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Electrochemical and ESR Studies of Au-Protein from *Micrococcus luteus*

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Abstract

Au-protein from *Micrococcus luteus*, with and without Au in active center, and chloroauric acid ($HAu^{III}Cl_4 \cdot 4H_2O$) with the addition of rutin, catechol, and riboflavin have been studied by means of electrochemistry and ESR . The redox potentials for Au-protein, as well as for the complexes Au-rutin and Au-catechol, have been measured, and ESR spectra of complexes Au-rutin and Au-catechol have been recorded. It has been shown that the Au atom binds to Au-protein via OH-groups of rutin. Flavin does not participate in gold binding. Au-protein is characterized by two peaks of cyclic voltammogram, –0.37 and –0.54 V. Au-protein with these potentials is able to function in the electron-transport chain of membranes between flavoproteins and quinones.

Index Entries: Gold-accumulating bacteria; Au-protein; NADH-oxidase; cyclic voltammogram; ESR.

Introduction

We found earlier *(1)* that gold-accumulating cells from *Micrococcus luteus* contain an Au-protein, a membrane-bound NADH-oxidase with gold in the active center. Fluorescence spectra showed *(2)* that Au-protein, in contrast to other NADH-oxidases, contains two organic cofactors, flavin and rutin (or quercetin, the analog of rutin). The gold ion in the active center affects rutin fluorescence, whereas flavin fluorescence remains unchanged.

Rutin belongs to the vitamin P family. Compounds of this family are polyphenols and can bind metals *(3)*. They are known to be strong antioxidants and stabilizers of cell membranes in biology *(3,4)*.

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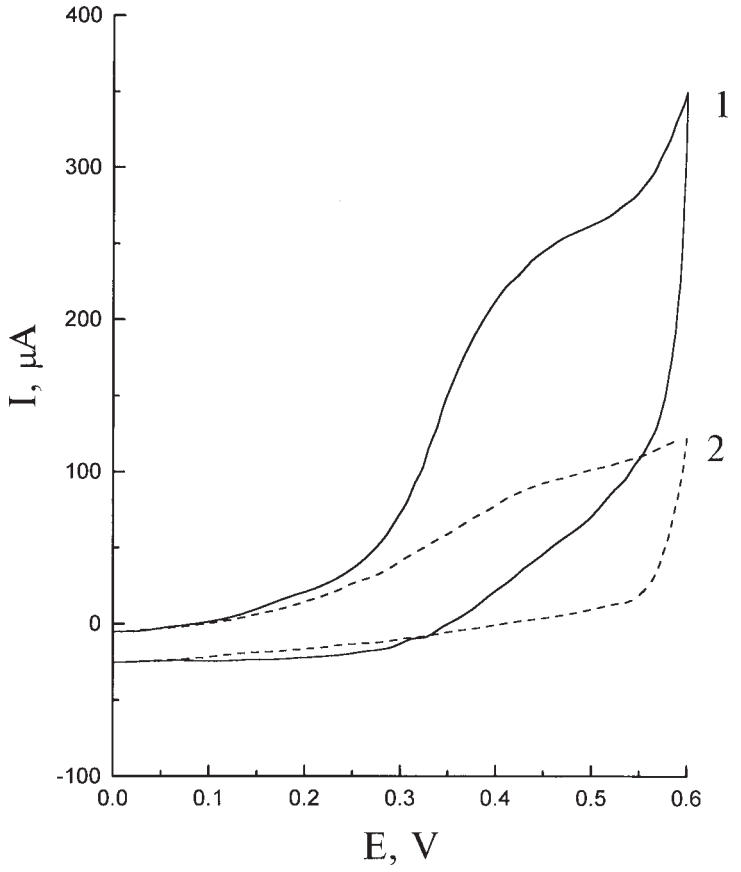


Fig. 1. Cyclic voltammograms for 1 m*M* rutin (1) and 0.1 m*M* protein without Au (2) in 0.1 *M* Tris-HCl buffer (pH 8.0) on a gold electrode.

In the present work we studied by means of electrochemistry and ESR the solutions of Au-protein, with and without Au in the active center, and chloroauric acid ($HAu^{III}Cl_4 \cdot 4H_2O$) with the addition of rutin, catechol, and riboflavin.

