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Electrochemical behavior of phytochelatins and related peptides at the hanging mercury drop electrode in the presence of cobalt(II) ions

Vlastimil Dorčák[a,b,*], Ivana Šestáková[c]

[a]Faculty of Agrobiology, Food and Natural Resources, Czech University of Agriculture, Prague 6-Suchdol CZ-165 21, Czech Republic
[b]Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, Brno CZ-612 65, Czech Republic
[c]J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, Prague 8 CZ-182 23, Czech Republic

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In memory of Professor Pavel Mader

Abstract

Direct current voltammetry and differential pulse voltammetry have been used to investigate the electrochemical behaviour of two phytochelatins: heptapeptide (γ-Glu–Cys)$_3$–Gly and pentapeptide (γ-Glu–Cys)$_2$–Gly, tripeptide glutathione γ-Glu–Cys–Gly and its fragments: dipeptides Cys–Gly and γ-Glu–Cys at the hanging mercury drop electrode in the presence of cobalt(II) ions. Most interesting results were obtained with direct current voltammetry in the potential region of − 0.80 V up to − 1.80 V. Differential pulse voltammetry of the same solutions of Co(II) with peptides gives more complicated voltammograms with overlapping peaks, probably in connection with the influence of adsorption at slow scan rates necessarily used in this method. However, in using Brdička catalytic currents for analytical purposes, differential pulse voltammograms seem to be more helpful. Presented investigations have shown that particularly the prewave of cobalt(II) allows distinguishing among phytochelatins, glutathione, and its fragments.

Keywords: Phytochelatins; Cysteine peptides; Voltammetry; Cobalt(II) prewave; Brdička reaction; Hydrogen catalysis

1. Introduction

The polarographic catalytic hydrogen evolution in mixtures of cobalt salts and cysteine-containing proteins was discovered by Brdička in 1933 [1]. He showed that the actual active components in the above system are electrochemically generated divalent cobalt ions and cysteine or cystine residua, with the latter giving free thiol groups upon their reduction at the mercury electrode. The catalytic hydrogen evolution in mixtures of cobalt salts and cysteine-containing proteins has been met with great interest and has been utilized in a number of ways [2–7]. Today, the modified Brdička procedure is used particularly for determination of animal metallothioneins with a high content of cysteine [8–15]. Insufficient selectivity is, in metallothioneins analysis, substituted by sample pretreatment, which removes other high molecular mass SH-containing proteins. The signal obtained from compounds of lower molecular mass is, under conditions used, negligible and is effectively independent of the presence of heavy metals [8].

Despite this broad range of practical applications, the method is still to be considered as based largely on empirical findings and recent statement [16] that "new interpretation of the existing results is presented, leading to the complete elucidation of the (Brdička catalytic) mechanism" has to be taken with great care. Previous investigations in systems containing cobalt(II) and cysteine-like compounds dealt with prewave of cobalt [17] and adsorption phenomena [18]. There are several papers concerning

* Corresponding author. Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, Brno CZ-612 65, Czech Republic. Tel.: +420 541 517 193; fax: +420 541 211 293.
E-mail addresses: dorcak@ibp.cz (V. Dorčák), ivana.sestakova@jh-inst.cas.cz (I. Šestáková).

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the Brdička reaction of low molecular thiols, both on dropping mercury electrode (DME) [19–21] and hanging mercury drop electrode (HMDE) [22].

Similar systems have been studied concerning both structural and mechanistic aspects in the presence of Ni(II) ions [23–29]. Replacement of Co(II) ions by Ni(II) ions has similar, but much less effect.

Besides Brdička response, two other catalytic phenomena could be observed, depending on type of thiol. "Peak P" at about −1.2 V is observed on the voltammograms recorded at low rates of potential scan [22,30]. Otherwise, at a more negative potential region, "peak H" appears in solutions containing [Lys^8]-vasopressin [31] or metallothionein [32], with or without Co ions, when HMDE is polarized by reducing current using chronopotentiometric stripping analysis (CPSA). This peak corresponds to the prenatrium catalysis of hydrogen evolution observed in polarography and voltammetry.

