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Research Article

Effect of UV Irradiation on Interactions of α-Lipoic Acid with Free Radicals

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Changes of antioxidant properties of α-lipoic acid (LA) after UV irradiation were studied. LA is the typical drug used in diabetic neuropathy. Quenching of free radicals is an important factor of therapy by using this substance. α-Lipoic acid is exposed to UV irradiation during the storage. The aim of our studies was to examine the effect of UV irradiation on the interactions of LA with free radicals. The α-lipoic acid was irradiated by UVA 315–400 nm light during 10 to 110 minutes by intervals of 10 minutes. The electron paramagnetic resonance spectroscopy was used as the experimental technique. The antioxidant properties of LA were spectroscopically confirmed. The strong effect of UV irradiation on interactions of α-lipoic acid with free radicals was observed. It was pointed out that interactions of LA with free radicals decrease after its exposition on UV. The interactions of LA with free radicals were higher after the sample irradiation during 10 minutes than for the samples irradiated longer (20–110 minutes). The results are important for problems connected with photomedicine; they pointed out that α-lipoic acid should not be stored on UV exposition. Application of electron paramagnetic resonance spectroscopy to characterize interactions of pharmacological substance with free radicals was confirmed.

1. Introduction

Application of electron paramagnetic resonance (EPR) in medicine and pharmacy is known. EPR spectroscopy was applied to examine conditions of photodynamic therapy [1], cells [1], skin [2], radiative [3] and thermally [4, 5] sterilized drugs, and binding of drugs to melanin biopolymers and synthetic melanins [6, 7]. EPR is used to study free radicals in the samples and interactions of diamagnetic samples with paramagnetic reference [8, 9]. The paramagnetic samples containing free radicals reveal EPR spectra with intensities proportional to their concentration. The types of free radicals and their properties may be obtained from the EPR line parameters. Interactions of diamagnetic samples without EPR signals may be tested by measuring EPR lines of the paramagnetic reference, for example, DPPH. The ability of the diamagnetic samples to interactions with free radicals is proportional to the quenching of the EPR line of the paramagnetic reference [8, 9]. Such interactions of diamagnetic substance with free radicals were studied in this work.

The aim of this work was to examine the effect of UV irradiation on the interactions of α-lipoic acid (LA) with free radicals. LA is used as the antioxidant [10, 11], so the knowledge about changes of the quenching of free radicals by its interaction after UV irradiation is a very interesting problem in photomedicine and pharmacy. The spectroscopic results may be used in the determination of storage conditions of LA. The best conditions of storage should not change the antioxidant power of LA. α-Lipoic acid should effectively quench free radicals.

2. Experimental

2.1. Samples. α-Lipoic acid was studied. LA is a natural molecule, which exists in our diet mainly in animal food such as meat and liver and at low or undetectable levels in plant food such as potatoes [12, 13]. α-Lipoic acid is also considered beneficial when it is used as a food supplement. Its antioxidant function has been previously reported and several studies have revealed its protective effects in cases such as

Table 1: Parameters A_1, A_2, A_3, ΔB_1, ΔB_2, and ΔB_3 of the analyzed EPR spectra of DPPH. The parameters are defined in Figure 1. The times of UV irradiation of the sample were written in minutes.

Sample	A_1 (a. u.) [±0.02]	A_2 (a. u.) [±0.02]	A_3 (a. u.) [±0.02]	ΔB_1 (mT) [±0.02]	ΔB_2 (mT) [±0.02]	ΔB_3 (mT) [±0.02]
DPPH—reference	2.99	4	2.92	0.55	1.64	2.97
Nonirradiated lipoic acid	0.03	0.03	0.03	0.41	1.23	2.17
UV-irradiated lipoic acid (10 minutes)	0.58	0.64	0.51	0.40	1.74	3.21
UV-irradiated lipoic acid (20 minutes)	1.06	2.44	1.56	0.43	1.68	3.13
UV-irradiated lipoic acid (30 minutes)	1.05	2.45	1.65	0.39	1.71	3.21
UV-irradiated lipoic acid (40 minutes)	1.11	2.50	1.60	0.47	1.67	3.31
UV-irradiated lipoic acid (50 minutes)	1.11	2.31	1.55	0.43	1.73	2.97
UV-irradiated lipoic acid (60 minutes)	1.09	2.43	1.56	0.40	1.77	3.14
UV-irradiated lipoic acid (70 minutes)	1.07	2.39	1.65	0.45	1.71	3.19
UV-irradiated lipoic acid (80 minutes)	1.04	2.44	1.56	0.41	1.71	3.13
UV-irradiated lipoic acid (90 minutes)	1.11	2.50	1.61	0.43	1.75	3.24
UV-irradiated lipoic acid (100 minutes)	1.02	2.29	1.50	0.42	1.63	2.94
UV-irradiated lipoic acid (110 minutes)	1.05	2.09	1.42	0.44	1.49	2.84

