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ORIGINAL PAPER

Development of an amperometric sensor for simultaneous determination of uric acid and ascorbic acid using 2-[bis(2-aminoethyl)amino]ethanol, 4,4′-bipyridine bridged dicopper(II) complex

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Abstract Binuclear copper complex (2-[bis(2-aminoethyl)amino]ethanol, 4,4′-bipyridine bridged dicopper(II) complex) was grafted onto the surface of a glassy carbon electrode (GCE) using the cyclic voltammetric method in a phosphate buffer solution (PBS). The modified electrode resulted in efficient electrocatalytic activity for anodic oxidation of uric acid (UA) and ascorbic acid (AA) via a substantial decrease in anodic over-potentials for both compounds. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) using this modified electrode result in two well-resolved anodic waves for the oxidation of UA and AA in mixed solution, making possible the simultaneous determination of both compounds. Linear analytical curves were obtained in the ranges 5.0–300.0 μM and 5.0–160.0 μM for UA and AA concentrations through DPV methods, respectively. The detection limits were 2.0 μM of UA and AA. This electrode was used for UA and AA determinations in urine samples with satisfactory results.

Keywords Binuclear copper complex · Uric acid · Ascorbic acid · Modified electrode · Cyclic voltammetry · Differential pulse voltammetry

1 Introduction

Electrochemical determination of bioactive molecules has been intensively studied over the past decades. Among them, uric acid (UA) is the primary end product of purine metabolism in the human body. Analysis of UA concentration in biological systems provides crucial information regarding its metabolic and immune functions. Earlier research has shown that extreme abnormalities of UA levels are symptoms of several diseases (e.g. gout, hyperuricaemia and Lesch–Nyhan syndrome) [1, 2]. Therefore, determination of UA has attracted great interest. Although several methods have been used for UA detection, electrochemical methods are more practical for in vivo and in vitro detection. However, electrochemical detection of UA suffered from the strong influence of ascorbic acid (AA) oxidation. Since AA and UA co-exist in biological fluids, such as blood and urine, and can be oxidized at nearly the same potential by unmodified electrodes, the result is an overlapped voltammetric response making it extremely difficult to distinguish them [3, 4]. Therefore, it is important to develop a technique to selectively detect UA in the presence of AA in routine assay. Use of chemical modified electrodes is most promising for this task. Accordingly, different modified electrodes were fabricated for detection of UA and AA [5–14]. However, modified electrodes have their unique advantages and limitations. There is a need for the development of a simple, reliable and efficient sensor with enhanced characteristics for sensing of UA and AA simultaneously.

Transition metal complexes are well known as electron mediators in the electrocatalytic oxidation of some biological compounds [15–17]. Our group has designed and synthesized a series of copper complexes bridged by imidazole or 4,4′-bipyridine with 2-[bis(2-aminoethyl)amino]ethanol (Bael) ligand, which provide model systems for some enzymes [18]. Redox behaviors of these copper complexes are interesting; however, have rarely been studied in detail, especially for use in the construction of modified electrodes as sensors

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[18, 19]. Accordingly, we fabricated a modified glassy carbon electrode (denoted Baelbp/CuGCE) with five-coordinated binuclear copper (II) complex $[Bael_2Cu_2biPy](ClO_4)_4$ by a cyclic voltammetric method in a phosphate buffer solution. The electrochemical behavior of dopamine and AA at this modified electrode was studied [20]. In this paper, as a continuation of our previous work [20] we focused on the electrochemical behavior of UA and AA on the surface of Baelbp/CuGCE. Due to a very good resolution of voltammetric waves of these two compounds in the mixed solutions, a differential pulse voltammetric technique was developed for detecting the amounts of UA and AA simultaneously. Finally the analytical performance of this sensor for simultaneous determination of uric and AAs in human urine samples is evaluated by the DPV method.

2 Experimental

2.1 Apparatus

All electrochemical experiments were carried out with a CHI 660A electrochemistry workstation (CHI, USA). A conventional three-electrode electrochemical system was used for all electrochemical experiments; this consisted of a working electrode, a platinum wire auxiliary electrode and a saturated calomel reference electrode (SCE). All potentials reported are versus SCE.