Materials and Methods

We used an electrochemical cell described in ref. *5* for recording of ESR spectra. The cell requires 0.6 mL of solution and permits ESR spectra to be recorded at controlled potentials. Working, auxiliary, and reference electrodes were made of gold, platinum, and silver, respectively. Electrochemical measurements were carried out in an argon atmosphere. Tris-HCl buffer (0.1 *M*, pH 8.0) was used as a solvent. Cyclic voltammograms (CV) were recorded by a potentiostat PI-50 with a programmer PR-8 at a rate of scan of 20 mV/s. ESR spectra were recorded by a SE/X 2544 radiospectrometer (Radiopan, Poznan) at a modulation of magnetic field of 0.01 mT and microwave power of 5 mW.

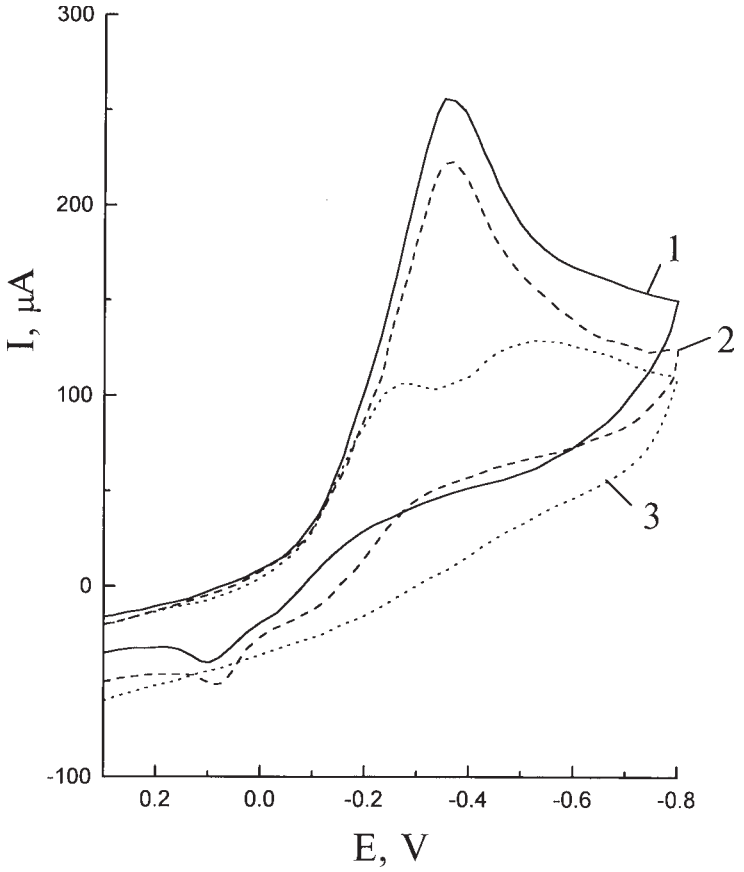


Fig. 2. Cyclic voltammograms for solutions of chloroauric acid with the different addition of rutin in 0.1 *M* Tris-HCl buffer (pH 8.0) on a gold electrode. (1) 0.5 m*M* $HAuCl_4 \cdot 4H_2O$ without rutin; (2) 0.25 m*M* $HAuCl_4 \cdot 4H_2O$ + 0.25 m*M* rutin; (3) 0.25 m*M* $HAuCl_4 \cdot 4H_2O$ + 2.5 m*M* rutin. For the convenience of data presentation, the current of curve 2 is doubled.

Au-protein, with and without gold, was isolated from *Micrococcus luteus* as described in *(1)*.

Results and Discussion

It has been found that CV for rutin and Au-protein without gold ion in the active center are similar (Fig. 1). For both samples, an irreversible anodic peak at +0.5 V is observed, and there are no peaks at negative potentials. Thus, rutin is apparently responsible for CV peak for Au-protein without gold.

