Protective properties of gold in the composition of nanoparticles and Au—rutin complexes

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Protective properties of gold nanoparticles and gold—rutin complexes were studied. Aurophilic bacteria *Micrococcus luteus* and methanotrophic bacteria *Methylococcus capsulatus* were studied. Gold—rutin nanoparticles and complexes protect the respiratory activity of the bacteria against toxins. Pretreatment of the cells with gold is more efficient than the treatment after the action of toxin.

Key words: bacteria *Micrococcus luteus*, bacteria *Methylcoccus capsulatus*, inhibitor, 1-naphthol, 2,2′-bipyridyl, gold, nanoparticles, respiratory activity, resistance.

Increased interest in the use of gold in chemistry, medicine, and biology is observed in the recent time. Special attention is given to gold nanoparticles[1—3] and gold complexes with natural compounds, including flavonoids.[4] This direction is one of the most important in green chemistry. As for the use of gold in medicine and biology, unique medicinal properties of this metal known from the ancient time (chrisotherapy) should be mentioned. Studies on the application of gold preparations for point diagnostics and treatment of such severe diseases as cancer and tuberculosis are carried out in many laboratories.[5]

In our opinion, it is also interesting that metalophilic organisms that assimilate gold including it in cell metabolism exist in nature.[6,7] We showed that in the presence of colloidal gold in an incubation medium aurophilic bacteria *Micrococcus luteus* incorporate the Au atoms into the active site of specific membrane-bound NADH oxidase, which was named Au-protein.[8] A specific feature of this protein is the presence of flavin and flavonoid cofactors.[9] It is the flavonoid cofactor that chelates gold. As a result, Au-protein gains a new function, *viz.*, the ability to oxidize methane and its homologs.[10,11]

Taking into account the medicinal properties of gold and the ability of aurophilic bacteria to include it in their metabolism, it seemed of interest to study the possibility of appearance of other new functions in bacteria cells under the action of gold, in particular, protection of biological systems against toxins. Since respiration is the main process providing vital activity to all living organisms, in the present work we studied the ability of Au—flavonoid nanoparticles and complexes to protect cells against poisons and toxins inhibiting respiratory activity.

Experimental

The studies were carried out on aurophilic microorganisms *Micrococcus luteus* and methanotrophs *Methylococcus capsulatus*. Inhibitors of the respiratory chain, 1-naphthol and 2,2′-bipyridyl, were used as toxins. The respiratory activity was determined by polarography using the Clark electrode.[12] The protein concentration was determined by the biuret method after the preliminary disintegration of cells and spheroplasts on an UZDN-1 sonicator at 22 kHz and 0 °C. The Au-protein was isolated from bacteria *Micrococcus luteus*.[8] The complexes and nanoparticles Au—rutin were prepared according to a method developed earlier.[4,13,14] Colloidal gold prepared by the Zsigmondy method[15] was used as the standard. Absorption spectra were recorded on a Specord M-40 spectrophotometer (Carl Zeiss).

The following reagents were used: $HAuCl_4 \cdot 4\,H_2O$, rutin, NADH (Sigma), 1-naphthol, 2,2′-bipyridyl (reagent grade), Tris-HCl buffer, pH = 8.0, and bidistilled water.

Results and Discussion

When studying the respiratory activity of bacteria *Micrococcus luteus*, we found that the respiratory activity of the cells increases by 20—25% in the presence of the Zsigmondy colloidal gold, Au—rutin nanoparticles, and the Au—rutin complex (Table 1, Fig. 1, curves *1—4*), indicating that gold is incorporated into the main membrane electron-transport chain (ETC) of the bacterial cell.[16]

To solve the problem of the protective properties of gold, we carried out studies on the influence of the classical ETC inhibitors, 1-naphthol and 2,2′-bipyridyl, on the respiratory activity of bacterial cells *Micrococcus luteus* and *Methylococcus capsulatus*. In the experiments with cells of *Micrococcus luteus* treated with 1-naphthol and 2,2′-bipyridyl, the oxygen uptake reduces by 35 and 30%, respec-