2.2 Chemicals and solutions

Dicopper complex ($[Bael_2Cu_2biPy](ClO_4)_4$) was prepared according to the literature [18]. UA and AA (from Lancaster) were used as received. All other chemicals were of analytical grade. Phosphate-buffer solutions (PBS) of different pH were prepared by mixing four stock solutions of 0.2 M H_3PO_4, KH_2PO_4, K_2HPO_4 and K_3PO_4. All aqueous solutions were prepared in double distilled, deionized water. High pure nitrogen was used for deaeration.

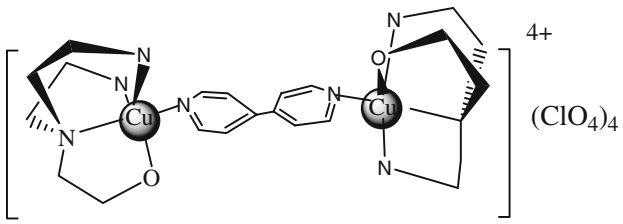
2.3 Preparation of Baelbp/Cu modified glassy carbon electrode

The preparation of the electrode was the same as that described elsewhere [20].

3 Results and discussion

3.1 Electrochemistry of Baelbp/Cu modified glassy carbon electrode

We have recently described the preparation of a Baelbp/Cu modified glassy carbon electrode (GCE) by a cyclic voltammetric method in phosphate buffer solution [20]. The structure of the dicopper complex is shown in Scheme 1.



Scheme 1 Structure of $[Bael_2Cu_2biPy](ClO_4)_4$ complex

The modification was conducted under cyclic voltammetric conditions, as shown in Fig. 1a. The cyclic voltammogram exhibited two pairs of anodic and cathodic peaks corresponding with two redox systems of the metal centers. This is possibly because the dicopper complexes have asymmetric interaction with the electrode surface, which causes the two metal centers to have redox reactions sequentially and thus two pairs of redox waves were formed corresponding to Cu(II,II)/Cu(II,I) and Cu(II,I)/Cu(I,I) respectively. The two-redox peak remained constant after the modified electrode was subjected to sonication in PBS for 15 min, verifying that the dicopper complex residues have immobilized on the GCE surface (Fig. 1b).

3.2 Electrocatalytic oxidation of single UA and AA

It was found that Baelbp/CuGCE has strong catalytic activity for oxidation of UA and AA. As can be seen in Fig. 2a, the cyclic voltammogram of UA at the modified electrode gave a well behaved anodic peak at 310 mV (curve a). However, only a relatively broad and irreversible anodic wave with a peak potential around 612 mV resulted at a bare GCE (curve b). It was well known that UA oxidation in aqueous media corresponds to an electron transfer process followed by fast chemical reactions [21]. Interestingly, a small but recognizable re-reducing peak appeared at about 267 mV in the reverse scan (curve a) even at a slow scan rate of 100 mV s^{-1}. A similar reverse peak was also observed at the 5-hydroxytryptophan modified GCE [22]. This is a result of a slow down effect on the following hydrolysis reactions, which can be attributed to the molecular interaction between the Baelbp/Cu and the UA molecules and/or its reaction intermediates.

The significant reduction in over-potential and improvement of peak shape indicate that the Baelbp/Cu has strong electrocatalytic activity toward UA oxidation. To investigate the reaction mechanism, scan rate dependent experiments were carried out for UA oxidation at the Baelbp/CuGCE, the result is shown in Fig. 3. The anodic