CV for pure chloroauric acid (Fig. 2, curve 1) shows an irreversible peak at –0.39 V and two peaks at –0.07 and +0.1 V at reverse scan of potential. The addition of rutin at a ratio rutin:Au = 1 shows CV close to that for pure chloroauric acid (curve 2). With a 10-fold excess of rutin, two irrevers-

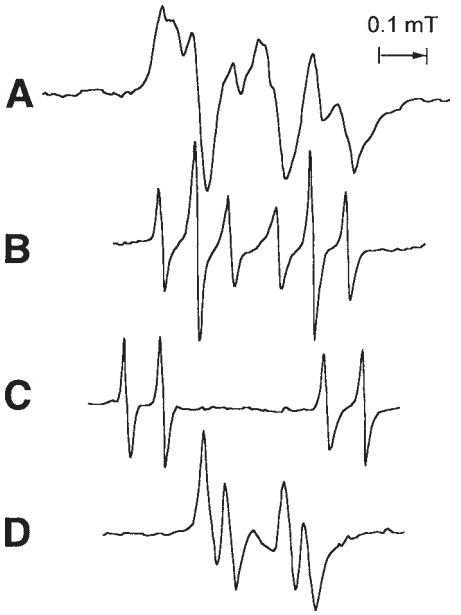


Fig. 3. ESR spectra of aqueous solutions of the mixture of rutin and chloroauric acid (1:1) **(A)** and pure rutin **(B–D)** at the room temperature. The concentration of rutin is 1 m*M*, the concentration of NaOH is 0.2 *M*. The spectra were recorded at different times after NaOH addition: spectra (A) and (B) were recorded just after NaOH addition, spectra (C) and (D) were recorded 10 and 20 min later, respectively.

ible peaks at –0.26 and –0.52 V and a weak peak at +0.5 V are observed, and the peak at –0.07 shifts by 100 mV to the cathodic region. These features of CV are explained by formation of Au^{III}–rutin complex, which is reduced at –0.26 and –0.52 V.

At pH 8.0, no ESR spectra were observed in solutions of pure rutin and Au-rutin mixture at controlled potentials in the electrochemical cell. Low concentration of radicals may be explained by their short life span. Indeed, CV at scan 20 mV/s reveals only irreversible peaks, i.e., products of the electrode reactions interreact with the components of solution.

ESR signals for rutin and Au-rutin complex were observed after alkalization of solutions to pH 12 both in the standard 1-mm tubes (Fig. 3) and in the elechtrochemical cell at controlled potentials (Fig. 4). We explain these ESR spectra by formation of complexes shown in Scheme 1. Formation of these complexes results in changes in densities of uncoupled electrons and, as a consequence, in hyperfine splittings from protons A and B. The splitting from Au (S = 3/2) is insignificant because the magnetic moment of Au nuclei is 60 times less than that of protons. Only a part of rutin structure is shown in Scheme 1.

For solution of rutin in the standard tubes three types of semiquinone radical anions are revealed at different times after NaOH addition (Fig. 3B–D). They are characterized by the following ESR parameters:

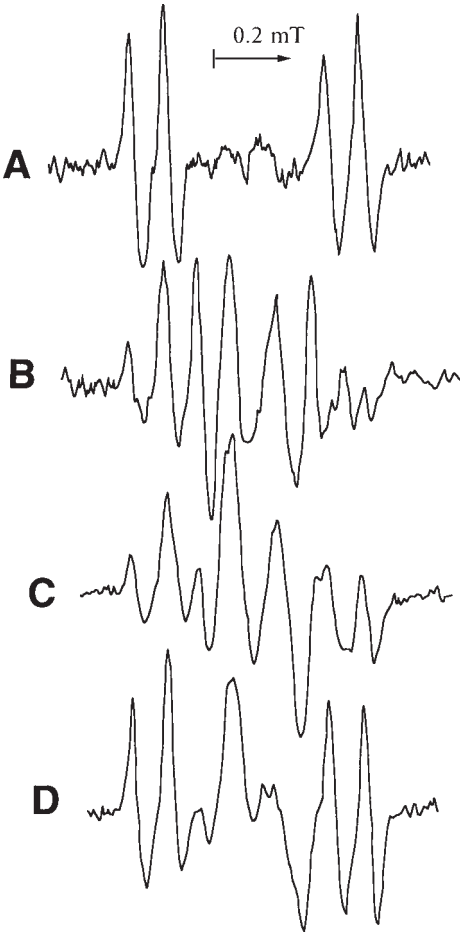
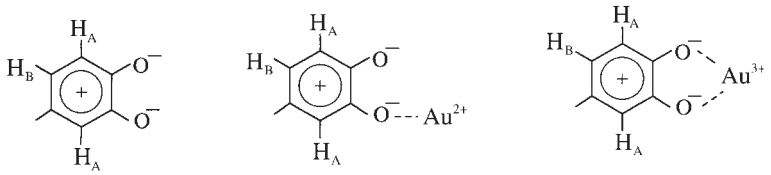