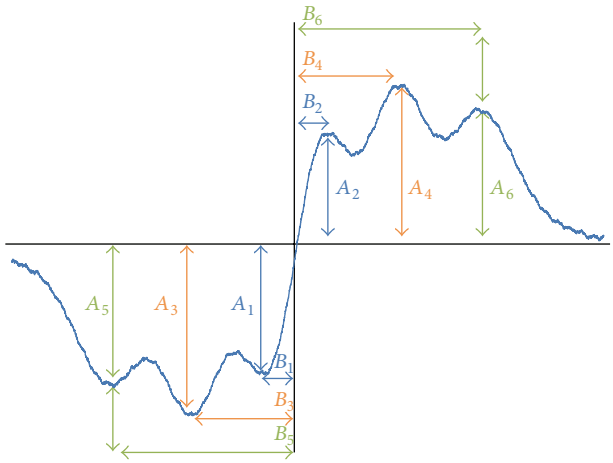

Figure 1: EPR spectrum of DPPH. The parameters of A_1–A_6 and B_1–B_6 were used to determine the values $A_1 = A_1 + A_2$, $A_2 = A_3 + A_4$, $A_3 = A_5 + A_6$, $\Delta B_1 = B_1 + B_2$, $\Delta B_2 = B_3 + B_4$, and $\Delta B_3 = B_5 + B_6$.

aging, diabetes mellitus, and vascular and neurodegenerative diseases. In all of them free radicals are involved [14, 15].

LA is a universal antioxidant that fights free radicals [10, 11]. It is a component soluble in aqueous and fat. It can protect the lipid membranes of cells and intercellular space. Water-soluble components have access to them [10, 11].

LA are mainly used in the treatment of diabetic complications such as diabetic neuropathy. LA strengthens nerves and improves metabolism and helps in absorption of insulin in diabetics, which increases sensitivity of muscle cells on this hormone [14].

α-Lipoic acids are used in treatment of diabetes and its complications and in liver diseases. Probably due to protection of nerve endings, they improve memory and support Alzheimer's disease treatment. LA should also be taken by elderly patients at risk of cataracts [14, 16].

Nonirradiated and UV-irradiated samples of α-lipoic acid were tested. The following times of irradiation: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 110 minutes were used, respectively. The irradiation was done by Medison 250 lamp with 4 radiators with power of 20 W. The UVA radiation with waves of λ: 315–400 nm was performed from the distance of lamp to the sample of 30 cm.

2.2. EPR Measurements. EPR spectra were measured for the samples placed in the thin-walled glass tubes with the external diameter of 1 mm. The measurements were done for the UV-irradiated α-lipoic acid (110 minutes) without DPPH, the 10% ethyl alcohol solution of DPPH, and the 10% ethyl alcohol solution of DPPH with the nonirradiated lipoic acid, respectively. DPPH—2,2-diphenyl-1-picrylhydrazyl—was used as the reference to examine interactions of α-lipoic acid samples with free radicals. The decrease of the EPR line

Table 2: The asymmetry parameters A_1/A_2, A_3/A_4, A_5/A_6, B_1/B_2, B_3/B_4, and B_5/B_6 of the analyzed EPR spectra of DPPH. The parameters are defined in Figure 1. The times of UV irradiation of the sample were written in minutes.

Sample	A_1/A_2 [±0.02]	A_1/A_2 [±0.02]	A_1/A_2 [±0.02]	B_1/B_2 [±0.02]	B_1/B_2 [±0.02]	B_1/B_2 [±0.02]
DPPH—reference	1.11	1.03	1.01	1.07	1.04	1.00
Nonirradiated lipoic acid	0.82	0.63	1.70	1.04	0.75	0.71
UV-irradiated lipoic acid (10 minutes)	1.54	1.33	1.24	1.00	0.94	1.02
UV-irradiated lipoic acid (20 minutes)	0.89	1.56	0.74	0.82	0.95	1.01
UV-irradiated lipoic acid (30 minutes)	0.79	1.08	0.58	0.69	0.92	0.94
UV-irradiated lipoic acid (40 minutes)	1.29	2.18	1.40	1.07	1.11	1.07
UV-irradiated lipoic acid (50 minutes)	0.90	0.80	0.91	0.63	0.95	0.91
UV-irradiated lipoic acid (60 minutes)	1.34	1.03	1.02	1.42	1.09	1.02
UV-irradiated lipoic acid (70 minutes)	0.96	1.30	0.94	1.04	1.00	0.94
UV-irradiated lipoic acid (80 minutes)	0.88	1.07	0.97	1.14	0.98	1.01
UV-irradiated lipoic acid (90 minutes)	0.90	0.96	0.78	0.88	1.01	1.01
UV-irradiated lipoic acid (100 minutes)	1.00	0.84	1.50	0.78	0.93	0.96
UV-irradiated lipoic acid (110 minutes)	0.69	1.25	0.68	0.92	1.22	1.01