No paper has described catalytic hydrogen evolution in the presence of Co or Ni and phytochelatins, which are the most abundant compounds from the metallothionein family in the plant kingdom. Therefore, in the present study, we have chosen two phytochelatins: pentapeptide (γ-Glu–Cys)$_2$–Gly (PC2) and heptapeptide (γ-Glu–Cys)$_3$–Gly (PC3); tripeptide glutathione γ-Glu–Cys–Gly (γECG) and its fragments: dipeptides Cys–Gly (CG) and γ-Glu–Cys (γEC), all of them having the possibility of being present simultaneously in the plant cell.

2. Experimental

2.1. Apparatus

All voltammetric measurements were carried out using PC-controlled Eco-Tribo Polarograph (PolaroSensors, Prague, Czech Republic). The three-electrode system consisted of a pen-type HMDE (PolaroSensors, Prague, Czech Republic) as a working electrode, Ag|AgCl|KCl_{sat} as a reference electrode, and Pt wire as a counter electrode. Purified nitrogen was used to deaerate solutions and keep them oxygen-free. Direct current voltammograms were recorded with 100 mV s^{-1} scan rate (except those recorded for scan rate dependences); differential pulse voltammograms were recorded with 10 mV s^{-1} scan rate, −50 mV pulse height, and 80 ms pulse width. For pH measurements, pH meter CPH 51 (Monokrystaly Turnov, Czech Republic) with a combined electrode was used.

2.2. Reagents

Cys–Gly (minimum 85%), γ-Glu–Cys (trifluoroacetate, purity 80%, peptide content 75%), and glutathione, reduced form (free acid, minimum 98%), were purchased from Sigma; phytochelatins PC2 and PC3 were prepared synthetically [33] in the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague. All chemicals for preparation of buffers were of suprapure grade (Merck). Cobalt(II) 1.000±0.002 g l^{-1} in 2% HNO_3 was from Astasol (Analytica Ltd., Prague, Czech Republic). For preparation of all solutions, bidistilled water was used.

2.3. Procedures

All measurements were carried out at laboratory temperature. The stock solutions of thiols in deaerated bidistilled water were prepared in 0.5 mmol l^{-1} concentration. The solutions were stored in a refrigerator. Fresh solutions of borate buffer, pH 8.2 and 9.4, with addition of 1.0 and 0.4 mmol l^{-1} cobalt(II) were prepared for each measurement. The working volume in the electrode cell was 10 ml and the thiol additions were from 20 to 2000 μl of stock solutions. Direct current and differential pulse voltammetric measurements were carried out in series in the same solution.

3. Results and discussion

Most interesting results were obtained with direct current voltammetry (DCV) in the potential region of −0.80 V up −1.80 V. The voltammograms illustrating observed peaks are presented in Fig. 1. When there is only cobalt(II) present, reduction peak in borate buffer, pH 8.2, is observed at −1.25 V; in buffer of pH 9.4 at −1.30 V. This peak (Co) corresponds to the reduction of cobalt(II) complexes with borate anions from the buffer. With the addition of peptides, peak Co decreases and new peaks appear at less negative potentials. Peaks denoted as I and II correspond to ligand-catalytic reduction of cobalt complexes with thiol ligand, as has been described earlier [34]. Further peaks (III for CG or IV for PC3) are observed in potential range from −1.10 V to −1.25 V. Finally, Brdička currents at a region of about −1.60 V, denoted here as peak B, are observed.

Replacement of DME by HMDE leads to a great increase of sensitivity of the measurement. At the same time, however, it brings an unexpected complication on how to measure the absolute height of the observed peaks. It is obvious from Fig. 1 that the response of the basic curve for blank with Co(II) demonstrates non-monotonous change upon gradual addition of thiol, thus making the determination of the "reference point" from which to read the (absolute) magnitude of the peak current very difficult. Hence, we decided to measure it from the zero current line in all cases.

From a comparison of DC voltammograms for five peptides (Fig. 2), it can be seen that voltammograms of PC2 or PC3 are different from those of other three peptides—only peak I is observed at both cobalt(II) concentrations used. With other three peptides, peak I first increases