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Table 1. Influence of gold sols and the Au—rutin complex on the respiratory activity of bacteria *Micrococcus luteus* and *Methylococcus capculatus* in the presence of toxins

Additive	*Micrococcus luteus*			*Methylococcus capculatus*		
	C_{O_2} /μmol L^{-1}	*A* (%)	*I* (%)	C_{O_2} /μmol L^{-1}	*A* (%)	*I* (%)
—	113.7	—	—	72.2	—	—
Zsigmondy sol	135.4	20	0	47.2	0	34.6
Au—rutin nanoparticles	135.4	20	0	57.1	0	20.8
Au—rutin complex	133.5	18	0	78.2	8.3	0
1-Naphthol	75.1	0	34.2	44.1	0	38.8
2,2′-Bipyridyl	78.2	0	29.4	50.0	0	30.7
Zsigmondy sol + 1-naphthol	135.4	0	0	55.5	0	23.0
Au—rutin nanoparticles + 1-naphthol	135.4	0	0	63.5	0	12.0
Au—rutin complex + 1-naphthol	117.0	0	14.0	69.4	0	4.0
1-Naphthol + Zsigmondy sol	130.1	0	0	52.5	0	27.0
1-Naphthol + Au—rutin nanoparticles	125.0	0	7.5	61.5	0	15.0
1-Naphthol + Au—rutin complex	123.0	0	10.0	72.5	0	0
Zsigmondy sol + 2,2′-bipyridyl	135.5	0	0	58.3	0	19.0
Au—rutin nanoparticles + 2,2′-bipyridyl	135.4	0	0	66.4	0	8.0
Au—rutin complex + 2,2′-bipyridyl	123.0	0	6.0	76.4	5.0	0
2,2′-Bipyridyl + Zsigmondy sol	132.0	0	2.5	52.8	0	27.0
2,2′-Bipyridyl + Au—rutin nanoparticles	122.5	0	9.5	57.7	0	20.0
2,2′-Bipyridyl + Au—rutin complex	119.3	0	12.0	76.4	5.9	0

Note. Composition of the sample: initial cells of *Micrococcus luteus* or *Methylococcus capculatus* + additives. The content of the components in the samples: 2.5 mL of 0.005 *M* Tris-HCl buffer, pH = 8.0; 2 mg of total protein. Inhibitors: 1-naphthol; 2,2′-bipyridyl in a concentration of $1 \cdot 10^{-4}$ mol L^{-1}, gold concentration $1 \cdot 10^{-5}$ mol L^{-1}. *A* is activation; *I* is inhibition.