of DPPH after addition of α-lipoic acid to the solution reflected the value of these interactions.

Electron paramagnetic resonance measurements were done by the use of an X-band (9.3 GHz) EPR spectrometer produced by RADIOPAN Firm (Poznań, Poland). The magnetic modulation of 100 kHz was used. Microwave frequency was measured by MCM101 recorder of EPRAD Firm (Poznań, Poland). The first-derivative EPR spectra were measured by the use of the Rapid Scan Unit of JAGMAR Firm (Kraków, Poland), which was connected with the EPR spectrometer. The numerical acquisition of the individual EPR spectra was done during 1 second. The EPR spectra were measured with microwave power of 2.2 mW. This low microwave power was used to avoid microwave saturation effect of EPR lines. The total microwave power produced by klystron was 70 mW, and the application of 15 dB attenuation made it possible to decrease the microwave power to 2.2 mW.

The spectral analyses of EPR spectra were done by the use of programs of JAGMAR Firm (Kraków, Poland) and LabVIEW 8.5 of National Instruments Firm.

The following parameters of EPR spectra: g-factors, A_1, A_2, A_3, ΔB_1, ΔB_2, and ΔB_3 were analysed. These parameters are defined in Figure 1. Parameters A_1, A_2, and A_3 are proportional to the free radical concentration in the samples. Parameters ΔB_1, ΔB_2, and ΔB_3, depend on magnetic interactions between free radicals in the samples. The higher values of ΔB_1, ΔB_2, and ΔB_3 correspond to stronger dipolar interactions [8, 9].

g-Factors were calculated from the paramagnetic resonance condition according to the formula [8]: $g = h\nu/\mu_B B_r$, where h—Planck constant, ν—microwave frequency, μ_B—Bohr magneton, and B_r—induction of resonance magnetic field.

3. Results and Discussion

The research which was conducted by us confirmed antioxidant properties of α-lipoic acid [10, 11]. The EPR spectra of DPPH in ethanol with addition of nonirradiated (a) and irradiated (b, c) LA are shown in Figure 2. The lineshape and parameters of the EPR spectra depend on the treatment of α-lipoic acid. The EPR spectrum of DPPH interacting with nonirradiated LA revealed the simplest shape (Figure 2(a)). The hyperfine structure of DPPH was visible for DPPH interacting with UV-irradiated LA (Figures 2(b) and 2(c)).

The comparison of the EPR spectra of the reference—DPPH in ethanol and DPPH after addition of α-lipoic acid to the solution—showed that the signal of DPPH free radicals decreases after reaction with LA. Interactions of LA with free radical molecule of DPPH with unpaired electrons localized on nitrogen atom caused the transformation of DPPH from paramagnetic to diamagnetic molecule. It was revealed as the quenching of EPR signal of DPPH. The antioxidant properties characterized also UV-irradiated α-lipoic acid independently on the time of exposition to electromagnetic waves.