Fig. 4. ESR spectra of aqueous solution of the mixture of rutin and chloroauric acid (10:1) at the room temperature and different potentials. The concentration of rutin is 2 m*M*, the concentration of NaOH is 0.2 *M*. **(A)** –0.12 V, **(B)** 0 V, **(C)** +0.2 V, **(D)** +0.4 V.

g = 2.0045, a'_A = 0.091 mT, a_B = 0.30 mT (shown in Fig. 3B), g = 2.0046, a_A = 0.091 mT, a_B = 0.52 mT (shown in Fig. 3C), g = 2.0043 , a_A = 0.044 mT, a_B = 0.10 mT (shown in Fig. 3D), where a_A and a_B are hyperfine splitting from nonequivalent protons A and B, a'_A is splitting from two equivalent protons. In the beginning (spectrum b), we see the splittings from three protons (two equivalent plus one proton), then we see the splitting only from two nonequivalent protons (spectra c and d). We attribute spectra b, c, and d to structures I, II, and III, respectively, where admixtures of cations serve instead of gold. For the mixture of chloroauric acid and rutin, the ESR spectrum immediately after addition of NaOH (Fig. 3A) seems to be a superposition of spectra b, c, and d. The ESR spectrum of this mixture decreases in time and almost disappears after half an hour.



I II III

Scheme 1. The structure of complexes of gold with radical anion of rutin at pH 12.0.

In the electrochemical cell, for the mixture of rutin with gold in the presence of NaOH, no ESR spectrum is observed at potentials more negative than –0.2 V; at higher potentials ESR spectra appear (Fig. 4). This means that a radical anion is formed at potentials close to 0 V. Parameters of spectrum 4a (g = 2.0045, a_A = 0.093 mT, a_B = 0.52 mT) are identical to those of spectrum 3c. Spectrum 4a may be attributed to structure II, spectra 4b–d are probably caused by a mixture of structures I–III. Note that Au^{3+} causes substantial changes in ESR spectra at rutin/Au^{3+} = 10; this means that only a small portion of rutin (less than 10%) is paramagnetic at pH 12.0.

To confirm the participation of two adjacent OH groups in the phenol ring of rutin in chelation of the Au atom, we recorded CVs and ESR spectra for chloroauric acid with the addition of catechol [Cat, $C_6H_4(OH)_2$] at pH 8.0 and 12.0 (Fig. 5). At pH 8.0 and Cat/Au = 1, in addition to the irreversible peak of reduction of Au^{3+}, the quasi-reversible (the difference between potentials of peaks in direct and reverse scans ΔE is equal to 110 mV) cathodic peak arises at +0.01 V. For pure Au^{3+} or catechol, no peaks are found at these potentials. The increase of catechol concentration from 0.2 to 2 m*M* (curves 1–3) is accompanied by the increase in the cathodic peak at +0.01 V and by the decrease in the peak of reduction of Au^{3+}. At Cat/Au^{3+} = 10 two overlaid cathodic peaks are observed at –0.37 and –0.54 V instead of the peak of reduction of pure Au^{3+}. Cathodic peaks at +0.01, –0.37, and –0.54 V are caused probably by reduction of different complexes Cat–Au^{3+}. At pH 12.0, the quasi-reversible cathodic peak at +0.01 V shifts to –0.1 V (curves 4) and becomes reversible. The value of ΔE (60 mV) testifies to reversible one-electron reduction of Au–Cat complex at –0.1 V.

