Resonance Effect of Microwaves on the Genome Conformational State of *E. coli* Cells

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The effect of low intensity microwaves on the conformational state of the genome of X-irradiated *E. coli* cells was studied by the method of viscosity anomalous time dependencies. It has been established that within the ranges of 51.62–51.84 GHz and 41.25–41.50 GHz the frequency dependence of the observed effect has a resonance nature with a resonance half-width of the order of 100 MHz. The power dependence of the microwave effect within the range of 0.1–200 μW/cm² has shown that a power density of 1 μW/cm² is sufficient to suppress radiation-induced repair of the genome conformational state. The effect of microwave suppression of repair is well reproduced and does not depend on the sequence of cell exposure to X-rays and microwave radiation in the millimeter band. The results obtained indicate the role of the cell genome in the resonant interaction of cells with low intensity millimeter waves.

Introduction

At present a significant body of evidence has been collected on the ability of microwaves in the millimeter range to bring about biological effects including those on the cellular level [1, 2]. It has been found that microwaves can influence the processes of gene expression [3–5]. The specific features of such interaction are dependence on frequency and also effectiveness of low intensity microwave radiation which does not result in significant heating of the irradiated object. One of the possible explanations of these facts accounts for the influence of millimeter waves on the genome conformational state [6]. The genome conformational state (GCS) is expressed as the space-topological organization of the entire chromosomal DNA, which is ensured, among other things, by the supercoiling of DNA and DNA protein bonds. The GCS changes play a significant role in all elementary genetic processes – transcription, replication, repair.

The hypothesis which accounts for the influence of millimeter radiation most evident in the case of stressed systems [1, 7] among them bioobjects subjected to ionizing radiation [6] has repeatedly been verified.

The influence of millimeter waves on the process of the GCS repair after *E. coli* K 12 cell exposure to X-rays was examined in this work. As a test for appearance and repair of changes in a chromosomal DNA we used the method of the anomalous viscosity time dependencies (AVTD) in cell lysates [6].

Materials and Methods

Microwave and X-ray irradiation

A block diagram of the experimental unit used for microwave irradiation of cell suspension is given in Fig. 1. A G4-141 generator served as the source of extremely high frequency electromagnetic radiation (EHF EMR). In the course of irradia-

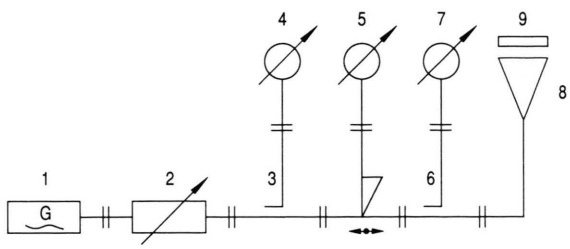

Fig. 1. Block diagram for microwave irradiation of cell suspension: 1 – EHF EMR generator; 2 – controlled attenuator; 3, 6 – directional coupler; 4 – frequency analyzer; 5 – measurement line (VSWR-meter); 7 – power meter; 8 – pyramidal horn; 9 – cell suspension.

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tion the frequency, the output power, as well as the voltage standing wave ratio (VSWR) were controllable. Frequency instability was 1 MHz, error in the measurement of the output power did not exceed 10% and the value of VSWR in the waveguide was not more than 1.6. Irradiation of a cell suspension (1.5 mm thickness) was carried out in Petri dishes, 50 mm in diameter, by means of a pyramidal horn having dimensions 40×50 mm^2.

The space distribution of the power density (PD) on the surface of the suspension was measured by means of a dipole EHF probe [8]. With the irradiation frequencies used the local PD values at the surface of the suspension differed by nearly an order of magnitude. But frequency changes of $\pm$ 200 MHz did not lead to significant changes of the pattern of PD distribution. At the same time frequency changes in a wide range (of the order of units GHz) could lead to a marked displacement of PD minima and maxima up to their inversion. In the event of parity of output power in the waveguide, the PD value, averaged over the whole surface under irradiation, did not change.

The specific absorption rate (SAR) was measured in two ways: by the acoustic method [9] and the calorimetric method. The suspension temperature was measured by a microthermocouple.

Cells were subjected to X-rays (XR) using a radiological unit RUP-150. The distance from the focus to the suspension was 40 cm, average radiation energy – 50 keV, dose rate 0.7 Gy/min. Microwave and X-irradiation of cells was carried out at ambient temperature.

