1} 6-BA were added to the Cu$^{2+}$ solution (Fig. 5B). This resulted from the complex that formed between 6-BA and the Cu$^{2+}$ produced by first-step reduction of Cu$^{2+}$.

With the increase of 6-BA concentration in the solution containing 2.0 × 10^{-4} mol L^{-1} Eu$^{3+}$, the oxidation potential of Eu$^{3+}$ was shifted to more positive values and the reduction potential was shifted to more negative values (Table 2), but no influence of 6-BA was found for the peak current of Eu$^{3+}$. This can be attributed to the effect of reversibility to Eu$^{3+}$ reduction by adsorption of 6-BA on the mercury electrode. In the obtained cyclic voltammograms, new peaks were not found. Moreover, the reduction potential of Eu$^{3+}$ clearly shifted to more negative values when 6-BA concentration was much lower than Eu$^{3+}$ concentration (Table 2). Therefore, we suggest that Eu$^{3+}$ does not form any complex with 6-BA.

As can be seen in Table 2, with the increase of 6-BA, the peak potential of Cd$^{2+}$ is shifted to more positive values and the $\Delta E_p$ is reduced by 0.02 V, which indicates that the adsorption of 6-BA catalyzes the reduction of Cd$^{2+}$ slightly. The reduction of Zn$^{2+}$ is irreversible. The peak potential is shifted to more positive values with the increase of 6-BA concentration and the $\Delta E_p$ is increased. However, the increase in $\Delta E_p$ is less obvious than that of Eu$^{3+}$. The reason is probably the smaller effect resulting from the weak adsorption of 6-BA at the reduction potential of Zn$^{2+}$; this reduction potential is more negative than that of Eu$^{3+}$.

Determination of 6-BA in bean sprouts

As an example of the analytical performance, the method was applied to the analysis of bean sprout samples. Two bean sprout samples from food markets were preconditioned according to the literature. The test solution obtained from the preconditioning were analyzed for 6-BA contents with the method presented above. The analytical results are listed in Table 3. To ascertain the correctness of the results, we spiked the samples with certain amounts of 6-BA; then the total values of 6-BA were detected. The recovery rates of the spiked samples were determined to be between 96 and 105% (Table 3). For further confirmation of the trustworthiness of the results obtained by the proposed method, sample 1 was determined by the high performance liquid chromatography method. The results for 6-BA content were 0.45, 0.46, 0.42, 0.39, and 0.42 μg g^{-1}, respectively. The paired t-test was applied and a t value of 0.141 was calculated, the t-tabulated value at the 95% confidence level and 8 degrees of freedom is 2.31. It can be concluded that the methods do not give significantly different values.

Conclusions

From this study we can conclude that 6-BA can be reduced irreversibly at a mercury electrode. Its reduction includes two pH-dependent processes, corresponding to reduction of the 1.6 and 3.2 NaC bonds (two electrons are added per bond). Each reduction stage consists of a preprotonation of the nitrogen atom at the electroactive site and a rapid two-electron transfer. The adsorption of 6-BA on the mercury electrode was verified and the rate constant of the reaction was obtained, about 0.84 s^{-1}. In addition, the reduction of 6-BA on the mercury electrode appears as a very promising analytical tool for the determination of 6-BA in real samples.

References

2. T. Buban, Plant Growth Regul., 2000, 32, 381.

Table 3 Analytical results of 6-BA in bean sprouts

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\rho$(Found, μg g^{-1})</th>
<th>$\rho$(Average found, μg g^{-1})</th>
<th>RSD, %</th>
<th>$\rho$(Added, μg g^{-1})</th>
<th>$\rho$(Found, μg g^{-1})</th>
<th>Rec. %</th>
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<tr>
<td>1</td>
<td>0.41, 0.43, 0.45, 0.42, 0.42</td>
<td>0.43</td>
<td>3.5</td>
<td>1.00</td>
<td>0.96</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>0.33, 0.34, 0.32, 0.32, 0.31</td>
<td>0.32</td>
<td>3.6</td>
<td>1.00</td>
<td>1.05</td>
<td>105</td>
</tr>
</tbody>
</table>

2.0 × 10^{-4} - 8.0 × 10^{-5} mol L^{-1}. The detection limit was 7.1 × 10^{-5} mol L^{-1} (based on a signal to noise ratio of 3). In order to improve the precision, repetitive measurements were carried out in 2.0 × 10^{-4} mol L^{-1} 6-BA. The results of eleven measurements showed a relative standard deviation of 4.4%.

$\Delta E_p$ is increased. However, the increase in $\Delta E_p$ is less obvious than that of Eu$^{3+}$. The reason is probably the smaller effect resulting from the weak adsorption of 6-BA at the reduction potential of Zn$^{2+}$; this reduction potential is more negative than that of Eu$^{3+}$.