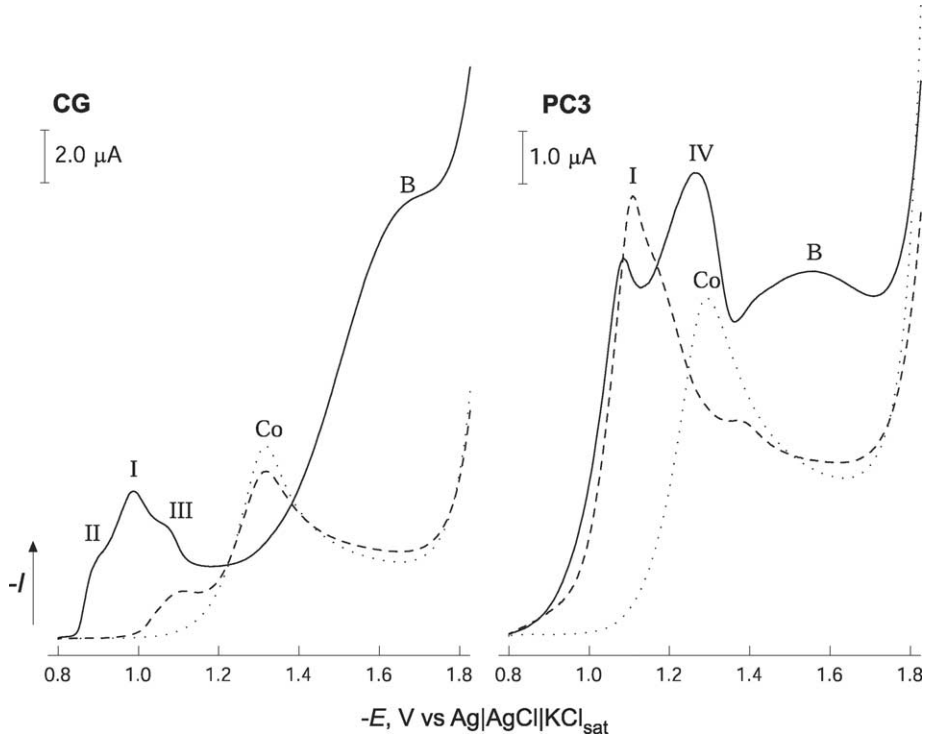

Fig. 1. Voltammograms of Co(II) in the presence of Cys–Gly or PC3 in borate buffer, pH 8.2. Dotted line, 1.0 mmol l^{-1} Co(II); dashed and solid line, added 5.0 μmol l^{-1} and 83.3 μmol l^{-1} of the assigned peptide, respectively. Co-reduction of Co(II) complexes with borate anions from the buffer; I, II, III, IV, B-peaks observed after peptide addition (for details, see text). Recorded by DCV on HMDE from −0.80 V at 100 mV s^{-1} scan rate.

together with the shift of peak potential towards less negative values and then peak II appears and increases.

At a lower cobalt(II):peptide concentration ratio of 5:1 (second row of Fig. 2), peak II is predominant for CG and γEC, and more pronounced at a higher cobalt(II):peptide ratio of 12:1 (first row of Fig. 2) for γECG. Therefore, the appearance of peak II under conditions used as in Fig. 2 (pH 8.2, 9.4; 0.4 mmol l^{-1} Co(II); 100 mV s^{-1} scan rate) can distinguish between phytochelatins and CG, γEC, or γECG.

Peak III of CG and γEC, and peak III/IV of γECG are observed only at higher peptide concentration, above 40 μmol l^{-1}. Whereas peak IV is observed even for the smallest addition of both phytochelatins and is increasing with an increase of Co(II) concentration (Figs. 2 and 3), peak V appears only on DC voltammograms of PCs at the highest concentrations used.

Voltammograms recorded at 1 and 50 mV s^{-1} scan rates (Fig. 4) indicate that a peak denoted as III, III/IV, or IV does not correspond to the same electrochemical process in all peptides studied. For CG and γEC, peak III corresponds to peak P [30], observed under similar conditions with homocysteine [22] and explained by catalytic evolution of hydrogen observed at HMDE (reaction in the adsorbed state). This is in agreement with high current increase, when the slow scan rate is used [22]. Only a slight increase of γECG peak III/IV was observed when the scan rate was reduced from 50 to 1 mV s^{-1}, whereas peak IV, characteristic of PC2 and PC3, has increased with increasing scan rate (Fig. 4). From its behaviour, it is possible to explain peak IV as the reduction of a stable PC–Co(II) complex, similarly as described for metallothioneins, where RS_2Co structure on HMDE surface is formed [16].

Differential pulse voltammetry (DPV) of the same solutions of Co(II) with peptides gives more complicated voltammograms with overlapping peaks, probably in connection with the influence of adsorption at slow scan rates necessarily used in this method. In order to reach a proper interpretation of the voltammetric data, a more detailed measurement, in connection with mathematical methods such as powerful multivariate data analysis techniques (e.g., a multivariate curve resolution with alternating least squares optimisation method, based on factor analysis techniques [33,35–41]), would be more successful procedure to separate mixed processes in such case.

