Protective and Inhibitory Effects of Brain Extracts on the Growth of Bacteria

Yu. A. Nikolaev, A. N. Erin, and G. T. Sukhikh

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The biological effects of the peptide and protein fractions of human brain extract are examined using an *Escherichia coli* culture as a simple and adequate test system. Both fractions bind superoxide radical and N-ethylmaleimide, the protein fraction being more active than the peptide fraction. In addition, the protein fraction inhibits the growth of the bacteria.

Key Words: *brain; extracts; antioxidative activity; bacteriostatic effect*

Brain extracts possess a wide spectrum of biological activities [2]. It was demonstrated that the protein and peptide fractions of human brain bind active oxygen forms, specifically, the superoxide radical. We used a chemical model of 6-hydroxydopamine autooxidation. The protective effect of the extracts was verified on live cells. A culture of *Escherichia coli* was selected as a test system, since these bacteria are a very convenient tool: they readily grow in culture, their metabolism is well known, and defense from active oxygen forms is an intrinsic part of their biology [3]. The $O_2^{\cdot}$ radical, which is generated in the lysosomes of phagocytic granulocytes, and N-ethylmaleimide (NEM), a well-known reagent for readily oxidized SH groups, were employed.

MATERIALS AND METHODS

Human brain extracts were prepared as described previously [2]. *E. coli* MC4100 culture derived from *E. coli* K-12 was grown in glucose minimal medium K120 [1] containing (g/liter): $K_2HPO_4 \times 3H_2O$ — 1, KH_2PO_4 — 3.12, $(NH_4)_2SO_4$ — 1.05, $MgSO_4$ — 0.4 mM, $(NH_4)_2SO_4 \times FeSO_4 \times 6H_2O$ — 60 μM, thiamine — 1 μg/ml, and glucose — 0.2%, or a complex LK medium (10 g/liter tryptone, 5 g/liter Oxoid yeast extract, and 6.4 g/liter KCl) in 75-ml flasks with 10-20 ml of the medium stirred at 100 rpm and 37°C. Cells in the exponential growth phase were used in all experiments. Cell growth was assessed by light absorbance in the suspension at 540 nm. $O_2^{\cdot}$ was generated in the phenazine methosulfate reaction (Reanal, final concentration 4 μM) with NADH (Chemapol, final concentration 25 μM) as described elsewhere [4]. NEM (Sigma) was used in a concentration of 10 μM. The extracts were added to the growing cultures to attain a final concentration of 1%. All experiments were repeated at least three times.

International Institute of Biological Medicine, Moscow

RESULTS

Preliminary experiments revealed that phenazine methosulfate in micromolar concentrations without adding NADH markedly inhibited the growth of bacteria in the minimal and rich media, probably, a reaction with endogenous NADH and production of $O_2^{\cdot}$. Bacterial growth was slightly inhibited by the addition of NADH.

Figure 1 shows that $O_2^{\cdot}$ suppressed the growth of *E. coli* in the minimal medium (from 1 to 0.3 h^{-1}) without arresting it. The addition of both peptide and protein extracts altered the process. The growth rate remained unchanged for 0.5 h with the peptide extract and for 1 h with the protein extract, after which it decreased to 0.2 h^{-1}, being lower in the presence

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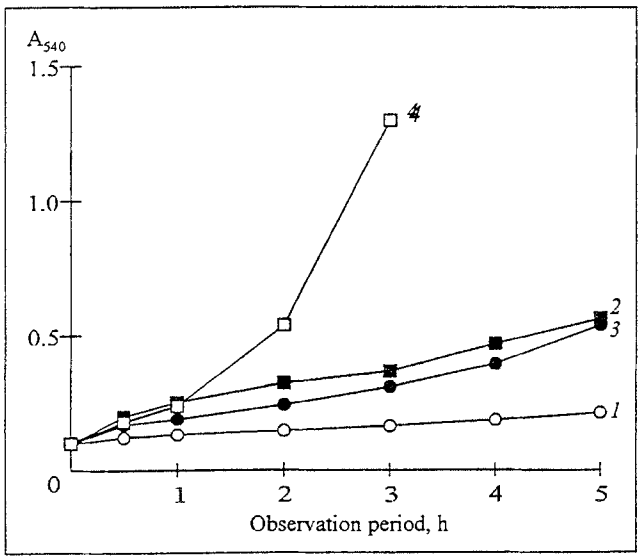

Fig. 1. Growth of *E. coli* in minimal medium with phenazine methosulfate (PMS)+NADH (*1*), PMS+NADH+protein extract (*2*), and PMS+NADH+peptide extract (*3*); *4*) control (no additives).