Preparation of bacterial cells for experiments and cell lysis

The following strains were used in the work: *E. coli* K12: AB1157 F^- thr-I ara-14 leu-B6 proA2 lacGI tsx-33 supE44 galK2 hisG4 rfbDI mgl-51 rpsL31 xyl-5 mtl-I argE3 thi-I λ^-rac$^-$; G62 F^+ proA23 lac-28 trp-30 his-51 rpsLR and also strain RM117 which is isogenic with strain AB1157. Cells were cultivated by standard methods in Luria broth or minimal medium M-9 [10]. The *E. coli* cultures used in the experiments were kept in spreadings on the Hottinger nutrient agar at 3–4 °C.

Before irradiation, cells from the night culture were resuspended in concentrations of $3 \div 9 \times 10^7$ cells/ml in a salt buffer M-9. Cells were kept under these conditions for 1 h before irradiation.

After irradiation, cells were lysated by gradually adding LET-lysozyme (LET-medium: 0.5 M Na_2 EDTA, 0.01 M Tris-HCl, pH 7) in a concentration of 3 mg/ml, LET-sarcosyl (2%) and LET-papain (3 mg/ml) with 10 to 15 min intervals between addition of each agent. 0.3 ml LET-lysozyme, 1.0 ml LET-sarcosyl, 0.7 ml LET-papain were added to 1 ml of cell suspension. The lysates were then kept in darkness at a temperature of 30 °C for 40 h, after that the AVTD were measured.

Method of anomalous viscosity time dependencies

This method is based on the fact that in solutions of high-polymer DNA, placed in a rotary viscosimeter, radial migration of DNA, which is a directed movement of macromolecules towards the inner cylinder of the viscosimeter (rotor), is observed [11].

Measurements were carried out in a rotary cylindrical viscosimeter with an automatic record of the rotor's rotation period [6]. In the unit used, the rotor was set in motion by a constant moment of force created by an external electromagnetic field.

Upon completion of the lysis the rotor was suspended on the meniscus of the lysate examined. Thereafter the lysate was placed in a thermostatically controlled (30 °C) jacket of the viscosimeter for measurement.

When the external electromagnetic field is switched on, the rotor starts moving. In the initial stage of measuring the rotor's rotation period (T), the lysate viscosity increases due to a radial migration of macromolecules. This results in an increased rotation period of the rotor since the period is proportional to the specific viscosity (Fig. 2, curve 1). After the DNA macromolecules had deposited on the external surface of the rotor the velocity of its rotation decreased to the value typical of a pure solvent. The dependence of the rotor's rotation period in the cell lysate on the time after the rotor's rotation starts (t) is called anomalous viscosity time dependence.

It should be noted here that AVTD cannot be observed in protein solutions, because radial migration doesn't take place in solutions of molecules with weights less than 10^6 D [11]. The param-

eters of the AVTD curve in the cell lysate are determined by the genome conformational state, *i.e.* by hydrodynamic parameters of chromosomal DNA macromolecules which in their turn depend on the DNA nativity, DNA association with various proteins, the microenvironment, *etc.* The rotor's maximum rotation period (T_{max}) which in this method is the most sensitive parameter characterizing the genome conformational state of *E. coli* cells, was obtained from the AVTD curve. The measurement error of the rotor's rotation period was 2%.

Results

Irradiation of *E. coli* cells with doses of 10–50 Gy leads to changes of the AVTD curve of the cell lysate (Fig. 2, curve 2). The major cause of these changes is the considerable decrease of T_{max}. After post-irradiated cell incubation for 90–120 min, depending on the dose of irradiation, an almost complete recovery of the AVTD curve (Fig. 2, curve 3) took place. This means that during this period the GCS of the irradiated cells returned to the control level. It is in this sense that we use the term "repair" of the genome conformational state.

In preliminary experiments the X-irradiated cells were exposed to microwaves in the regime of frequency switching. This was brought about within the range of about 200 MHz during 30–90 min. Fig. 2 (curve 4) shows the AVTD curve after cell irradiation within the frequency band of 51.60–51.78 GHz at PD = 3 mW/cm^2 for 90 min.