Reasonable Brdička response from all studied peptides was obtained in borate buffer, pH 9.4. No response was obtained for γECG at pH 8.2 with 0.4 or 1.0 mmol l^{-1} Co(II) ions, and for γEC at pH 8.2 with 1.0 mmol l^{-1} Co(II) ions. In contrast to γECG, γEC provided catalytic hydrogen currents at the Brdička potential region when Co(II) concentration was decreased to 0.4 mmol l^{-1}. At all studied conditions, CG manifested the highest response. One or two Brdička responses were observed by both voltammetric techniques. CG, PC2, and PC3 gave one response at about −1.60 V, whereas γEC and γECG gave two responses at

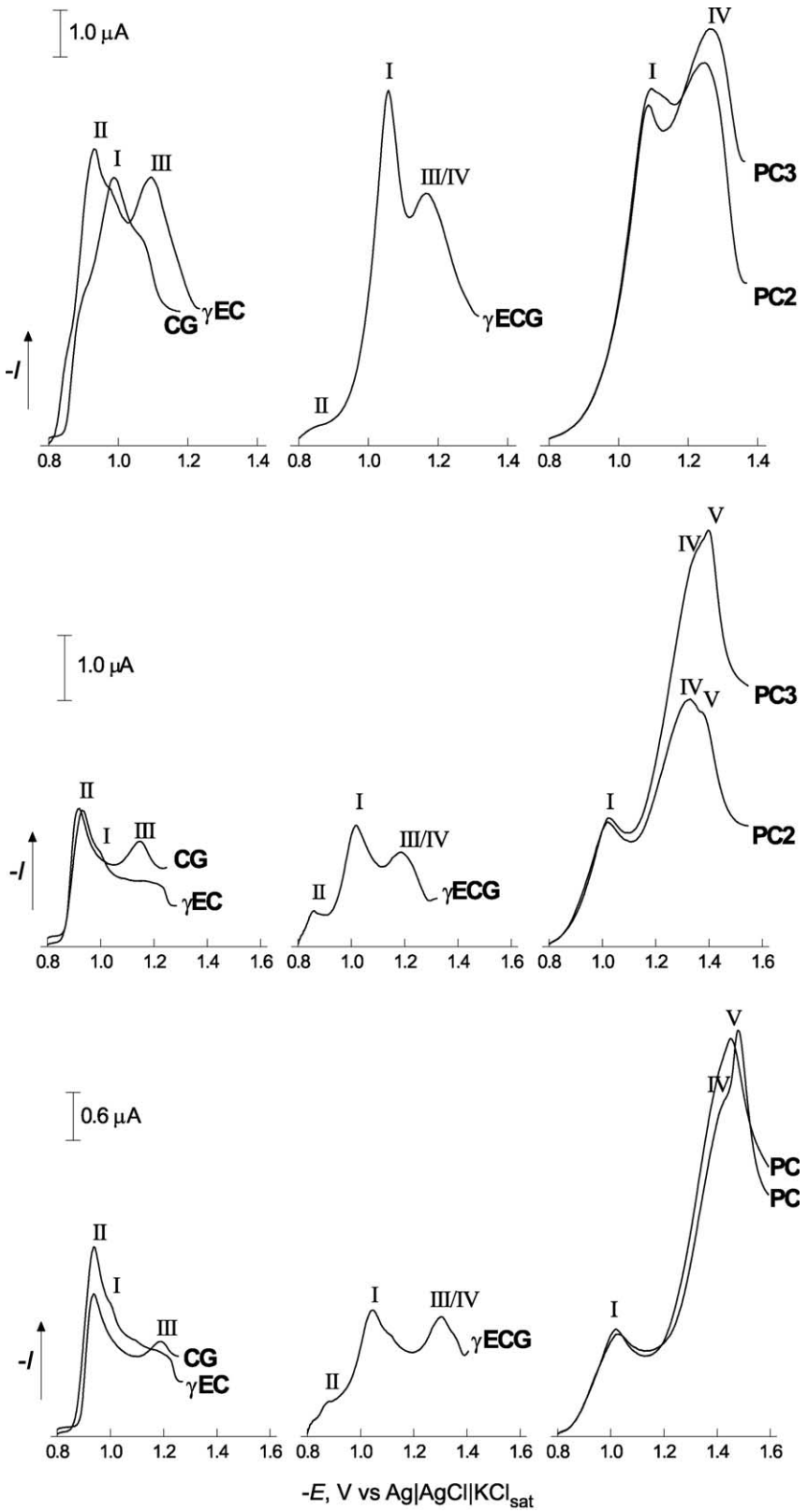

Fig. 2. Comparison of DC voltammograms for five peptides (83.3 μmol l^{-1}) recorded on HMDE from −0.80 V at 100 mV s^{-1} scan rate in borate buffer containing Co(II) ions. First row: pH 8.2, 1.0 mmol l^{-1} Co(II); second row: pH 8.2, 0.4 mmol l^{-1} Co(II); third row: pH 9.4, 0.4 mmol l^{-1} Co(II). Roman numerals designate individual peaks (for details, see text).

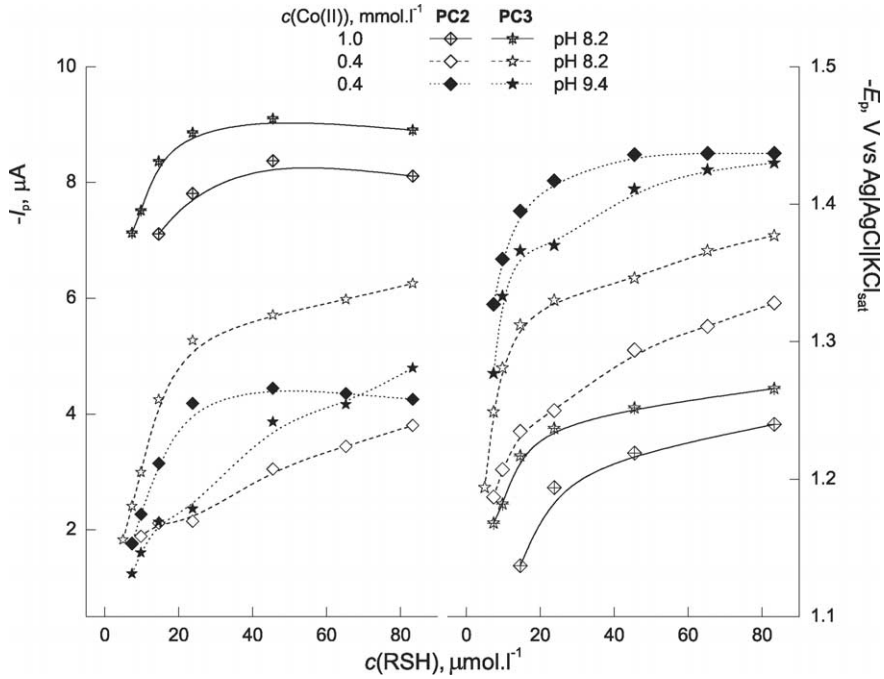