At pH 8.0, no ESR spectra were recorded for pure catechol and mixtures of catechol and Au^{3+}. At pH 12.0, ESR spectra were observed both in the standard tubes and in the electrochemical cell (Fig. 6). These spectra are characterized by the following ESR parameters: g = 2.0042, a'_A = 0.24 mT (shown in Fig. 6A), g = 2.0045, a'_A = 0.37 mT, a'_B = 0.077 mT (shown in Fig. 6B), g = 2.0045, a'_A = 0.37 mT, a'_B = 0.047 mT (shown in Fig. 6C). In the electrochemical cell, no ESR spectra were observed at potentials below –0.05 V. The ESR spectra are explained by hyperfine splittings from two pairs of equivalent protons, in accordance with the chemical structure of catechol at pH 12.0 $C_6H_4(O^-)_2$, and, as in the case of rutin, by changes in

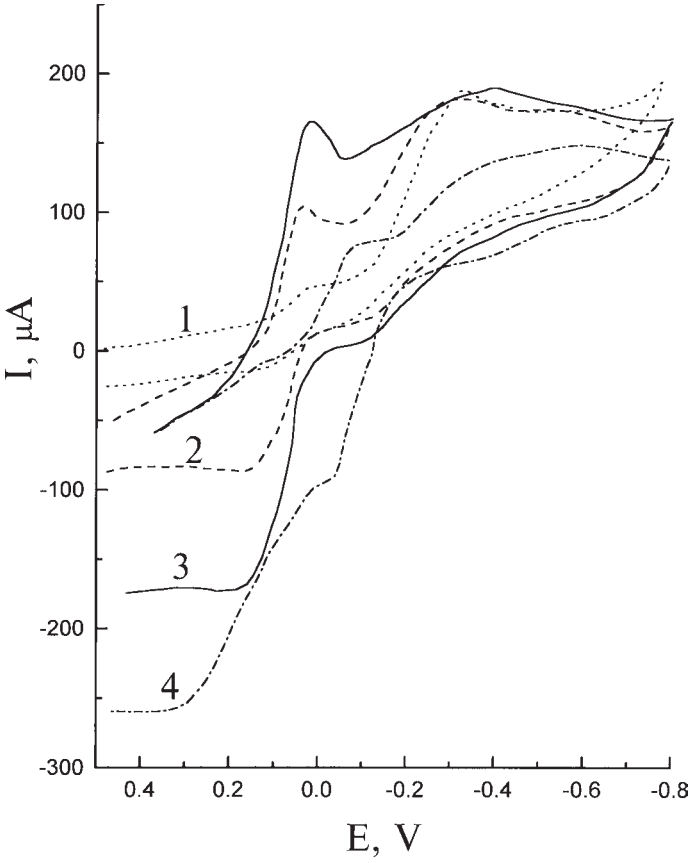


Fig. 5. Cyclic voltammograms for solutions of chloroauric acid with different concentration of catechol in 0.1 *M* Tris-HCl buffer on a gold electrode. (1) 0.2 m*M* $HAuCl_4 \cdot 4H_2O$ + 0.2 m*M* Cat (1:1), pH 8.0; (2) 0.2 m*M* $HAuCl_4 \cdot 4H_2O$ + 1 m*M* Cat (1:5), pH 8.0; (3) 0.2 m*M* $HAuCl_4 \cdot 4H_2O$ + 2 m*M* Cat (1:10), pH 8.0; (4) 0.2 m*M* $HAuCl_4 \cdot 4H_2O$ + 2 m*M* Cat (1:10), pH 12.0.

electron densities of uncoupled electrons due to formation of the Cat–Au complex.

CV for Au-protein with Au in the active center (Fig. 7, curve 1) reveals two irreversible overlaid peaks at –0.37 and –0.54 V and no peaks at anodic potentials (whereas for Au-protein without Au the peak at +0.5 V is observed). Two CVs are reproduced in Fig. 7, for convenience of comparison, for the mixture of catechol and chloroauric acid at Cat/Au = 5 (curve 2) and for the mixture of rutin and chloroauric acid at rutin/Au = 10 (curve 3). The comparison of these CVs shows that the potentials of cathodic peaks for Au-protein and the complexes Au-rutin and Au-catechol are close. Therefore, Au ion in Au-protein binds to apoprotein via rutin, and potentials of Au ion in Au-protein are –0.37 and –0.54 V.

