Short communication

Ferrous-Ascorbate Complexes as Carriers of Nitric Oxide

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Abstract. Ferrous-ascorbate is known to form with nitric oxide paramagnetic nitrosyl ferrous-ascorbate complexes, Fe-AA-NO These complexes yield on EPR signal with g-factor close to 2 02 and an optical absorption spectrum with maxima at 340, 460, and 600 nm Fe-AA-NO complexes are unstable in the presence of oxygen Ferrous-ascorbate complexes promote $NaNO_2$ decay resulting in the formation of NO Nitric oxide is taken up by Fe-AA complexes to form paramagnetic ferrous-ascorbate nitrosyl complexes, Fe-AA-NO It is suggested that ferrous-ascorbate complexes can play the role of carriers of NO and, perhaps, O_2 in the blood plasma Nitrosyl ferrous-ascorbate complexes can also be the NO containing factor involved in the blood vessel relaxation (endothelium-derived relaxing factor, EDRF)

Key words: Nitrosyl ferrous-ascorbate complexes — Nitric oxide — Carriers — EDRF

It has been established earlier that many nitrocompounds are reduced in the animal organisms as well as in tissue homogenates to form nitric oxide (Shubin and Kuropteva 1983, Kuropteva and Pastushenko 1985, Zhumabaeva et al 1987, Kuropteva et al 1991) Probably, NO production is an important factor of the nitrocompounds activity at the organism level A number of works have appeared in recent years concerning the role of nitric oxide as a factor involved in basic processes in man and animals (Palmer et al 1987, Moncada et al 1989, Moncada and Higgs 1990) Also, L-arginine has been shown to be the endogenous NO source (Palmer et al 1988, Moncada et al 1989, Moncada and Higgs 1990) NO is known to be the principal constituent of a factor regulating blood vessel relaxation (endothelium-derived relaxing factor, EDRF) and can act as a central nervous system messenger, cytotoxic action mediator in immunologically activated cells, etc (Moncada et al

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1988, Stuehr and Nathan 1989, De Vente et al 1990, O'Connor et al 1990, Beckman 1991, Drapier et al 1991) How these active molecules can be transferred to the sites of their action remains, however, unclear NO carriers (and EDRF, accordingly) have been shown to contain thiols (Palmer et al 1987, Ignarro 1990), and it was proposed that NO-carriers are Fe-S complexes, and Fe-S-NO complexes were suggested as the hypothetical structure of EDRF (Lancaster and Hibbs 1990, Vanin 1991) Actually, these complexes are easily formed and are sufficiently stable and easily registered by EPR technique In our opinion, however, Fe-S-NO complexes are too stable to play the carrier role They actually are formed in the organism but may play the role of NO scavangers These complexes may serve the elimination of excessive NO molecules from the organism or may represent NO storage

Herein, we present data concerning another type of complexes which can play the role as NO and, possibly, O_2 carriers ferrous-ascorbate complexes which form with NO nitrosyl ferrous-ascorbate complexes, Fe-AA-NO

Materials and Methods

The following chemicals were used $FeCl_3$ and $NaNO_2$ from REACHIM Company (Russia), L-ascorbic acid from Sigma (St Louis, MO, USA) The $FeCl_3$ AA $NaNO_2$ ratio in water solutions was 1 5 8 All solutions were prepared in argon atmosphere (argon blowing during 2 3 min) The experiments were performed at pH 6 5 8 0 $NaNO_2$ solution was added to the stock Fe-AA complexes solution and optical absorption spectra were immediately recorded Similar samples were frozen to liquid nitrogen temperature in the form of columns, 3 5 mm in diameter and 30 mm in length, to measure EPR spectra

EPR spectra were recorded with a Bruker ER-300 spectrometer at 77 K Optical absorption measurements were performed with a "Specord UV-VIS" spectrophotometer

Results and Discussion

Fig 1 (curve 1) shows the EPR spectrum of nitrosyl ferrous-ascorbate complexes (Fe-AA-NO) in argon atmosphere at 77 K Fe-AA-NO complexes have asymmetric EPR signal with g-factor close to 2 02 During air blowing through mixed solution or during incubation under an atmosphere, the EPR signal disappears Fig 1 (curve 2) shows for comparison the known EPR spectrum of Fe-S-NO nitrosyl complexes obtained by mixing $FeCl_3$ and reduced glutation solution with sodium nitrite

The optical absorption spectra of the same solutions are shown in Fig 2*A* Spectrum 2*A* 2 was recorded immediately after solution mixing (within 2–3 min), and Fig 2 *A* 3 shows the spectrum of the same sample recorded after 5 7 min Optical absorption spectrum 2 *A* 2 has three expressed absorption maxima 340, 460,

