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 an 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 NaN02 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, O2 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...
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 (Palmei et al 1987, Ignarro 1990), and it was proposed that NO-carrers are Fe-S complexes, and Fe-S-NO complexes were suggested as the hypothetical structure of EDRF (Lancaster and Hibbs 1990, Vann 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 scavengers. 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, O2 carriers. Fe-iron-ascorbate complexes which form with NO nitrosyl ferrous-ascorbate complexes, Fe-AA-NO.

Materials and Methods

The following chemicals were used: FeCl3 and NaNO2 from REACHIM Company (Russia), L-ascorbic acid from Sigma (St Louis, MO, USA). The FeCl3 AA NaNO2 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. NaNO2 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 an 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 FeCl3 and reduced glutation solution with sodium nitrite.

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

Nitric oxide carriers 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 2A 2. Spectra 2A 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 2A 3 and 2A 2. The difference spectrum is shown in Fig 2B; 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 FeCl₃ and AA mixture absorption spectra are represented in Fig 2A 1. Spectra 2A 2 and 2A 3 were recorded with the reference cell containing NaNO₂ solution.

As mentioned above, the paramagnetic Fe-AA-NO complexes are unstable in the presence of molecular oxygen, and then EPR signal disappeared after an blowing. There can be two explanations for this effect: either O₂ oxidizes AA and the complex disintegrates, or NO is substituted by molecular oxygen with the formation of diamagnetic Fe-AA-O₂ 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 NaNO₂ 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, Vannin 1991)
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