The closeness of redox potentials of Au-protein and rutin-Au complex, combined with our previous spectroscopic results *(2)*, shows that gold

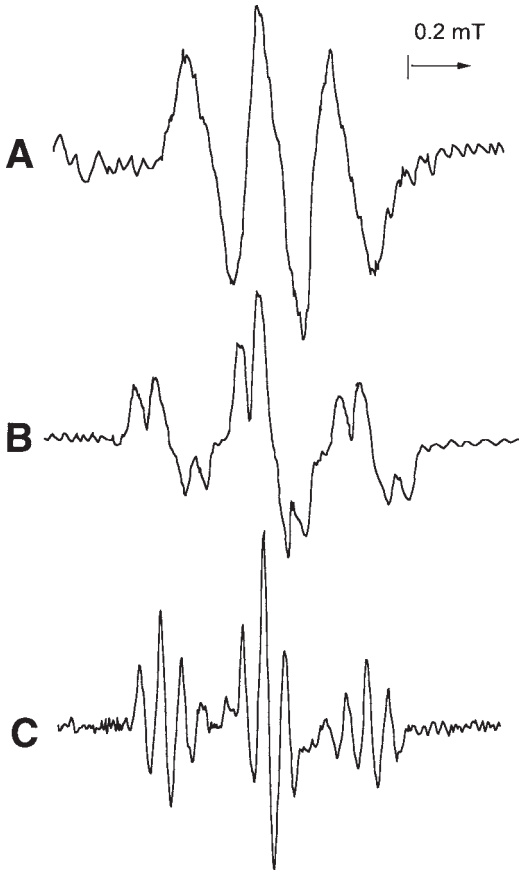


Fig. 6. ESR spectra of aqueous solutions of catechol and the mixture of catechol with Au^{3+} at pH 12.0 and the room temperature. **(A)** 2 m*M* Cat, **(B)** 2 m*M* Cat + 0.2 m*M* Au^{3+} (10:1), **(C)** 2 m*M* Cat + 0.2 m*M* Au^{3+} (10:1), E = +0.1 V. Spectra a and b were recorded in the standard tubes, spectrum c was recorded in the electrochemical cell after removal of dissolved dioxygen.

in both Au-protein and rutin-Au complex is chelated by two adjacent OH groups of the phenol ring of rutin. A small difference in values of potentials of cathodic peaks for Au-protein and complex Au-rutin can be easily explained if we accept that rutin in Au-protein is bound not only to gold but also to the protein matrix (apoenzyme).

For Au-protein with and without gold, no ESR spectra were recorded even after alkalization of solutions, probably, because rutin in these membrane proteins is inaccessible for alkali.

Au-protein contains flavin, besides rutin. Fluorescence spectra show *(2)* that flavin is not affected by gold. Therefore, it is interesting to perform an electrochemical and ESR study of chloroauric acid in the presence of riboflavin. Riboflavin CV (Fig. 8) reveals a reversible reduction of riboflavin at –0.54 V (ΔE = 60 mV). In the presence of riboflavin at ratio riboflavin/