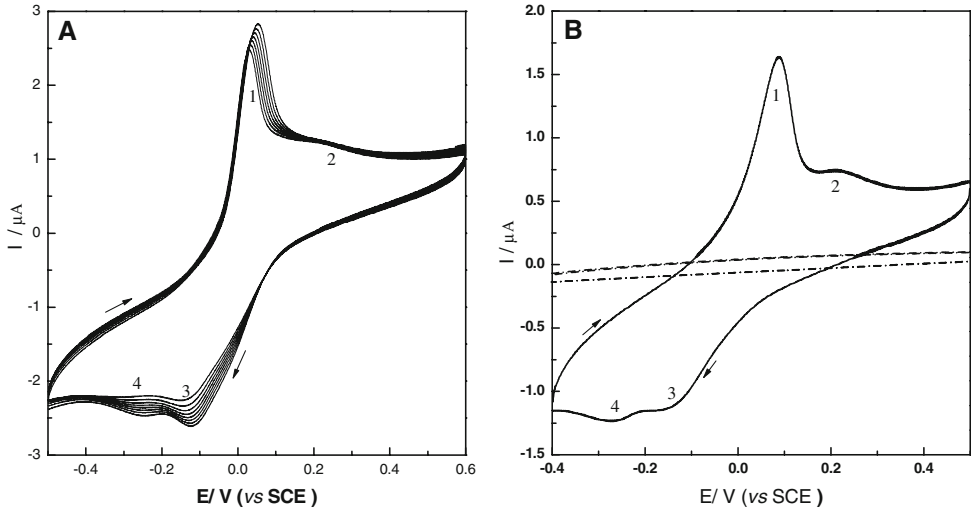


Fig. 1 (**a**) The multi-cycle CVs of GCE in PBS (pH 5.0) containing 0.1 mM [Bael $_2$Cu$_2$biPy](ClO$_4$)$_4$. (**b**) Cyclic voltammogram of Baelbp/CuGCE in PBS solution without dicopper complex. (Dashed curve indicates bare GC electrode) Scan rate 100 mV s^{-1}, taken from Ref. [20]

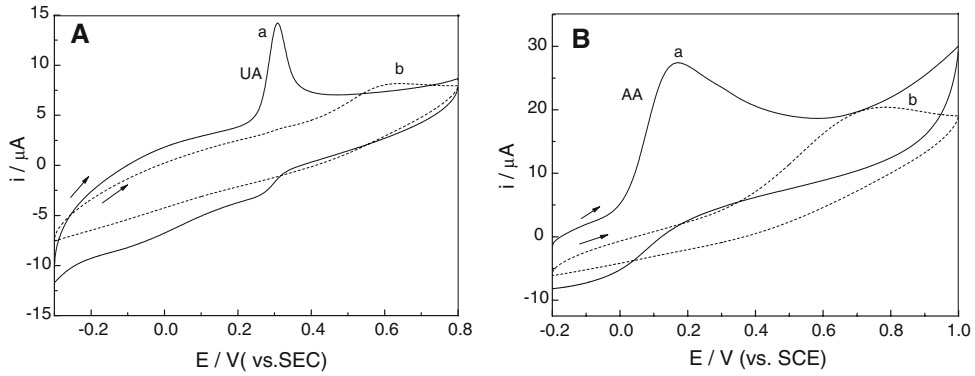


Fig. 2 CVs of 30 μM UA and 50 μM AA at Baelbp/CuGCE (**a**) and bare GCE (**b**) in 0.2 M PBS (pH = 6.8) respectively. Scan rate: 100 mV s^{-1}

peak current was proportional to the square root of scan rate in the range 10–200 mV s^{-1} (i_{pa} (μA) = −1.342 + 0.962$v^{1/2}$ (mV s^{-1})$^{1/2}$, $r = 0.999$), indicating diffusion controlled kinetics [23].

Electrocatalytic oxidation of AA was also found at Baelbp/CuGCE. Figure 2b shows the CVs of AA at the Baelbp/CuGCE (curve a) compared to a bare GCE (curve b). Nearly a 532 mV reduction in over-potential for AA oxidation was achieved by the Baelbp/CuGCE [20]. The CV peak of AA oxidation appeared at 160 mV, which is 150 mV more negative than that of UA oxidation. Thus, simultaneous determination of UA and AA could be conducted in a mixed solution by this modified electrode.