It can be seen that microwaves in this range effectively suppress repair of the GCS. To assess the microwave effect on the repair process after X-irradiation, we used the following ratio:

$$\varkappa = \frac{\bar{T}_{\text{max XR + I}} - \bar{T}_{\text{max eff}}}{\bar{T}_{\text{max XR + I}} - \bar{T}_{\text{max XR}}}$$

where:

$\bar{T}_{\text{max XR}}$ – the average maximum rotor's rotation period in the lysates of cells lysated immediately after X-irradiation;

$\bar{T}_{\text{max XR + I}}$ – the average maximum rotor's rotation period in the lysates of cells lysated after X-irradiation and subsequent incubation (I);

$\bar{T}_{\text{max eff}}$ – the average maximum rotor's rotation period in the lysates of cells subjected to EHF EMR during the radiation-induced repair. In two effective microwave ranges the $\varkappa$ dependencies on frequency were determined.

In these experiments, cells were irradiated with microwaves of a certain frequency for 5 to 15 min after X-irradiation. To assess the average value of the rotor's maximum rotation period ($\bar{T}_{max}$) in each of the experiments 3 AVTD measurements were made. Significance level was determined by the Student's t-test. The extent and results of a standard experiment are given in Table I.

Fig. 3 and Fig. 4 present the $\varkappa$ dependence in the ranges examined: 51.62–51.84 GHz (strain

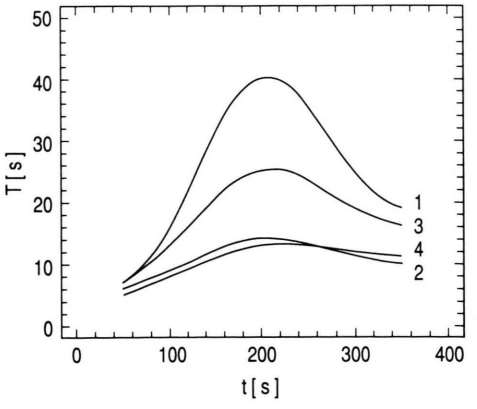

Fig. 2. Anomalous viscosity time dependencies of *E. coli* G62 cell lysates: 1 – control; 2 – X-irradiation (30 Gy); 3 – XR and incubation (90 min); 4 – XR and incubation under the influence of EHF EMR.

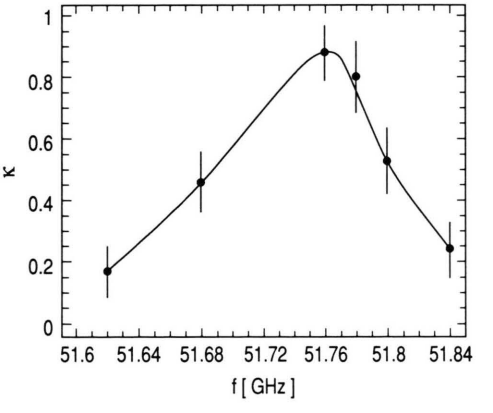

Fig. 3. Frequency dependence of EHF EMR effect on radiation-induced repair of the GCS of *E. coli* RM117 cells (20 Gy; 15 min, 3 mW/cm^2).

Table I. Values of the maximum rotor's rotation period derived from AVTD curves obtained in lysates of *E. coli* AB1157 cells, lysated after X-irradiation (20 Gy), subsequent incubation or irradiation with EHF EMR (200 μW/cm²) in the course of incubation.

Type of effect	EMR frequency [GHz]	Duration of EHF EMR irradiation [min]	T_{max} [S]	$\bar{T}_{max} \pm SE^*$ [S]	Significance level as compared with XR + I
Control	–	–	51.1 35.1 47.8	44.8 ± 4.8	$p < 0.03$
XR	–	–	7.4 7.2 6.5	7.0 ± 0.3	$p < 0.0001$
XR + I	–	–	28.1 24.7 25.7	26.2 ± 1.0	–
	41.25	10	14.0 12.3 12.1	12.8 ± 0.6	$p < 0.0004$
	41.30	10	7.2 6.4 7.2	6.9 ± 0.3	$p < 0.0001$
XR + EMR + I	41.35	10	8.9 10.1 10.1	9.7 ± 0.4	$p < 0.0002$
	41.40	10	9.7 11.2 12.2	11.0 ± 0.7	$p < 0.0004$
	41.45	10	16.2 17.3 16.6	16.7 ± 0.3	$p < 0.001$
	41.50	10	15.2 16.2 15.6	15.6 ± 0.3	$p < 0.0006$

* Standard error.