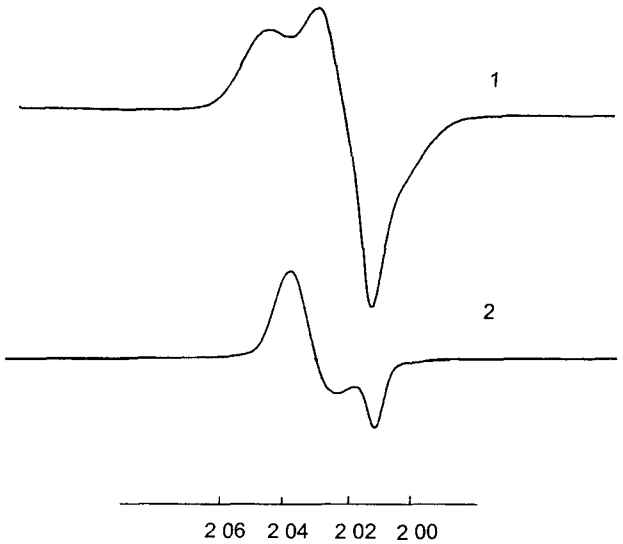

Figure 1. ESR spectra of nitrosyl complexes 1 – ferrous-ascorbate Fe-AA-NO, 2 – ferrous-thiol Fe-S-NO Settings microwave power 20 mW, magnetic field modulation 4 G, temperature 77 K Magnetic field values are given in the g-factor units

and 600 nm It can be assumed that this spectrum is due to Fe-AA-NO complexes recorded by EPR method (Fig 1 curve 1) in the same conditions as the optical spectrum in Fig 2*A* 2 Spectrum 2*A* 3 is the sum of absorptions of two complexes that of Fe-AA-NO and of a new formed, the spectrum of which was obtained as the difference between absorption spectra 2*A* 3 and 2*A* 2 The difference spectrum is shown in Fig 2*B*, it has an absorption maximum in the visible region at 400 nm This complex does not yield any EPR signal It should be noted that ascorbic acid has no absorption in the wavelength range studied, and $FeCl_3$ and AA mixture absorption spectra are represented in Fig 2*A* 1 Spectra 2*A* 2 and 2*A* 3 were recorded with the reference cell containing $NaNO_2$ solution

As mentioned above, the paramagnetic Fe-AA-NO complexes are unstable in the presence of molecular oxygen, and then EPR signal disappeared after air blowing There can be two explanations for this effect either O_2 oxidizes AA and the complex disintegrates, or NO is substituted by molecular oxygen with the formation of diamagnetic Fe-AA-O_2 complex We obtained the data supporting the latter suggestion (unpublished data)

Thus, we could show that ferrous-ascorbate complexes Fe-AA can be formed at pH values close to physiological ones These complexes promote $NaNO_2$ decay giving rise to NO Nitric oxide is taken up by Fe-AA complexes, resulting in the formation of paramagnetic nitrosyl ferrous-ascorbate complexes Fe-AA-NO, with

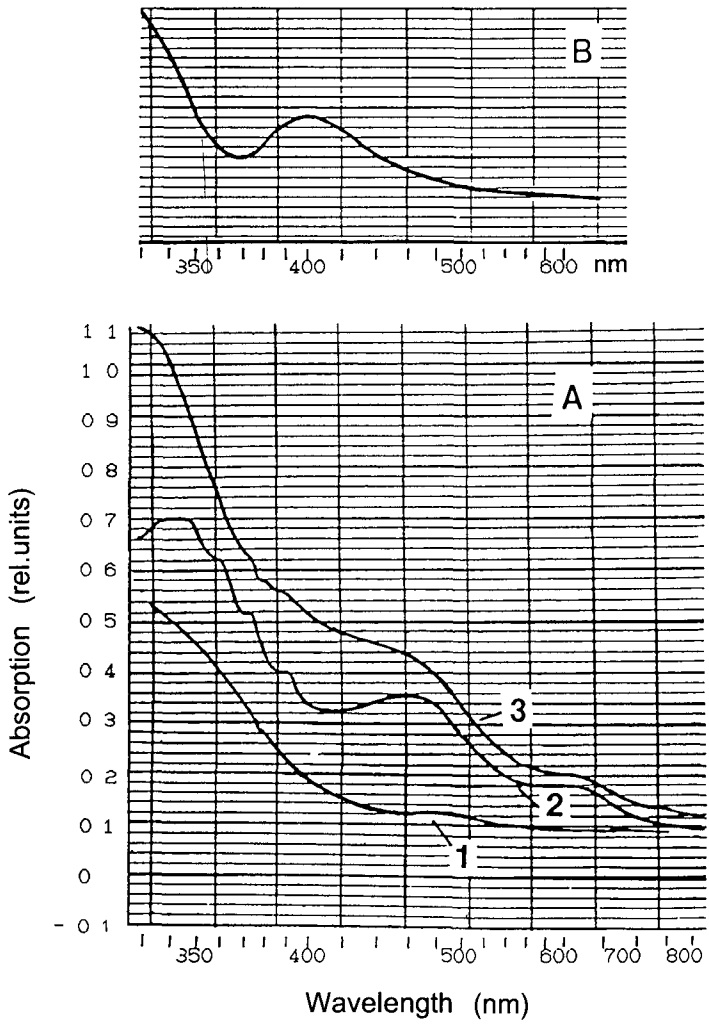

Figure 2. Optical absorption spectra *A* 1 – $FeCl_3$ and ascorbic acid solution (Fe-AA complexes), *A* 2 – after addition of sodium nitrite to *A* 1, *A* 3 – 5–7 min after recording of *A* 2 (in air) *B* – difference spectrum between *A* 3 and *A* 2

g-factor near 2 02 In our opinion, ferrous-ascorbate complexes can play a role as carriers of NO and, perhaps, O_2 in the blood plasma Ferrous-ascorbate nitrosyl complexes can also be the NO containing factor that is involved in the blood vessel relaxation (endothelium-derived relaxing factor, EDRF), the structure of which is widely discussed (Palmer et al 1987, Moncada et al 1989, Ignarro 1990, Moncada and Higgs 1990, Vanin 1991)

Acknowledgements. The authors wish to thank prof L A Blumenfeld for useful discussion

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Final version accepted January 28, 1997