3.3 Electrocatalytic oxidation of UA and AA in a mixture

Since UA and AA have nearly similar oxidation potential at most solid electrodes, separate determination of these species is a problem due to their overlapped signals. To establish a sensitive and selective method for the quantification of UA and AA, the Baelbp/CuGCE was studied. Figure 4 shows the CV and DPV responses of UA and AA in a mixed solution at Baelbp/CuGCE in comparison with a

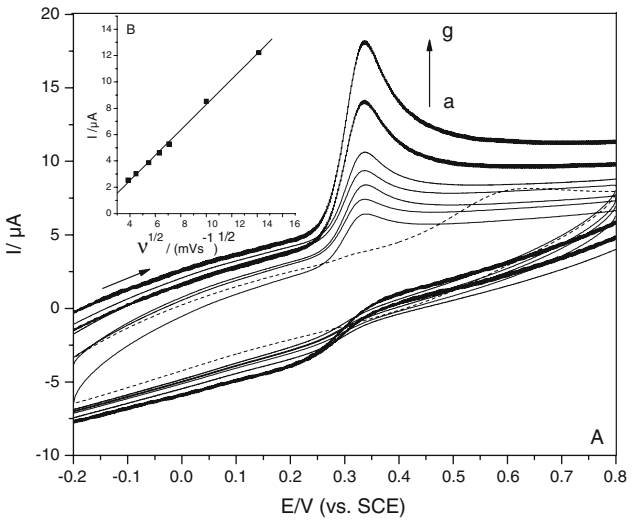


Fig. 3 (**a**) CVs for oxidation of 30 μM UA at Baelbp/Cu modified GCE in 0.2 M PBS (pH 6.8) at scan rates of 10, 20, 30, 40, 50, 100 and 200 mV s^{-1} (inner to outer). (Dashed curve indicates 30 μM UA at bare GC electrode at scan rate of 100 mV s^{-1}) (**b**) Plots of peak currents versus square root of scan rates

bare GCE. Figure 4a shows that AA and UA responses were resolved into two well separated CV peaks at 153 and 339 mV at Baelbp/Cu modified GCE (curve a), and one

broad and overlapped anodic peak at about 719 mV at the bare GCE (curve b). However, as shown in Fig. 4b, better-resolved peaks were obtained by the DPV technique, which gave two peaks at 104 and 327 mV with almost a flat baseline for the oxidation of UA and AA respectively. The 213 mV peak separation was even larger than the separation of the CV peak, which was large enough to determine AA and UA individually and simultaneously.

3.4 Effect of pH on the oxidation of UA and AA in mixture

Figure 5 shows the pH effect on the DPV peak current in the pH range 3–8. The current maximum appeared at pH 6.8 for both the UA and AA determinations. Further increase in pH caused a slight decrease in the peak currents. Therefore, the optimum solution pH selected was 6.8. In addition, all anodic peak potentials for the oxidation of AA and UA shifted negatively with increase in pH, demonstrating that protons participate in the electrode reactions.

3.5 Simultaneous determination of DA and AA

Figure 6a shows DPV recordings at various UA concentrations with 60 μM AA under the optimized conditions at the Baelbp/CuGCE. The peak current of UA increased as the UA concentration increased with the concentration of AA held constant. Similarly, as shown in Fig. 7, when the concentration of UA remained constant the oxidation peak current of AA was proportional to its concentration.

Under optimum conditions in the DPV mode, the catalytic peak current was linearly related to UA and AA concentrations (Figs. 6b and 7b). The analytical parameters for the simultaneous determination of UA and AA are listed in Table 1.

It can be seen that the electrochemical response peaks for UA and AA oxidation at the Baelbp/CuGCE are clearly separated from each other when they co-exist in pH 6.8 PBS. It is therefore possible to determine UA and AA

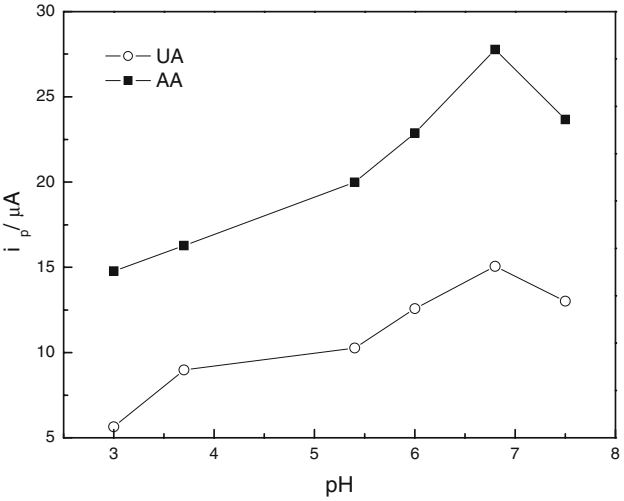


Fig. 5 Effect of pH on the peak currents for the oxidation of UA and AA. Concentrations: 30 μM UA, 50 μM AA