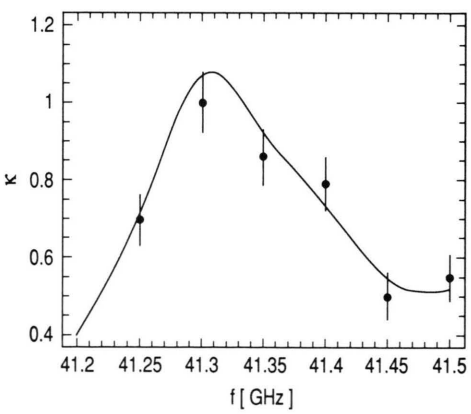

Fig. 4. Frequency dependence of EHF EMR effect on radiation-induced repair of the genome conformational state of *E. coli* AB1157 cells (20 Gy; 200 μW/cm², 10 min).

RM117) and 41.25–41.50 GHz (strain AB1157). It is clear that in both ranges this dependence has a resonance nature with a resonance half-width of the order of 100 MHz and resonance frequencies of 51.76 GHz and 41.32 GHz respectively. In the first instance the cell exposure to EHF EMR was carried out at PD = 3 mW/cm². The SAR value, estimated by acoustic and calorimetric methods, amounted to 17 mW/g and 22 mW/g respectively. Heating of the cell suspension, when irradiated, did not exceed 1 °C. The $\varkappa$ dependence on frequency within the range of 41.25–41.50 GHz was studied at PD = 200 μW/cm² with heating not exceeding 0.1 °C. It should be noted that heating of a cell suspension by 5 °C for 10 min right after the X-irradiation did not lead to suppression of repair

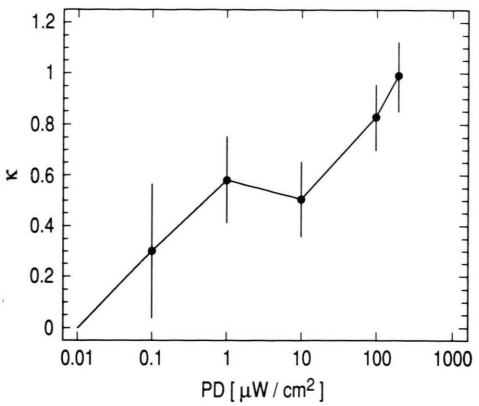

Fig. 5. Dependence of suppression effectiveness of radiation-induced GCS repair on microwave PD (strain AB1157, 20 Gy; 41.32 GHz, 5 min).

processes. We also studied the dependence of suppression of radiation-induced GCS repair on PD of the microwave exposure at the 41.32 GHz frequency. The power dependence of $\varkappa$ is shown in Fig. 5. Starting with a PD of 1 $\mu W/cm^2$, irradiation for 5 min significantly suppressed GCS repair.

As pointed out above, Fig. 3 shows a frequency dependence within the 51.62–51.84 GHz range for RM 117 strain. But this microwave irradiation was effective in repair suppression for the other strains used: AB1157 and G62. Altogether 11 experiments were carried out, each revealing statistically significant suppression of repair processes by microwaves at frequencies of this resonance.

An EHF EMR effect on the genome conformational state was also discovered in the case of inverse sequence of cell exposure to microwaves and X-rays. Irradiation of cells with EHF EMR at the 51.78 GHz frequency (that is close to that of resonance) before X-irradiation prevented the process of radiation-induced repair (Table II).

Table II. Values of the maximum rotor's rotation period in cell lysates after a combined effect EHF EMR (3 mW/cm^2, 51.78 GHz, 30 min) and XR (30 Gy) on *E. coli* RM 117 cells.

Type of effect	$\bar{T}_{max} \pm SE$ [S]	Significance level as compared with XR + I
Control	17.1 ± 0.9	$p < 0.04$
XR	6.9 ± 0.1	$p < 0.02$
XR + I	12.5 ± 1.4	–
EMR + XR + I	7.2 ± 0.2	$p < 0.003$

