Peroxide-Eliminating Oxidoreductases as Biosensors of Antioxidant Components of Medicinal Plants

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A simple *in vitro* test system based on the use of peroxide-eliminating oxidoreductases (glutathione reductase, glutathione peroxidase) is described. The system allows detection of antioxidant properties of aqueous extracts from raw medicinal plants. The system was used for testing water extract of Baikalskii-6 tea. Antioxidant and, presumably, adaptogenic activity of this tea was detected. In addition to activation of glutathione reductase and glutathione peroxidase, the preparation inhibits erythrocyte catalase and therefore should not be used for detection of antioxidant activity in *in vitro* screening tests.

Key Words: *Baikalskii-6 tea; H_2O_2; antioxidants; oxidoreductases*

The use of enzymes for the creation of test systems for *in vitro* characterization of bioactive substances in complex mixtures of natural origin is limited by unpredicted molecular effects of their metabolites [9]. The components of plant extracts, for example, O-quinoline derivatives and polyphenols belonging to compounds slowly metabolized in mammalian cells [10] are also known as allosteric effectors of some kinases [14] and oxidases [6], which implies the possibility of detection of these compounds with enzyme-based *in vitro* test systems.

Water extract of Baikalskii-6 tea contains an appreciable (up to 8% dry weight) fraction of polyphenols, which largely determines the antioxidant effects of this preparation previously used for the therapy of experimental nephropathies [1]. The contribution of enzymes to antioxidant activity of drugs are often related to their effects on hydrogen peroxide and lipid metabolism [12,13], and therefore the group of oxidoreductase of the peroxide metabolism might serve as the basis for the creation of a new biosensory system for the detection of antioxidant properties of polyphenol-containing extracts from raw medicinal plants. Possible relationship between polyphenols and activation of antiperoxide metabolism [11] makes the development of this test system particularly important.

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MATERIALS AND METHODS

Antioxidant activity of the summary preparation of Baikalskii-6 tea water extract was *in vitro* evaluated using three peroxide-eliminating oxidoreductases: glutathione peroxidase (EC 1.11.1.9, GP), catalase (EC 1.11.1.6, CAT), and glutathione reductase (EC 1.6.4.2, GR). Crystalline preparations of GP from calf kidney (Boehringer Mannheim), CAT from sheep erythrocytes (Sigma), and GR from rabbit liver (PL-Biochemicals) were used. Specific activity (U/mg protein) of each enzyme was measured under optimal conditions during 40-min incubation at 30°C in appropriate substrate-containing buffers for GP [2], CAT [8], and GR [5]. The total protein content in the solution was measured by photocolorimetry after Bradford [3]. When measuring activities of the studied enzymes [2,5,8] in parallel samples by traditional methods, the initial concentrations of substrates 6-7-fold surpassed the saturating concentrations. Water extract of Baikalskii-6 tea was prepared and used as described previously [1].

The content of water extract in the incubation medium (0.5-250 μg/ml) corresponded to its doses

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providing the nephroprotective effect of the preparation in experimental toxic nephropathies [1]. This dose interval permits evaluation of physiologically significant antioxidant effect under conditions of significant inhibition of the key oxidases *in vitro* [10,11], which is essential for the selection of biosensor enzymes of antioxidant activity [9,12].

The significance of differences in control—experiment pairs was evaluated by Dunnett's nonparametric method for $n<8$ [7]. Measurements were carried out 6-8 times for all series.

RESULTS

Glutathione peroxidase and reductase meet the requirements to biosensors of antioxidant activity [9, 13]: these enzymes are significantly activated by components of the test object (Fig. 1). Synchronous activation of GP and GR unambiguously indicates the presence of antiperoxide defense: GP-dependent utilization of H_2O_2 and GR-catalyzed formation of active SH glutathione are important steps in elimination of peroxide products from higher animal cell energy metabolism [11]. Inhibition of CAT detected in our study (Fig. 1) is described as an effect of tannins and short polyphenols of tea leaf water extracts, concomitant with their antibacterial and adaptogenic effects [4].

Direct effect of herb alkaloids (digitalis, convallaria, *etc.*) on CAT activity, similarly to the effect of tannin and polyphenol, are not always due to antioxidant properties of these substances, whose biological effects manifest mainly in enzyme systems of glutathione and lipoperoxide metabolism [4,9,13]. Our findings (Fig. 1) are in line with previously published data on low efficiency of CAT as an antioxidant biosensor, due to nonspecific inhibition of this enzyme with aromatic and heterocyclic metabolites [4,10,11].

This paper presents the first experience gained in the use of GR for biosensor detection of antioxidant activity in multicomponent mixtures. Glutathione peroxidase was previously used for evaluating the capacity of synthetic hydroxypyrrolidines as peroxide radical traps in aging ascomycete cultures [9]. We selected both enzymes for testing due to their capacity to reduce the level of peroxides in cells in a state of "oxidative shock" [4,13].

Attempts of some authors to use NAD-dependent oxidoreductase [9,10] and cytochrome oxidase preparations [4,11] for screening of xenobiotics for antioxidant properties failed. However, our experiments revealed a relationship between this group of enzymes and regulation of cell redox homeostasis, depending on natural metabolites of aromatic [10] and carotenoid [4] groups.

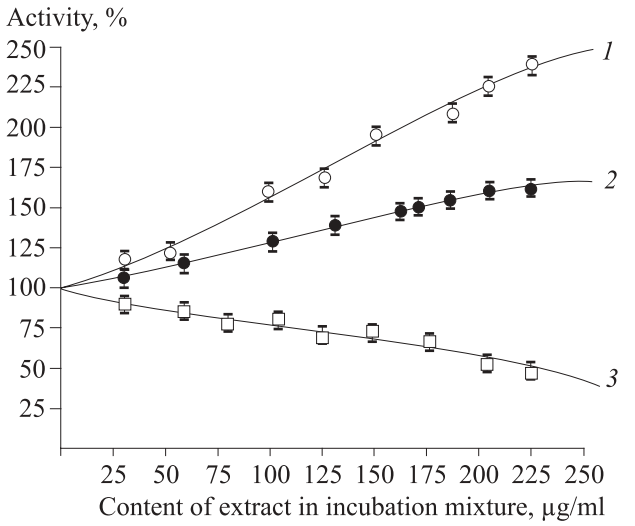

Fig. 1. Activity of peroxide-eliminating oxidoreductases in *in vitro* test systems containing aqueous extracts of Baikalskii-6 tea. *1*) glutathione peroxidase; *2*) glutathione reductase; *3*) catalase.

Our findings (Fig. 1) suggest that GR and GP can be effectively used in test systems for *in vitro* studies of raw medicinal plant components and dosage forms from these plants.

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