Fig. 3. Comparison of concentration dependences of peak IV height and potential of phytochelatins (PC2 and PC3) at different experimental conditions. Read from DC voltammograms recorded in borate buffer, pH 8.2, with 0.4 and 1.0 mmol l^{-1} Co(II) ions, and pH 9.4 with 0.4 mmol l^{-1} Co(II) ions. HMDE, scan from −0.8 V, scan rate 100 mV s^{-1}.

−1.45 V and −1.60 V using DCV. Two responses were observed for all peptides by DPV at a potential of about 0.1 V, more positive than in DCV (Fig. 5).

With increasing peptide concentration, the increase of cathodic current at B2 potential region is observed at first, and later the formation of peak B1. Peak B1 is shifted to a more negative potential, whereas peak B2 potential is almost changeless. Brdička response of both phytochelatins is overlapped by formation of predominant peaks IV. This effect was minor at higher Co(II) concentration and lower pH. There was no significant influence of peak III formation in the case of CG, γEC, and γECG as on DC and DP voltammograms (Figs. 2 and 5). DP voltammograms seem to be more helpful in using the Brdička reaction for analytical purposes. Dependences of Brdička responses B1 and B2 as a function of peptide concentration are shown in Fig. 6. Particularly, peak B2 was chosen for linear regression in order to obtain well-fitted concentration