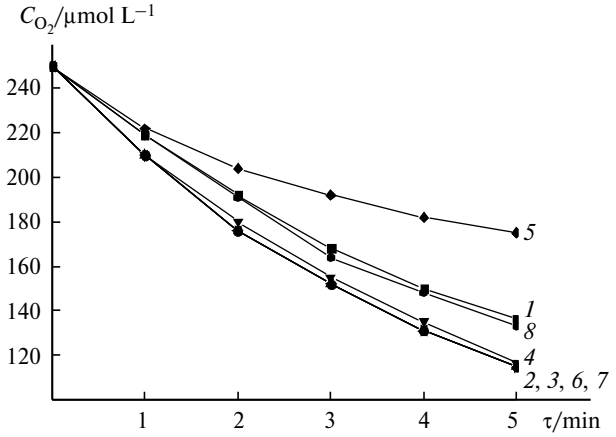

Fig. 1. Influence of gold sols and the Au—rutin complex on the respiratory activity of bacteria *Micrococcus luteus* in the absence and in the presence of 1-naphthol: *1*, bacteria *Micrococcus luteus*; *2*, bacteria *Micrococcus luteus* + Zsigmondy′s sol; *3*, bacteria *Micrococcus luteus* + Au—rutin nanoparticles; *4*, bacteria *Micrococcus luteus* + Au—rutin complex; *5*, bacteria *Micrococcus luteus* + 1-naphthol; *6*, bacteria *Micrococcus luteus* + Zsigmondy′s sol + 1-naphthol; *7*, bacteria *Micrococcus luteus* + Au—rutin nanoparticles + 1-naphthol; and *8*, bacteria *Micrococcus luteus* + Au—rutin complex + 1-naphthol. The content of the components in the samples: 2.5 mL of 0.005 *M* Tris-HCl buffer, pH = 8.0; 2 mg of total protein; $1 \cdot 10^{-4}$ *M* 1-naphthol; $1 \cdot 10^{-5}$ *M* gold.

tively (see Table 1 and Fig. 1, curve *5*). The data in Table 1 and Fig. 1 show that the preliminary treatment of bacteria *Micrococcus luteus* with the gold-containing preparations (the Zsigmondy sol, Au—rutin nanoparticles, and the Au—rutin complex) results in blocking of the inhibitor effect (Fig. 1, curves *6*—*8*). The protective effect of gold remains at the level of 90% if the order of introducing gold and the inhibitors into the incubation medium is reversed (when the cells are first subjected to the action of the inhibitors and then, 5 min later, gold is introduced, see Table 1 and Fig. 2, curves *6*—*8*).

Based on the results obtained, we may conclude that Zigmondy sol Au—rutin nanoparticles and complexes enhance the respiratory activity of the aurophilic cells and also favor acquiring resistance against toxins.

It was necessary to establish whether the fact of protective effect of gold against toxins is common for various cells or characteristic of aurophilic bacteria *Micrococcus luteus* only. For this purpose we carried out analogous experiments with methane-oxidizing bacteria *Methylococcus capsulatus*. It is seen from Table 1 and Fig. 3 (curves *1*—*3*) that, unlike cells of *Micrococcus luteus*, colloidal gold (the Zsigmondy sol) and Au—rutin nanoparticles induce the inhibition of the respiratory activity instead of the activation of this process. At the same time, the introduction of the Au—rutin complex intensified respiration in cells of *Methylococcus capsulatus* by 8—10%

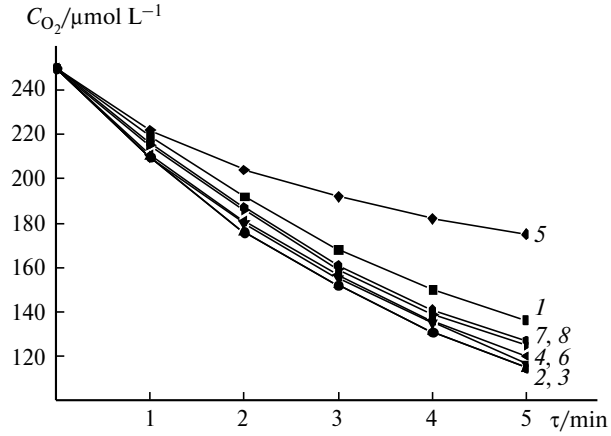

Fig. 2. Influence of gold sols and the Au—rutin complex on the respiratory activity of bacteria *Micrococcus luteus* after the treatment with 1-naphthol: *1*, bacteria *Micrococcus luteus*; *2*, bacteria *Micrococcus luteus* + Zsigmondy´s sol; *3*, bacteria *Micrococcus luteus* + Au—rutin nanoparticles; *4*, bacteria *Micrococcus luteus* + Au—rutin complex; *5*, bacteria *Micrococcus luteus* + 1-naphthol; 6, bacteria *Micrococcus luteus* + 1-naphthol + Zsigmondy´s sol; *7*, bacteria *Micrococcus luteus* + 1-naphthol + Au—rutin nanoparticles; and *8*, bacteria *Micrococcus luteus* + 1-naphthol + Au—rutin complex. The content of the components in the samples: 2.5 mL of 0.005 *M* Tris-HCl buffer, pH = 8.0; 2 mg of total protein; $1 \cdot 10^{-4}$ *M* 1-naphthol; $1 \cdot 10^{-5}$ *M* gold.

