GENETICS

Antimutagenic Activity of Wheat Polypeptides in Human Cells Exposed to Cadmium Chloride

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Antimutagenic effects of polypeptides isolated from *Triticum kiharae* wheat plantule extracts have been studied on human cells exposed to cadmium chloride. The most effective polypeptide Tk-AMP-BP β-purothionin exhibited higher antimutagenic activity than wheat water extract and another peptide isolated from the same wheat species, Tk-AMP-γ2 defensin; it also produced a pronounced antioxidant effect. This polypeptide can be used as a preventive agent for reducing the mutagenic potential of some environmental pollutants and for correction of human diseases associated with the defense system defects.

Key Words: *antimutagens; cadmium chloride; plant polypeptides*

Use of antimutagens is one of the main methods for protection of human cells from carcinogen/mutagens. A characteristic feature of antimutagen activities is a wide spectrum thereof: free radical capture, stimulation of xenobiotic detoxication systems, modulation of transcription factors, reparation system, *etc.*

Some antimutagens have exhibited geroprotective and anticarcinogenic effects and worked as adaptogens, modulating the cytokine production via modulation of some genes' expression [2]. Synthetic compounds, for example, 1,4-dihydropyridine [4,8], plastoquinone [9] derivatives, afobasole [5], and plant extracts [1,12] are used as antimutagens.

On the other hand some antibacterial plant polypeptides with high stress resistance, for example, *Triticum kiharae* whet [11], have exhibited antimutagenic activity in human cells exposed to cadmium chloride [7].

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We have compared antimutagenic effects of two polypeptides extracted from *T. kiharae* wheat – Tk-AMP-BP β-purothionin and Tk-AMP-γ2 defensin – and water extract of plantules from which these polypeptides were extracted. The mechanisms of antimutagenic activity are evaluated by antioxidant activity of the most effective antimutagen Tk-AMP-BP pyrothionine in comparison with *T. kiharae* wheat extract.

MATERIALS AND METHODS

The study was carried out on donor cultured lymphocytes. β-Purothionin Tk-AMP-BP and Tk-AMP-γ2 defensin were isolated from *T. kiharae* wheat as described previously [11]. Antimutagens were added 24 h before the mutagen (5×10^{-6} M cadmium chloride, 18-20 h), after which DNA ruptures were counted in the cells treated and not with mutagens. Antimutagen effects were evaluated by protection coefficient:

$$K_3 = \frac{(c\text{-}t)\text{-}(c'\text{-}t')}{(c\text{-}t)} \times 100\%,$$

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where c and t are the contents of double-stranded DNA in the control and mutagen-treated cells, respectively; c' and t' are the corresponding values in cells with antimutagen [3]. The antioxidant effects of the resultant compounds were studied in cell-free radical-generating system $Fe^{2+}+H_2O_2$ by evaluating luminol-dependent chemiluminescence. The reaction system contained 600 µl mixture of 1 mM $FeCl_2$ and 1 mM luminol, 100 µl 50 mM H_2O_2, and 50, 100, or 200 µl of the test substance. For control 1×PBS (pH 7.4) was used. Antioxidant ionol (10 mM) served as the positive control. The reaction was carried out in 1xPBS (pH 7.4). Chemiluminescence was measured for 25 min after reaction initiation on a BioOrbit 1251 chemiluminometer using MultiUse 2.01 software.

RESULTS

The data on the efficiency of antimutagenic protection with wheat plantule extract in comparison with aloe extract, a well-known antimutagenic agent in radiation exposure [10], are summed up in Table 1. Both extracts used in certain concentrations exhibited antimutagenic activity in a comparable range. No relationship of any kind between the antimutagen concentration and its effect was detected, which was in line with previous data [6]. Importantly that an increase of the studied antimutagen concentration in experiments with aloe was not paralleled by an increase of antimutagenic activity, while experiments with wheat extracts showed even a lower efficiency of higher concentrations of the preparation.

Antimutagenic activities of the two polypeptides isolated from wheat plantule extracts with efficiency higher than the initial wheat plantule extract or aloe extract (control antimutagen) were summed up in Table 2. Purothionin Tk-AMP-BP was superior to defensin; moreover, it exhibited higher activity at lower concentrations (20-40 µg/ml) than defensin (80 µg/ml). This prompted us to study the antioxidant activity of purothionin in comparison with wheat plantule extract (Fig. 1). Purothionin and wheat plantule extract exhibited a pronounced antioxidant activity, somewhat higher in the extract. It was due to the fact that any plant extract contained components with antioxidant activities: flavonoids, quercetins, *etc.* The data indicated that Tk-AMP-BP purothionin was characterized by antioxidant activity essential for cell defense from mutagens.

Hence, antibacterial polypeptides have exhibited antimutagenic activity in human cells exposed to cadmium chloride. This approach, the use of plant polypeptides as antimutagens for humans, seems to be promising for regions with high basal radiation or for some types of chemical production, in accidents involving exposure to radiation or chemical mutagens. Presumably, these antimutagens can be used as preventive drugs (anticarcinogens) in humans at a high risk of cancer, for example in cases with documented changes in oncogenesis suppressor genes. In addition, these antimutagens can serve as correctors in the treatment of some diseases associated with deficient cellular defense system. And these polypeptides can serve as preventive means in tobacco-smokers, particularly in cases with the genotype characterized by zero variants of detoxication genes.

TABLE 1. Coefficients of Human Cell Protection from Cadmium Chloride (5×10^{-6} M; %) by *T. kiharae* Wheat and Aloe Extracts

Substance concentration*	Wheat	Aloe
3 mg/ml	50	49
6 mg/ml	73	67.5
12 mg/ml	23	69

Note. *Weight of tissue taken for extraction, in conversion to 1 ml water.

TABLE 2. Coefficients of Human Cell Protection from Cadmium Chloride (5×10^{-6} M; %) by Antibacterial Peptides

Polypeptide concentration	Tk-AMP-BP β-purothionin	Tk-AMP-γ2 defensin
10 µg/ml	74	48
20 µg/ml	85	52
40 µg/ml	85	59
80 µg/ml	88	79

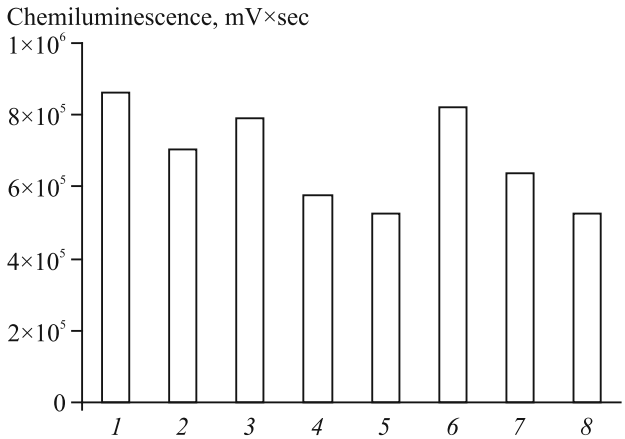

Fig. 1. Effects of wheat plantule extract and Tk-AMP-BP β-purothionin on luminol-dependent chemiluminescence. *1*) control; *2*) ionol, 10 mM; *3*) Tk-AMP-BP β-purothionin, 50 µl/ml; *4*) 100 µl/ml; *5*) 200 µl/ml; *6*) wheat plantule extract, 50 µl/ml; *7*) 100 µl/ml; *8*) 200 µl/ml.

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