Microwave irradiation enhances peroxidase activity of cytochrome c

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Abstract

Apoptosis, a programmed cell death, plays an essential role in embryonic development, tissue homeostasis and various pathological conditions including inflammation and ischemia. Cytochrome c-driven cardiolipin (CL) oxidation in mitochondria membrane appears to be one of the key events at the initial stage of mitochondria-dependent apoptosis. Here, by using model system of cytochrome c (cyt c) and phospholipid membranes we demonstrated that microwave irradiation can stimulate cyt c-dependent oxidation of substrates in the water and membranes. Our results suggest that microwave irradiation may elevate sensitivity of cells to apoptotic stimuli.

1. Introduction

In apoptosis cell proceeds through several steps, which include initiation of the death program, activation of intracellular proteases, fragmentation of intracellular material and organelles, plasma membrane "blebbing", expression of death signals on the surface and finally, the engulfment of apoptotic cell and blebs by neighboring healthy cells and professional phagocytes. Thus, carried by this well-orchestrated death pathway cells are removed in controllable manner without release of noxious cellular constituents in surrounding tissue, without induction of inflammation and autoimmune responses [1].

In last decade it became apparent that mitochondria often play a central role in apoptosis. On one hand, mitochondria supply machinery involved in apoptosis with required energy; on the other hand, this organelle can produce reactive oxygen species (ROS), which participates in apoptosis development, and can release pro-apoptotic proteins, which directly initiate programmed cell death [2].

It has been demonstrated recently that mitochondrial protein cytochrome c can form complex with cardiolipin, a unique mitochondrial phospholipid. Upon complex formation with unsaturated cardiolipin, cyt c turns from a mitochondrial electron carrier into a peroxidase which can oxidize small reducing substrates, protein tyrosines and CL itself. Hydrogen peroxide (H_2O_2), a reactive oxygen species, is a co-substrate and initiator of this catalytic oxidation. Accumulation of cardiolipin hydroperoxide, one of the major products of this reaction, induces detachments of cyt c from the membrane and stimulates mitochondrial outer membrane permeabilization [3]. These events lead to the release of pro-apoptotic proteins including cyt c from mitochondria into cytosole and to the induction of apoptotic program. Thus cyt c, when attached to inner membrane of mitochondria, can sense ROS and can transduce it into death signal via catalytic CL oxidation.

Microwave irradiation has been shown to affect various biological processes even at relatively low irradiation power. In particular, EHF irradiation can increase conformational flexibility of proteins, stimulate enzymatic activity, induce lipid peroxidation and affect cell proliferation [4-7]. Research success made in last decades in this area has stimulated development of new instruments and approaches for the therapy of skin and internal diseases in Russia. However, mechanisms of biological and therapeutic effects of microwave irradiation remain poorly understood.

Here, we hypothesized that microwave irradiation may stimulate peroxidase activity of cyt c and, thus, can affect cell death pathways. Peroxidase activity of cyt c is largely depends on the protein folding and on the accessibility of active center (heme) to substrates. Binding of this globular protein to the membrane is associated

with a partial protein unfolding, opening of heme crevice and augmentation of peroxidase activity by nearly two orders of magnitude. Our hypothesis is based on the observations that microwave irradiation can increase interface convection in water and can affect protein conformational stability [4-6]. Later effect may stimulate binding of the protein to a membrane and increase accessibility of heme to substrates, while former process can hasten chemical reactions at the membrane interface. Stimulation of Na^+ ion transport in live cells is an example of EHF irradiation effect, which was explained by increase interface convection in water rather by integral heating of the sample [4,5]. Thus, in addition to well recognized thermal effect [8], microwave irradiation can induce specific convectional and conformational effects on membrane-bound catalyst, and hence, significantly alter the course of the reaction.

We prepared membrane-bound and membrane free (globular) cyt c by using purified protein and small unilamellar liposomes (SUV) made from synthetic phospholipids. We analyzed peroxidase activity of cyt c in different states by using an array of fluorescence probes, which adequately reflect oxidation of hydrophobic (e.g., unsaturated lipids) and hydrophilic compounds. Significant enhancement of cyt c-catalyzed substrate oxidation was found upon microwave irradiation. An investigation of microwave irradiation effect on the programmed cell death induced by various stimuli is in progress in our lab.

2. Experimental procedures

Gunn diode tuned to 39 GHz has been used as a source of EHF irradiation. Electromagnetic waveguide (section of 7.2x3.4 mm^2) was designed to control an output and irradiation frequency. Samples in the volume of 50 μl were irradiated in the quartz cuvette in the immediate vicinity of the waveguide horn. Irradiation of samples was carried out for 10 min; an output was in the range of 0.1-10 mW/cm^2. Temperature in the samples was measured by using microthermodetector MT-4MO (Russia).

SUV were prepared from phosphatidylcholine (PC) alone or PC and CL (1:1). Individual phospholipids, stored in chloroform, were mixed and dried under nitrogen. Tris buffer (10 mM, pH 7.0) was added to obtain a final lipid concentration of 4mM, and then lipids were mixed in vortex and sonicated five times for 30 s on ice. Liposomes were used immediately after preparation.

Membrane-bound horse heart cyt c was prepared by incubating purified protein with CL-containing SUV at protein/lipid molar ratio of 1:100 and 1:500 for 10 min at room temperature.

Cyt c-catalyzed oxidation of Amplex red (AR) was determined on Hitachi F-2500 spectrofluorophotometer (Japan) using an excitation wavelength of 550 nm and an emission wavelength of 584 nm. C11-BODIPY oxidation was estimated by using excitation/emission wavelengths of 575 nm and 595 nm respectively for its reduced form and an excitation wavelength of 505 nm and an emission wavelength of 522 nm and 550 nm for the oxidized form.

3. Results

Nonfluorescent hydrophilic molecule Amplex red is converted into fluorescent resorufin upon oxidation. By using fluorescence analysis we observed cyt c-dependent oxidation of AR in the presence of H_2O_2. Membrane-bound cyt c was three times more effective catalyst than free cyt c. EHF irradiation enhanced rate and yield of AR oxidation by cyt c in both states in a dose dependent manner. At highest output of 10 mW/cm^2, the magnitude of the effect was ~40%. Of note, temperature of the sample increased by 5^oC at this power. Comparable results were obtained upon integral sample heating in a water bath.

C_{11}-BODIPY is a hydrophobic ratio probe for lipid peroxidation. We found that it was efficiently oxidized in the presence of H_2O_2 by membrane-bound cyt c, while free cyt c was at least 40 times less effective. EHF irradiation enhanced C_{11}-BODIPY oxidation by membrane-bound cyt c by 20%. Small elevation in oxidation by free cyt c was not statistically reliable.

We conclude that EHF irradiation at frequency of 39 GHz does not facilitate cyt c binding to membranes lacking CL. However, this irradiation can enhance oxidation of hydrophilic substrates by cyt c and may promote phospholipid oxidation by its membrane-bound form.

4. References

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