(Fig. 3, curve *4*), which can be due to different valent states of gold in the nanoparticles and Au—flavonoid com-

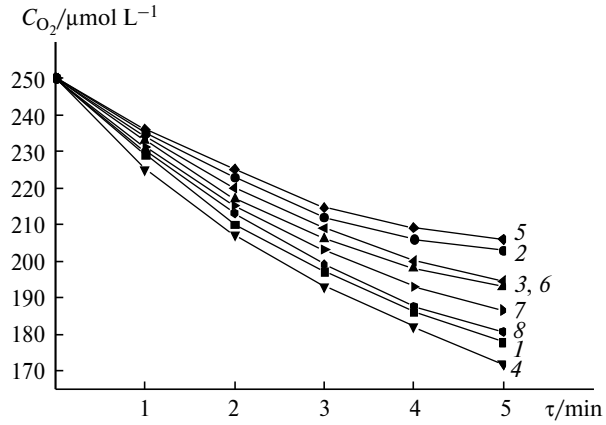

Fig. 3. Influence of gold sols and the Au—rutin complex on the respiratory activity of bacteria *Methylococcus capsulatus* in the absence and in the presence of 1-naphthol: *1*, bacteria *Methylococcus capsulatus*; *2*, bacteria *Methylococcus capsulatus* + Zsigmondy´s sol; *3*, bacteria *Methylococcus capsulatus* + Au—rutin nanoparticles; *4*, bacteria *Methylococcus capsulatus* + Au—rutin complex; *5*, bacteria *Micrococcus luteus* + 1-naphthol; 6, bacteria *Micrococcus luteus* + 1-naphthol + Zsigmondy´s sol; *7*, bacteria *Methylococcus capsulatus* + Au—rutin nanoparticles + 1-naphthol; and *8*, bacteria *Methylococcus capsulatus* + Au—rutin complex + 1-naphthol. The content of the components in the samples: 2.5 mL of 0.005 *M* Tris-HCl buffer, pH = 8.0; 2 mg of total protein; $1 \cdot 10^{-4}$ *M* 1-naphthol; $1 \cdot 10^{-5}$ *M* gold.

plexes and to different permeabilities of cell walls for the gold nanoparticles and complexes.

When the inhibitors 1-naphthol and 2,2´-bipyridyl are introduced into cells of *Methylococcus capsulatus* treated with gold, no further decrease in the respiratory activity was observed (see Table 1 and Fig. 3, curves *6*—*8*). Gold nanoparticles exert a dual effect on methane-oxidizing cells of *Methylococcus capsulatus*: gold decreases the level of cell respiration and, at the same time, protects them from toxins.

Since the *Micrococcus luteus* and *Methylococcus capsulatus* cells possess the common function, *viz.*, the ability to activate methane,[8] we studied the possibility of recombination of the Au-protein from *Micrococcus luteus* and the synthetic Au—rutin complex with cell membranes of *Methylococcus capsulatus* in order to use them as protection against toxins. As is known, membranes of *Methylococcus capsulatus* contain methane monooxygenase, the enzyme, which like Au-protein from *Micrococcus luteus* is NADH oxidase. However, cells of *Methylococcus capsulatus* are incapable of transforming colloidal gold. Moreover, as can be seen from Table 1, for this bacterium, unlike *Micrococcus luteus*, gold nanoparticles are an respiratory inhibitor.

The experiments on the recombination of the components from *Micrococcus luteus* with *Methylococcus capsulatus* membranes showed (Fig. 4, curves *1*—*3*) that the Au-protein and the Au—rutin complex interact with the *Methylococcus capsulatus* membranes, which is expressed in reduction of the level of oxygen uptake by the membranes. Nevertheless, this recombination increases the resistance of the *Methylococcus capsulatus* membranes against the inhibitors of the respiratory chain, 1-naphthol and 2,2´-bipyridyl (see Fig. 4, curves *4*—*6*). This fact can be explained by the interaction of gold of the Au-protein from *Micrococcus luteus* and of the model Au—rutin complex with the active site of methane monooxygenase of *Methylococcus capsulatus* or by the conformational changes in the membrane under the action of the gold-containing preparations. These data agree with the results of studies of intact cells of *Micrococcus luteus* and *Methylococcus capsulatus*, which suggest that the protective effect of gold, especially in the complex with the flavonoid (rutin), is common for the studied microorganisms.