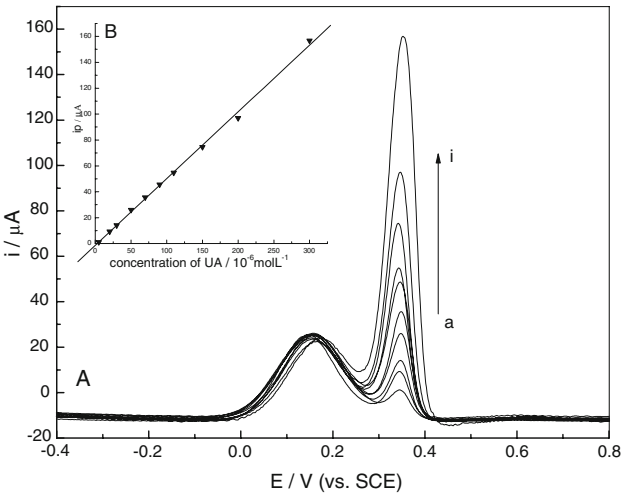
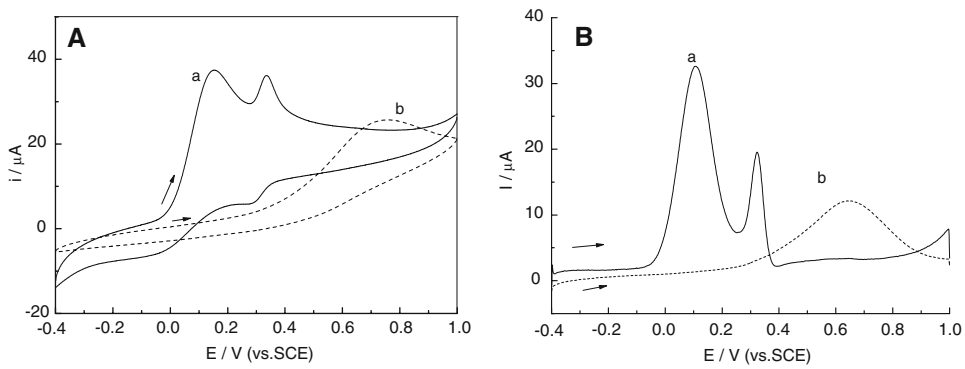


Fig. 6 (**a**) Differential pulse voltammogram of UA at various concentrations (1) 5; (2) 20; (3) 30; (4) 50; (5) 70; (6) 90; (7) 110; (8) 150; (9) 200; (10) 300 μM in presence of 60 μM AA at the surface of Baelbp/CuGCE. (**b**) Plot of electrocatalytic peak currents (from **a**) versus UA concentration. Solution conditions: pH 6.8 PBS. Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms

Fig. 4 (**a**) CVs of a mixture of 30 μM UA and 50 μM AA in PBS (pH 6.8) at Baelbp/CuGCE. (a) and a bare GCE (b). Scan rate 100 mV s^{-1}. (**b**) DPV of 30 μM UA and 50 μM at Baelbp/CuGCE (a) and a bare GCE (b) in PBS (pH 6.8). Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms



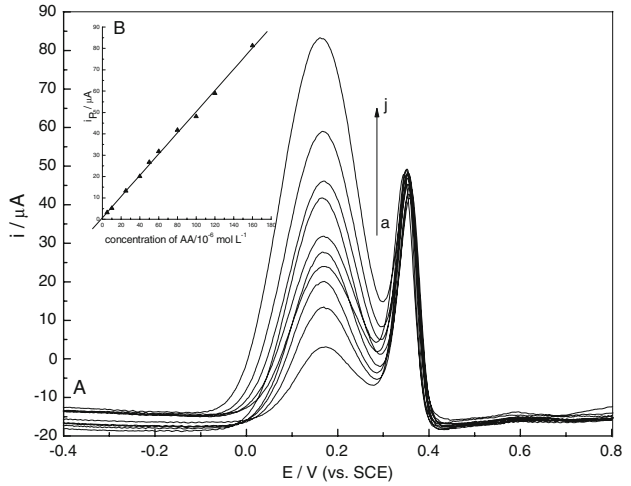


Fig. 7 (**a**) Differential pulse voltammogram of AA at various concentrations (1) 5; (2) 10; (3) 25; (4) 40; (5) 50; (6) 60; (7) 80; (8) 100; (9) 120; (10) 160 μM in presence of 90 μM UA at the surface of Baelbp/CuGCE. (**b**) Plot of electrocatalytic peak currents (from **a**) versus AA concentration. Solution conditions: pH 6.8 PBS. Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms

simultaneously in samples at a Baelbp/CuGCE. The relative standard deviations (RSD) ($N = 7$) for determination of 30 μM UA and 50 μM AA were 2.5 and 2.7%, respectively, showing excellent repeatability.

3.6 Interferences

As previously indicated, an intrinsic property of the modified electrode could substantially differentiate UA and AA. Therefore, the interference from AA can be neglected. Other influences from common co-existing substances on the determination of 30 μM UA or 50 μM AA were investigated. No significant interference was found for the following compounds: NaCl (300), $CaCl_2$ (200), citric acid (800), glucose (200), tryptophan (100), cysteine (100) and tyrosine (200), where the numbers in brackets are the concentration ratios.

3.7 Stability

Baelbp/CuGCE was stable and reproducible. Repeatability in the construction of the modified electrode was evaluated by constructing five modified electrodes and determining the sensitivity obtained for each. The reproducibility expressed as RSD was 3.2%. However, the electrode had to be treated to remove adsorbed contaminants to maintain repeatability. Fortunately, the modified electrode can be renewed by CV scans in 0.1 M PBS in the potential window 0.0 ~ 0.8 V after each experiment. Generally 20 cycles regenerated clean background CV curves, and the electrode was ready for the next experiment or for storage.

The long-term storage stability of the Baelbp/CuGCE was investigated under specific storage conditions (exposure to air, ambient temperature). The current response decreased 5% over the first 7 days and by 15% for the following one month.

Table 2 Analytical results of urine samples

	Urine samples	Original (μM)	Added (μM)	Found[a] (μM)	Recoverage (%)	Total value[b] (mg l^{-1})
1	UA	36.2	10	45.8	96	478
			20	56.4	101	
			30	67.9	106	
	AA	4.25	15	19.8	104	56.2
			25	28.0	95	
			35	40.1	102	
2	UA	34.6	10	44.1	95	457
			20	54.9	102	
			30	64.2	99	
	AA	5.27	15	20.5	102	69.7
			25	29.2	96	
			35	40.9	102	

[a] The average of five repeated measurements

[b] The total value (average of three measurements) was obtained by multiplying the detected value and the appropriate dilution factor

3.8 Real samples analysis

We examined the applicability of the modified electrode for determination of UA and AA in urine samples. The urine samples were diluted with PBS (pH 6.8) before the measurements to prevent the matrix effect of real samples. Results are listed in Table 2. In order to evaluate the validity of the modified electrode for the determination of UA and AA, recovery studies were carried out on samples to which known amounts of UA or AA standards were added. Results obtained by this method agree well with the values reported by other researchers [6, 22].

Table 1 Analytical parameters for simultaneous determination of UA and AA

Analyte	Linear range (μM)	Linear regression equation I (μA), C (μM)	Correlation coefficient	Detection limit (μM)
UA	5.0–300.0	i_{Pa} (μA) = −1.354 + 0.516C	0.9990	2.0
AA	5.0–160.0	i_{Pa} (μA) = 0.759 + 0.496C	0.9991	2.0

4 Conclusions

The electrocatalytic behavior of Baelbp/CuGCE with regard to UA and AA oxidation was investigated. The Baelbp/Cu modified GCE is sensitive and stable for UA and AA determination. Moreover, the modified electrode can be successfully applied for determination of UA and AA simultaneously in urine samples using the DPV technique. The simple fabrication procedure, wide linear range, low detection limit, high stability and good reproducibility for repeated determinations indicate that this electrode is an attractive candidate for practical applications.

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