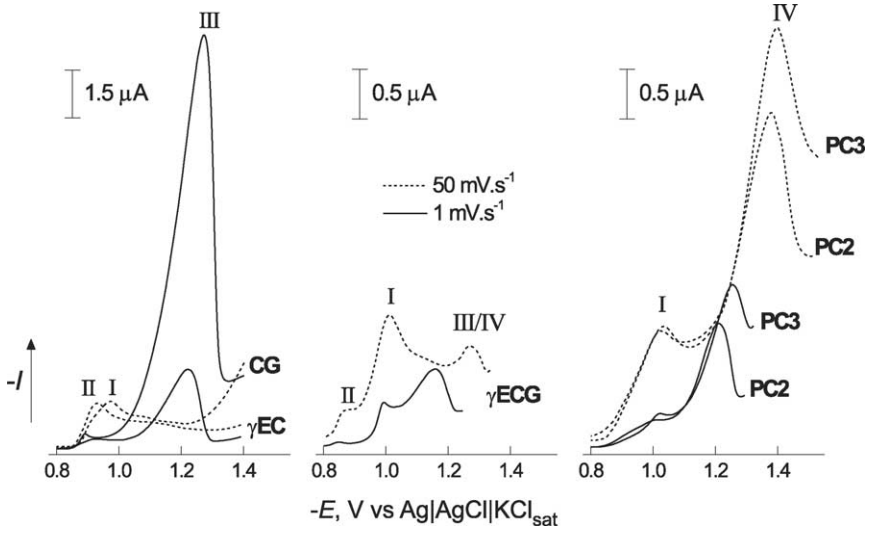

Fig. 4. Comparison of DC voltammograms recorded at 1 and 50 mV s^{-1} scan rates on HMDE from −0.80 V in borate buffer, pH 9.4, containing 0.4 mmol l^{-1} Co(II) ions and 45.5 µmol l^{-1} individual peptides.

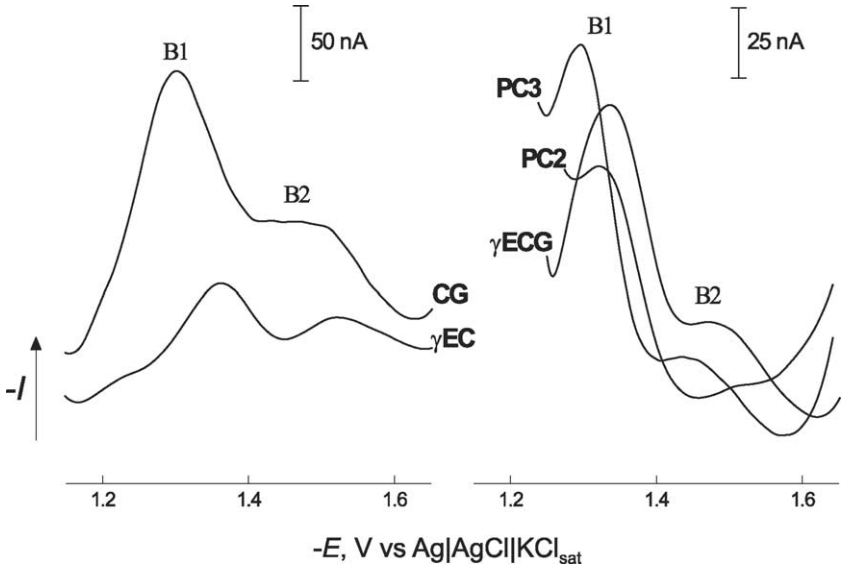

Fig. 5. DP voltammograms of five catalysts with two Brdička responses (B1 and B2) recorded in borate buffer, pH 9.4, containing 0.4 mmol l^{-1} Co(II) ions and indicated peptides: CG, 7.4 μmol l^{-1}; γEC and γECG, 83.3 μmol l^{-1}; PC2, 5.0 μmol l^{-1}; PC3, 23.8 μmol l^{-1}.

intervals with a correlation coefficient of fit near 1. Chosen concentration intervals are listed in Table 1.

4. Conclusion

It is obvious from this study that the Brdička phenomenon is not restricted to the ammonia buffers only, as claimed in Ref. [16]. The absence of its essential role was demonstrated long time ago [42]. Large Brdička catalytic hydrogen currents occur also, for example, in borate, tris, 4-hydroxybenzoate, phenolate, and further types of buffers (decisive for their resulting magnitude, besides the nature of the concrete thiol and buffer, and the value of pH). Microdissociation constants of thiols studied here also have a substantial influence on coordination equilibria in the thiolate cobalt(II) mixtures. Formation of the Co(II)–thiolate complexes is a necessary prerequisite for the appearance of both the cobalt(II) prewave and the Brdička catalytic hydrogen wave.