Discussion

It is generally accepted that biological membranes are receptors of chemical and electromagnetic signals. Can this premise alone explain those resonance bioeffects which can be seen when cells are subjected to low-intensity millimeter radiation? This resultant effect can change such important biological parameters as velocity of cell division [1, 2] or processes of gene expression [3, 5]. It would seem that the simplest answer to the question of the target of microwave resonance effect is that the target is the cell membrane whose properties determine frequencies of resonant interaction. Indeed, in a number of model studies microwave effects were detected that had been caused by a change in the ion membrane transport [13–15]. But the microwave "membrane" effects examined did not depend on the EMR frequency and therefore do not permit explanation of the resonance effect on the processes of cell development and gene expression. It appeared to us that a promising explanation of these observations could be supplied by the notion of the role of the genome conformational state in forming cell's resonance response to the millimeter wave exposure. In other words, we assumed that parameters of the GCS, *i.e.* space-topological organization of chromosomal DNA, determine resonance frequencies. In such an event the GCS would be sensitive to the effect of millimeter waves of certain frequencies.

In order to provide support for this supposition we used the method of anomalous viscosity time dependencies in cell lysates, which has a high sensitivity to the GCS change [6]. Changes in the AVTD can be detected even with an X-ray dose of 10 cGy when less than one single-strand DNA break is induced per *E. coli* genome. This result already made it possible to assume that the AVTD method is sensitive not only to damages of the sugar-phosphate bonds of the DNA secondary structure. The AVTD sensitivity to other changes of the genome conformation, particularly those caused by DNA-protein bonds, was confirmed by the experiments we carried out [16]. The results obtained in the course of our work indicate that repair of the genome conformational state of bacterial cells after ionizing irradiation is highly sensitive to the resonance effect of millimeter waves.

The microwave effect discovered cannot be explained by trivial heating. This was borne out by

many of the results obtained. First, there were effective PD of about 1 $\mu W/cm^2$, while SAR amounted to 10 $\mu W/g$ is not enough for a noticeable heating of the irradiated suspension over 5–15 min. Second, heating of the cell suspension by 5 °C for 10 min during the postradiative incubation has no influence on the restoration. Finally, the PD averaged over the irradiated surface did not depend on the frequency within the limits of the observed resonances (± 200 MHz).

There is hardly any doubt that destabilization of repair and probably other protein complexes with DNA is the central event of the molecular-biological mechanism preventing the GCS repair. Surprisingly, this effect may be obtained even if cells are subjected to EMR with resonance frequency before X-irradiation. This result means that a cell, irrespective of whether or not it was X-irradiated, retains the microwave resonance effect for a certain period. It is especially important to stress that this memory is realized at the level of the genome conformational state. This inference is supported by the fact that after a 5–10 min EMR effect on X-irradiated cells, the prevention of GCS repair persists for at least an hour and a half of the subsequent incubation.

The discovered frequency dependence of the effect, especially the half-width of resonances (100 MHz), is similar in character to that which had been obtained when studying the gene expression of repressed λ-prophage operon in lysogenic *E. coli* cells [3, 5]. In our view, this coincidence is one more argument in favour of the supposition of the role of the genome conformational state in the resonance response of bacterial cells to a millimeter wave effect.

In general, a chain of events seems to be involved in this interaction. At the first stage, microwaves interact with cell membranes. It is likely that the signal in the membrane intensifies and is received in the DNA through the point (points) where DNA is attached to the membrane. We believe that there are parameters of DNA or its selected sites, including those bound with proteins, that determine the resonance frequencies of electromagnetic waves capable of influencing the genome conformational state through the membrane.

One cannot exclude the possibility that the primary targets of millimeter wave action are proteins, which take part in maintaining the structural and functional integrity of chromosome DNA [18, 19]. Then changes in the GCS registered by the AVTD method will be defined by the influence of EHF EMR on the function of these proteins. By affecting the GCS through the processes of molecular interaction the microwaves may give rise to changes of DNA secondary structure, changes in elementary genetic processes: transcription, replication, repair and recombination. Consequently, it is possible to record the final biological effect at the cell level: modification of gene expression down to derepression of operons [2–5], changes in the velocity of DNA synthesis and in cell division [1, 17].

It is worth noting that cells of all the *E. coli* strains used (AB1157, RM117, G62) were sensitive to EMR of the 51.62–51.84 GHz frequency band. The first two of these strains are isogenic by known markers. As to the third strain, it differs from the previous ones by a number of markers. For instance, G62 cells have no mutations in the gene of acetylornithine deacetylase or other genes whose products take part in the biosynthesis of arginine and therefore are not auxotrophic on this amino acid. It is possible that structural genes whose mutations determine differences in the strains used have no relationship with a mechanism of resonance interaction. But it appears likely to us that resonance frequencies are determined by regulatory nucleotide sequences and their mutual position within cellular DNA.