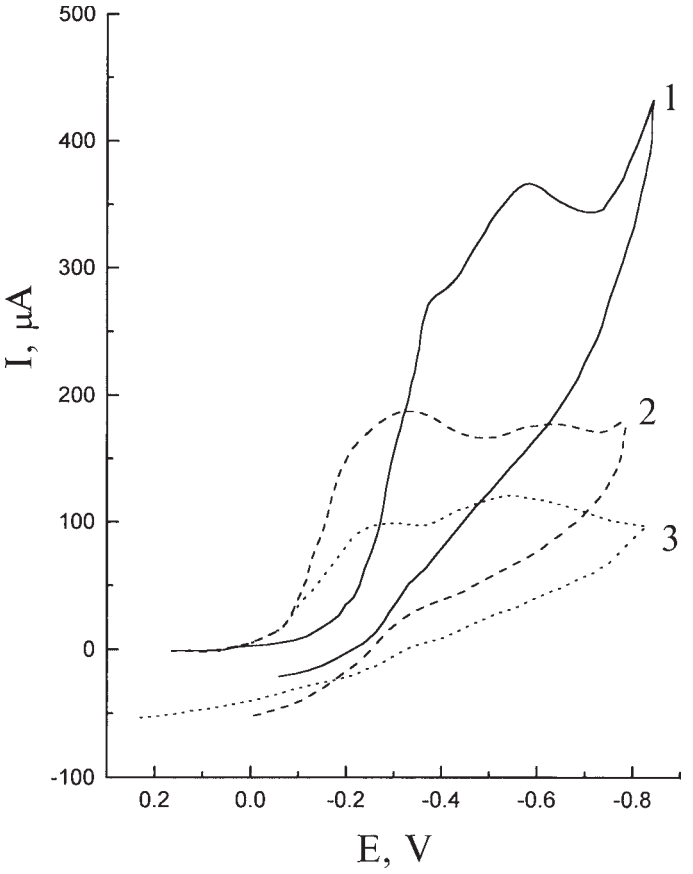


Fig. 7. Cyclic voltammograms for Au-protein (1) and the mixtures of chloroauric acid with catechol (2) and rutin (3) in Tris-HCl buffer (0.1 *M*, pH 8.0) on a gold electrode. (1) Au-protein, 0.5 m*M*; (2) 0.1 m*M* $HAuCl_4 \cdot 4H_2O$ + 0.5 m*M* Cat (1:5); (3) 0.25 m*M* $HAuCl_4 \cdot 4H_2O$ + 2.5 m*M* rutin (1:10).

Au^{III} = 10, we found a superimposition of CVs for riboflavin and chloroauric acid, i.e., riboflavin does not interact with Au^{3+}.

Thus, the redox potentials for Au-protein from *Micrococcus luteus*, as well as for the complexes Au-rutin and Au-catechol have been measured, and ESR spectra of complexes Au-rutin and Au-catechol have been recorded. We show that Au binds to Au-protein via OH-groups of rutin. Au-protein is characterized by two peaks of CV, –0.37 and –0.54 V. After reduction of these potentials to a standard hydrogen electrode, potentials E^o of Au-protein are –0.13 and –0.3 V. Au-protein with these potentials is able to function in the electron-transport chain of membranes between flavoproteins and quinones *(6)*. It should be noted that the presence of quercetin in the active center of Au-protein is less probable, because its ability to chelate metals is lower than that for rutin. Flavin does not participate in gold binding.

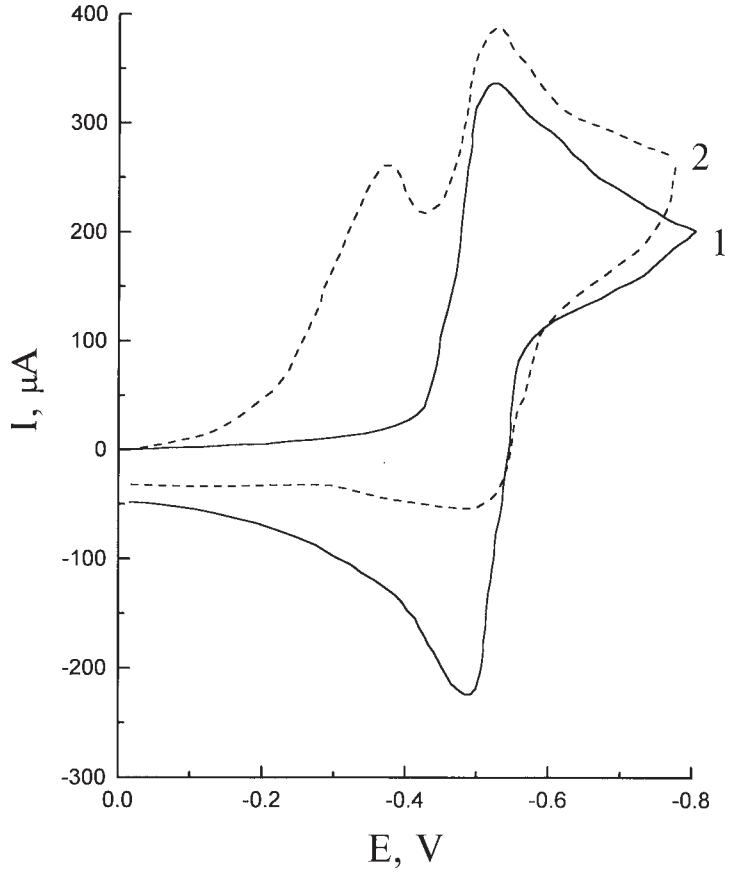


Fig. 8. Cyclic voltammograms for the mixtures of riboflavin and chloroauric acid in Tris-HCl buffer (0.1 *M*, pH 8.0) on gold electrode. (1) 1 m*M* riboflavin, (2) 0.2 m*M* $HAuCl_4 \cdot 4H_2O$ + 2 m*M* riboflavin (1:10).

Acknowledgment

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