The conditions were found when the pattern of cobalt(II) prewave allows to distinguish between PCs, γECG, and its fragments. The part of prewave denoted as peak II appears only for γECG and its fragments, CG and γEC. Further differences were also found in the formation of peaks before the Brdička response. While the peak III corresponding to

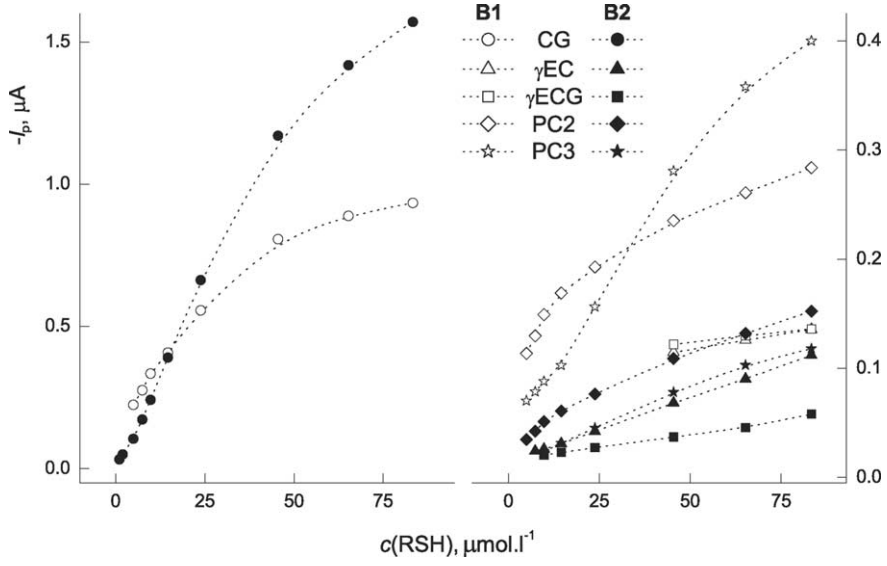

Fig. 6. Comparison of concentration dependences of two Brdička responses (B1 and B2) of five catalysts. Peak heights read from DP voltammograms recorded in borate buffer, pH 9.4, containing 0.4 mmol l^{-1} Co(II) ions and indicated peptides.

Table 1
Selected concentration intervals with correlation coefficients of five catalysts chosen for the linear regression of the second Brdička maximum (B2) at given conditions

pH	c(Co(II)) [mmol l^{-1}]	c(RSH) [μmol l^{-1}] (R)				
		CG	γEC	γECG	PC2	PC3
8.2	1.0	5.0–23.8 (0.9976)	–	–	7.4–83.3 (0.9999)	5.0–23.8 (0.9981)
8.2	0.4	5.0–23.8 (0.9999)	7.4–45.5 (0.9975)	–	1.0–14.6 (0.9967)	5.0–23.8 (0.9975)
9.4	0.4	5.0–23.8 (0.9999)	5.0–83.3 (0.9998)	9.8–83.3 (0.9968)	–	9.8–65.2 (0.9992)

hydrogen evolution catalysed by labile complexes arising at HMDE during slow scan rates is restricted to CG and γEC only, peak IV of PCs is probably due to reduction of stable Co(II)–peptide complexes. Peak III/IV for γECG represents the borderline between those two cases.

All studied peptides provided reasonable Brdička response in borate buffer, pH 9.4, with 0.4 mmol l^{-1} Co(II) ions. DPV on HMDE seems to be more helpful in using the Brdička reaction for their analytical determination. The concentration intervals were found where particularly the current of the second Brdička maximum is linearly proportional to the peptide concentration, and also the conditions where γEC and γECG behave as catalytically inactive. It can be exploited in the determination of PCs in real samples.

From our results, it follows that, before formulating the optimal experimental conditions under which the potential of the Brdička phenomenon can be fully exploited, reconsideration of the overall situation at the surface of the electrode, beginning from potentials of the cobalt(II) prewave [17], must be first made, which would be based on new experiments using contemporary means (electrochemical instrumentation and approaches).

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