MECHANISM OF THE ANTIOXIDATIVE ACTION OF CARNOSINE

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The antioxidative protection system of the cell is a complex mechanism including specialized enzyme systems (superoxide dismutase, catalase, glutathione peroxidase) and also low-molecular-weight compounds, namely lipophilic and water-soluble antioxidants. Besides their antioxidative function proper, water-soluble antioxidants also are involved in the regeneration of lipophilic antioxidants. This interaction between lipophilic and water-soluble antioxidants has been well studied with respect to vitamins E and C [3]. In particular, it has been shown that ascorbate, which itself is a weak antioxidant, forms a powerful synergic couple with α-tocopherol (the principal component of vitamin E), for it can regenerate the radical form of α-tocopherol.

It has recently been demonstrated that carnosine is a natural water soluble antioxidant [1]. Although the antioxidative effects of carnosine (including its synergism with α-tocopherol) in various membrane systems have been well described, the molecular mechanism of this action is far from having been studied [1]. Accordingly the aim of the present investigation was to compare the antiradical activity of Carnosine and vitamin C.

EXPERIMENTAL METHOD

Carnosine and α-tocopherol were obtained from "Serva" (West Germany), sodium ascorbate from "Fluka" (West Germany), and 2,2-diphenyl-1-picrylhydrosyl (DPPH) from "Aldrich" (USA); the solvents were of Soviet origin and of the chemically pure grade. The concentrations of the reagents were chosen on the basis of preliminary tests, and also of published data [3]. EPR spectra of the test samples were recorded on a small EPR spectrometer Makers [2] (made at the "Svetlana" Leningrad Optico-Electronic Instrument) in capillary tubes with a diameter of 1 mm. The reaction of formation of the semiquinone α-tocopherol radical was carried out at –90°C and the spectrum of the radical was recorded under the same conditions.

EXPERIMENTAL RESULTS

The mechanism of the antiradical action of vitamin C includes its ability to interact both with water-soluble radicals and with radicals of lipid nature, including the α-tocopherol radical. These properties of vitamin C have been demonstrated in various systems [3-5]. The most conclusive results have been obtained by the use of the stable free radical DPPH.

The results in Fig. 1 show that addition of sodium ascorbate to a solution of DPPH reduces the latter, and this is accompanied by "quenching" of the characteristic multiplet spectrum of DPPH. Meanwhile, addition of carnosine to this system caused no similar changes in the character of the spectrum indicating that carnosine does not interact directly with highly reactive free radicals.

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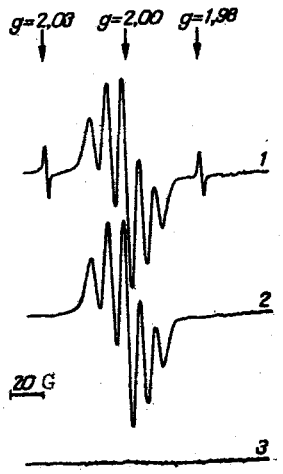

Fig. 1

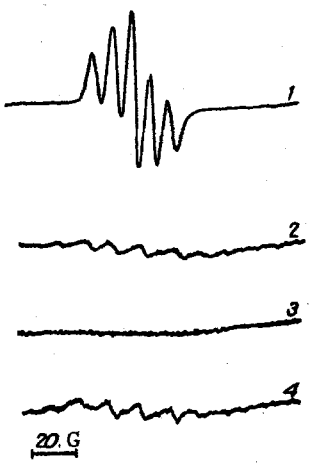

Fig. 2

Fig. 1. EPR spectra of DPPH in alcoholic solution ($5 \cdot 10^{-4}$ M): 1) control, 2) alcoholic solution of carnosine ($5 \cdot 10^{-4}$ M), 3) alcoholic solution of sodium ascorbate ($5 \cdot 10^{-4}$ M). Arrows indicate position of reference lines of Mn^{2+} (g = 2.03 and 1.98) and reference line of crystalline DPPH (g = 2.00).

Fig. 2. Character of change in EPR signal in response to successive addition of $5 \cdot 10^{-4}$ M alcoholic solution of DPPH (1) and $5 \cdot 10^{-4}$ M alcoholic solution of α-tocopherol (2). Signals 3 and 4 reflect state of radical form of α-tocopherol, 2) addition of either $5 \cdot 10^{-4}$ M sodium ascorbate or $5 \cdot 10^{-4}$ M carnosine solution respectively. Spectra were recorded at —90°C.

The antioxidative properties of carnosine likewise cannot be explained by the ability of carnosine to regenerate the radical form of the natural antioxidant α-tocopherol. In fact, as follows from data in Fig. 2, on interaction of α-tocopherol with DPPH a semiquinone radical of α-tocopherol is formed, which has a characteristic multiplet EPR spectrum. On addition of ascorbate, reduction of the semiquinone radical is observed, and is reflected in disappearance of the multiplet. Unlike ascorbate, carnosine cannot reduce the semiquinone radical of α-tocopherol. This result means that the antioxidative synergism of α-tocopherol and carnosine cannot be explained by the ability of the latter to regenerate the radical form of vitamin E.

It is thus clear from all these results that the antioxidative activity of carnosine is based on molecular mechanisms that are basically different from those of ascorbate and are not associated with the manifestation of antiradical activity. An explanation of these mechanisms must await further research.

LITERATURE CITED

1. A. A. Boldyrev, Biokhimiya, **51**, No. 12, 1930 (1986).
2. P. M. Solozhenkin, Electron Paramagnetic Resonance in Analysis of Matter [in Russian], Dushanbe (1986), pp. 248-250.
3. J. E. Packer, T. F. Slater, and R. L. Willson, Nature, **278**, 737 (1979).
4. J. L. Redpath and R. L. Wilson, Int. J. Radiat. Biol., **23**, No. 1, 51 (1973).
5. R. H. Schuler, Radiat. Res., **69**, 417 (1977).