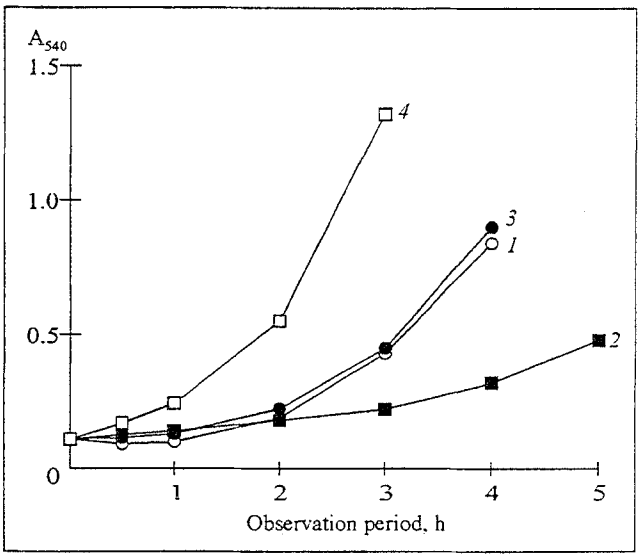

Fig. 2. Growth of *E. coli* MC4100 in minimal medium with N-ethylmaleimide (NEM, *1*), NEM+protein extract (*2*), and NEM+peptide extract (*3*); *4*) control (no additives).

of the protein extract. Thus, the brain extracts bound $O_2^{\cdot}$ and protected bacterial cells from it. However, this protection is limited, and after 0.5 to 1 h it was no longer observed, probably due to exhaustion (oxidation) of the antioxidant molecules. The protective effect was not observed in the complex medium.

A different time course of the culture growth is characteristic of the oxidative shock caused by NEM. After the reagent had been added, the cell growth stopped immediately; some cells were lyzed. After a period of adaptation and destruction of NEM, the growth was resumed at the same rate (Fig. 2, *1*). No cell lysis was observed in the presence of the peptide extract; active growth started somewhat earlier than in the control. The addition of the protein extract protected *E. coli* culture from this oxidant more effectively: the culture continued to grow, although slower, which is explained as follows. Protein extract inhibits the growth of *E. coli*. Figure 3 shows that protein extract diluted 1:100 (as in test variants) markedly decreased growth rate (from 1.0 to 0.65 h^{-1}). The protein extract in the concentration 1.5% decreased the growth rate to 0.2 h^{-1}. Therefore, despite the protective action of protein extract, the growth of culture with NEM was slower than in the control. In a complex medium, the peptide extract did not protect the culture from NEM, and the protective effect of the protein extract was very weak (data not shown).

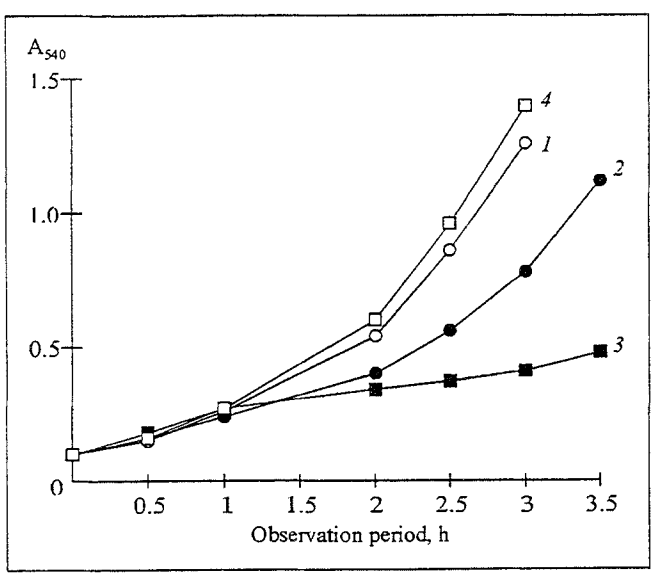

Fig. 3. Growth of *E. coli* MC4100 in minimal medium with 1% protein extract (*2*), 1.5% protein extract (*3*), and peptide extract (*4*): *1*) control (nothing added).

Thus, brain protein and peptide extracts possess antioxidative activity. Culture of *E. coli* can be used as a test object for characterization of these extracts. A bacteriostatic effect of brain protein fraction has been revealed.

REFERENCES

1. W. Epstein and B. S. Kim, *J. Bacteriol.*, **108**, No. 2, 639-644 (1971).
2. A. Fine, *Cell Transplant.*, **3**, No. 2, 113-145 (1994).
3. H. M. Hassan and I. Fridovich, *J. Bacteriol.*, **129**, No. 3, 1574-1583 (1977).
4. M. Nishikimi, N. A. Rao, and K. Yagi, *Biochem. Biophys. Res. Commun.*, **46**, No. 2, 849-854 (1972).