The respiratory chain of bacteria localized in cell membranes and the respiratory chain of higher organisms localized in mitochondria are similar in composition and, hence, it can be assumed that the protective properties of gold combined with flavonoids would be common for prokaryotes and eukaryotes. Since rutin pertains to the group of vitamins P, which strengthen walls of blood vessels,[17] the addition of rutin—gold nanoparticles to drugs, in particular, to Troxevasin (troxerutin) can enhance their efficiency in curing vascular and articulation diseases.

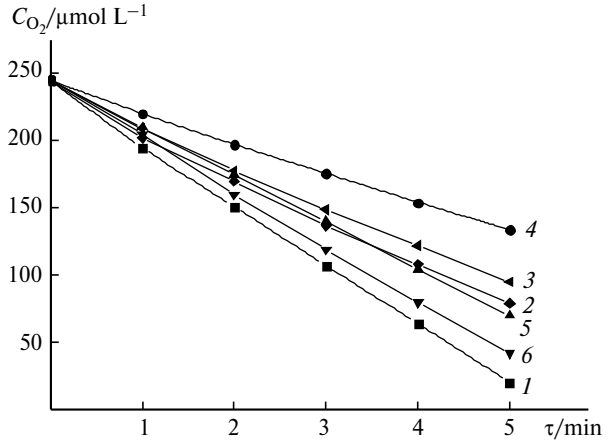

Fig. 4. The oxygen uptake by the membrane components of *Methylococcus capsulatus* (MCMC) recombined with the Au-protein from *Micrococcus luteus* and the model complex Au—rutin before and after the treatment with 1-naphthol: *1*, MCMC + NADH + buffer saturated with CH_4 and O_2; *2*, (MCMC + Au-protein) + NADH + buffer saturated with CH_4 and O_2; *3*, (MCMC + Au—rutin) + NADH + buffer saturated with CH_4 and O_2; *4*, MCMC + 1-naphthol + NADH + + buffer saturated with CH_4 and O_2; *5*, (MCMC + Au-protein) + + 1-naphthol + NADH + buffer saturated with CH_4 and O_2; and *6*, (MCMC + Au—rutin) + 1-naphthol + NADH + buffer saturated with CH_4 and O_2. The content of the components in the samples: 2.5 mL of 0.005 *M* Tris-HCl buffer, pH = 8.0; MCMC is 2 mg of total protein; gold concentration $1.2 \cdot 10^{-6}$ *M* (in the composition of Au-protein and Au—rutin); CH_4 $5 \cdot 10^{-4}$ mol L^{-1}, O_2 $5 \cdot 10^{-4}$ mol L^{-1}, and 1-naphthol $1.2 \cdot 10^{-5}$ mol L^{-1}.

Thus, gold as a constituent of the Au—rutin complexes and nanoparticles manifests the protective effect against the inhibitory effect of 1-naphthol and 2,2′-bipyridyl. The resistance to toxins in the presence of gold is manifested not only in cells capable of assimilating gold but also in other biological systems. Pretreatment of cells with gold is more efficient compared to the cases where the treatment carried out after the action of a toxin. It is of note that the protective mechanism of the action of gold to reach the same effect can differ for different organisms. For certain microorganisms, this is just a chemical reaction of an inhibitor with gold due to which the amount of free inhibitor decreases and, correspondingly, the inhibition effect decreases.[18] For other microorganisms, in particular, for *Micrococcus luteus*, the main ETC is branched at the level of cytochrome b_{558} and an additional alternative chain of electron transfer to oxygen[16] involving Au-protein is formed, which enhances the resistance of the cells against toxins.

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