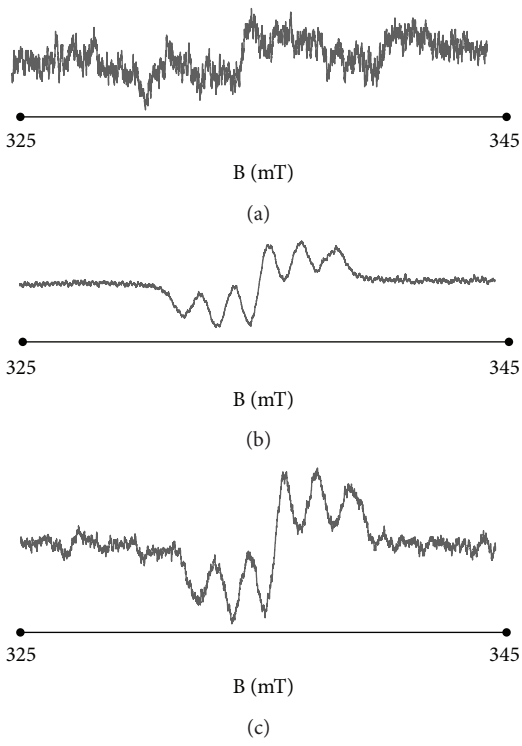

Figure 2: EPR spectra of DPPH with lipoic acid nonirradiated (a) and UV irradiated during 10 (b) and 110 (c) minutes. B is the magnetic induction of the field produced by electromagnet of the spectrometer.

The parameters A_1, A_2, A_3, ΔB_1, ΔB_2, and ΔB_3 of the analyzed EPR spectra of DPPH in ethanol and DPPH in ethanol after addition of nonirradiated and UV-irradiated α-lipoic acid are presented in Table 1.

The values of the parameters A_1, A_2, and A_3 of the three signals in the EPR spectra of DPPH depended on the concentration of DPPH free radicals in the ethanol solution. The calculation of these parameters is shown in Figure 1. The parameters A_1, A_2, and A_3 were the lowest for nonirradiated A_1, A_2, A_3 α-lipoic acid in ethanol solution of DPPH. These parameters were higher for all the UV-irradiated LA located in ethanol solution of DPPH. It indicated that nonirradiated LA stronger interacted with free radicals of DPPH. Our EPR examination pointed out that nonirradiated LA has the relatively stronger antioxidative properties. After UV irradiation these properties were weaker.

The interactions of α-lipoic acid with free radicals of DPPH depended on the time of UV irradiation of the samples (Table 1). The short UV irradiation of α-lipoic acid during 10 minutes quenched sharply its interactions with free radicals. The A_1, A_2, and A_3 parameters increased relatively to the nonirradiated LA. The values of A_1, A_2, and A_3 increased for the next time of UV irradiation of LA, which was 20 minutes. The prolongation of UV irradiation time longer than 20 minutes (30–110 minutes) did not considerably change the values of A_1, A_2, and A_3 parameters (Table 1). It indicated that the long UV irradiation did not change the antioxidative properties of LA.

The results (Table 1) of the effect of UV irradiation on interactions of α-lipoic acid with free radicals pointed out that α-lipoic acid should not be exposed to UV. The longer periods of time of exposition of this substance to UV irradiation may damage its therapeutic effects on living organisms. The quenching of free radicals in tissues after UV irradiation will be lower, and the free radical toxic effects may be developed. The storage of α-lipoic acid in the darkness without UV is proposed.

The high values of the parameters ΔB_1, ΔB_2, and ΔB_3 were obtained for the EPR spectra of DPPH with nonirradiated and UV-irradiated LA in the ethanol solution (Table 1). It indicated that strong dipolar interactions exist between unpaired electrons of DPPH. Such strong interactions are possible for low distances between unpaired electrons [8].

The individual signals in the EPR spectra of DPPH were unsymmetrical. Almost all asymmetry parameters: A_1/A_2, A_3/A_4, A_5/A_6, B_1/B_2, B_3/B_4, and B_5/B_6 were notequal (Table 2). Such nonsymmetrical shape was visible for both nonirradiated and irradiated α-lipoic acid samples. The character of magnetic interactions was similar in these samples.

The performed electron paramagnetic resonance studies confirmed usefulness of EPR spectroscopy in pharmacy and photomedicine. It was shown that EPR as the physical method of analysis may be easily applied in practice. Application of this spectroscopy to examine interactions of pharmaceutical substances with free radicals was confirmed. This method may be important in practical tests of the storage conditions of the samples for medicine. In this work, α-lipoic acid was tested, but this method may be used for the other major diamagnetic or paramagnetic substances.

4. Conclusions

X-band electron paramagnetic resonance studies of UV-irradiated α-lipoic acid (LA) confirmed or pointed out the following:

(1) The antioxidant properties of α-lipoic acid were confirmed. LA quenched free radicals of the reference—DPPH.

(2) The strong effect of UV irradiation on interactions of LA with free radicals strongly changed after UV irradiation. These interactions were weaker for the irradiated samples.

(3) The interactions of α-lipoic acid with free radicals depend on time of irradiation. They were higher after sample irradiation during 10 minutes than for the samples irradiated longer (20–110 minutes).

(4) Application of electron paramagnetic resonance spectroscopy to examine interactions of drug with free radicals was confirmed.

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