The results obtained in this work are in accordance with the physical models predicting the existence in living systems of discrete resonance states corresponding to the millimeter band of an electromagnetic field [18, 19].

A further experimental confirmation of the genome's role in giving rise to these discrete states and the existence of selection rules on helicity for transitions between them will be made public at a later date.

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[1] W. Grundler, U. Jentzsch, F. Keilmann, and V. Putterlic, in: Biological Coherence and Response to External Stimuli (H. Frohlich, ed.), pp. 65–85, Springer Verlag, Heidelberg 1988.
[2] E. Postow and M. L. Swicord, in: CRC Handbook of Biological Effect of Electromagnetic Fields (C. Polk and E. Postow, eds.), pp. 425–460, CRC Press, Inc., Boca Raton 1986.
[3] S. J. Webb, Phys. Lett. **73A,** 145 (1979).
[4] F. Kremer, C. Koschnitzke, L. Santo, P. Quick, and A. Poglitsch, in: Coherent Excitation in Biological Systems (H. Frohlich and F. Kremer, eds.), pp. 10–20, Springer Verlag, Berlin 1983.
[5] K. V. Lukashevsky and I. Ya. Belyaev, Med. Sci. Res. **18,** 955 (1990).
[6] Ye. D. Alipov, I. Ya. Belyaev, D. I. Yedneral, D. M. Izmailov, K. V. Lukashevsky, L. K. Obukhova, O. V. Okladnova, and V. S. Shcheglov, in: Proceedings of the Workshop on Genetic Effects of Charged Particles (K. G. Amirtaev and S. Kozubek, eds.), pp. 150–160, JINR, Dubna 1989 (in Russian).
[7] S. P. Sitko, E. A. Andreev, and I. S. Dobronravova, J. Biol. Phys. **16,** 71 (1988).
[8] G. T. Butkus, K. K. Mikalauskas, and A. S. Pausha, in: Medicobiological Aspects of Millimeter Radiation (N. D. Devyatkov, ed.), pp. 230–234, IRE Ac. Sci. U.S.S.R., Moscow 1987 (in Russian).
[9] I. G. Polnikov and A. V. Putvinsky, Biofisika **33,** 893 (1988) (in Russian).
[10] J. Miller, Experiments in Molecular Genetics, p. 373, Mir, Moscow 1976 (in Russian).
[11] E. L. Uhlenhopp, Biorheology **12,** 137 (1975).
[12] V. D. Iskin, Yu. V. Zavgorodny, N. M. Yatsenko, L. K. Silina, E. V. Stepula, A. W. Medvedovsky, B. G. Rice, and S. V. Rudenko, Biological Effects of Millimeter Waves. Review. A. Deposited Manuscript, p. 75, All-Union Institute of Scientific and Technical Information of the U.S.S.R., Moscow 1987 (in Russian).
[13] V. M. Shtemler, in: The All-Union Symposium with Foreign Participants: Theoretical and Applied Aspects of Usage of the Millimeter Electromagnetic Irradiation in Medicine. Abstracts (S. P. Sitko, ed.), pp. 114–115, TRC "Otklik", Kiev 1989 (in Russian).
[14] R. N. Khramov, Ye. M. Kobrinsky, A. K. Filippov, and N. I. Porotikov, ibid., pp. 49–50 (in Russian).
[15] I. Yu. Petrov and O. V. Betsky, Dokl. Acad. Nauk U.S.S.R. **305,** 474 (1989) (in Russian).
[16] Ye. D. Alipov, I. Ya. Belyaev, V. P. Kryuchkov, and V. N. Lystsov, in: Proceedings of the Eight Balkan Biochemical and Biophysical Days, pp. 1–2, Institute of Atomic Physics, Bucharest 1990.
[17] I. Ya. Belyaev, Ye. D. Alipov, V. S. Shcheglov, K. V. Lukashevsky, and V. N. Lystsov, in: Proceedings of Tenth International Biophysics Congress, p. 549, Vancouver 1990.
[18] S. P. Sitko, V. I. Sugakov, Dokl. Acad. Nauk Ukr. S.S.R. 6 B, 63 (1984) (in Russian).
[19] F. Keilmann, Z. Naturforsch. **41c,** 795 (1986).