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Dinitrosyl iron complexes with natural thiol-containing ligands in aqueous solutions: Synthesis and some physico-chemical characteristics (A methodological review)

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ABSTRACT

Two approaches to the synthesis of dinitrosyl iron complexes (DNIC) with glutathione and L-cysteine in aqueous solutions based on the use of gaseous NO and appropriate S-nitrosothiols, viz., S-nitrosoglutathione (GS-NO) or S-nitrosocysteine (Cys-NO), respectively, are considered.

A schematic representation of a vacuum unit for generation and accumulation of gaseous NO purified from the NO_2 admixture and its application for obtaining aqueous solutions of DNIC in a Thunberg apparatus is given. To achieve this, a solution of bivalent iron in distilled water is loaded into the upper chamber of the Thunberg apparatus, while the thiol solution in an appropriate buffer (pH 7.4) is loaded into its lower chamber. Further steps, which include degassing, addition of gaseous NO, shaking of both solutions and formation of the Fe^{2+}-thiol mixture, culminate in the synthesis of DNIC.

The second approach consists in a stepwise addition of Fe^{2+} salts and nitrite to aqueous solutions of glutathione or cysteine. In the presence of Fe^{2+} and after the increase in pH to the physiological level, GS-NO or Cys-NO generated at acid media (pH < 4) are converted into DNIC with glutathione or cysteine. Noteworthy, irrespective of the procedure used for their synthesis DNIC with glutathione manifest much higher stability than DNIC with cysteine.

The pattern of spin density distribution in iron-dinitrosyl fragments of DNIC characterized by the d^7 electronic configuration of the iron atom and described by the formula $Fe^{+}(NO^{+})_2$ is unique in that it provides a plausible explanation for the ability of DNIC to generate NO and nitrosonium ions (NO^{+}) and the peculiar characteristics of the EPR signal of their mononuclear form (M-DNIC).

Contents

Abbreviations: BPDS, bathophenanthroline disulfonate; B-, M- or T-DNIC, binuclear, mono- or tetra-nuclear dinitrosyl iron complex; Cys-NO, S-nitrosocysteine; EPR, electron paramagnetic resonance; GSH, glutathione; GS-NO, S-nitrosoglutathione; MGD, *N*-methyl-D-glucamine dithiocarbamate; MNIC, mononitrosyl iron complex; RS-NO, S-nitrosothiol.

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1. Introduction

The large body of evidence gained in the past decade unambiguously demonstrates that water-soluble dinitrosyl iron complexes (DNIC) with natural thiol-containing ligands (cysteine, glutathione, thiol-containing proteins, etc.), both endogenous and prepared by chemical synthesis, may function as a "working form" of nitric monoxide (NO), one of the most universal regulators of metabolic processes in animals and man. Incorporation of NO into DNIC provides effective protection of NO from deleterious effects of superoxide anions along with predominant accumulation of NO in protein-bound DNIC and targeted delivery of NO to cells and tissues (for the most part, within the composition of low-molecular DNIC, etc) [1–12].

This paper is a brief survey of the state-of-the-art in the synthesis of DNIC with natural thiol-containing ligands with special emphasis on the methodological approaches used therein, the electronic and spatial structures of DNIC responsible for their ability to donate neutral NO molecules and nitrosonium ions (NO^+) in aqueous solutions.

2. Two approaches to the synthesis of DNIC with natural thiol-containing ligands in aqueous solutions

2.1. General concepts

There exist two main approaches to the synthesis of DNIC with natural thiol-containing ligands (cysteine, glutathione, thiol-containing proteins, etc.) in aqueous solutions where gaseous NO purified from nitrogen dioxide (NO_2) by low-temperature sublimation is used as a source of NO [13,14] or S-nitrosothiols (RS-NO), viz., S-nitrosoglutathione (GS-NO) and S-nitrosocysteine (Cys-NO) [15,16] obtained by S-nitrosation of acidified aqueous solutions of appropriate thiols with nitrous acid (HNO_2) [17].

Fig. 1 represents a schematic diagram of a vacuum unit for obtaining gaseous NO purified from the nitrogen dioxide (NO_2) admixture; its design enables accumulation of NO in the evacuated glass Vessel 6 which we use for DNIC synthesis. NO synthesis in the evacuated Vessel 1 is performed using a reaction of Fe^{2+} in 30% HCl with nitrous acid formed by dropwise addition of a saturated solution of sodium nitrite to the solution of HCl (Vessel 2). The gaseous NO generated thereupon is passed sequentially through a saturated solution of NaOH to remove NO_2 (Vessel 3) and sulfuric acid (Vessel 4) to eliminate water, after which the NO-NO_2 mixture is fed into Vessel 5 and frozen at liquid nitrogen temperature. After formation of the blue-green-coloured liquid phase (NO + NO_2, 5–10 ml) accumulated on the bottom of Vessel 5, the NO-NO_2 flow into Vessel 5 is stopped to allow fast accumulation of NO evaporated from the bottom of Vessel 5 in the degassed Vessel 6. The evaporation begins after demounting of the Dewar vessel with liquid nitrogen from Vessel 5 and the increase in the liquid phase temperature of the NO-NO_2 mixture from $-196^{\circ}C$ to $-141^{\circ}C$, which initiates the transition of NO from the liquid to the gaseous phase.

It is important that the level of the liquid in Vessels 3 and 4 is kept low to ensure the unobstructed passage of the NO-NO_2 mixture to Vessel 5 through the alkaline solution and sulfuric acid. The layer thickness of the frozen gas mixture, i. e. the distance between the inner tube and the vessel wall in Vessel 5, must not exceed 15 mm, otherwise the temperature of the freezing mixture near the tube will exceed the liquid nitrogen temperature and thus initiate the inflow of the non-frozen NO-NO_2 mixture into Vessel 5, as a result of which the gas pressure may increase to an undesirable level.

In Vessel 6, the pressure of gaseous NO is controlled with the help of a mercury manometer and must not exceed 1 atm (740–760 mm Hg). After reaching this level, the intense accumulation of gaseous NO in Vessel 6 is stopped.

The transition of the bulk of NO from the liquid to the gaseous phase after cessation of the pressure boost in Vessel 6 and the subsequent increase in the temperature of the frozen fluid containing only NO_2 (which has a bright violet colour in the liquid state) to $-22^{\circ}C$ initiates the transition of NO_2 into the gaseous phase, which has a characteristic brown colour. In its turn, blockade of the NO_2 ingress into Vessel 6 initiates accumulation of pure gaseous NO in Vessel 6 due to low-temperature sublimation of the NO-NO_2 mixture in Vessel 5.

However, in the case of long-term (≥2–3 weeks) storage of NO in Vessel 6 the purification of gaseous NO from the NO_2 admixture by low-temperature sublimation did not ensure the appearance of NO_2 in this vessel. Under these conditions, the NO-NO_2 mixture was formed as a result of disproportionation of NO molecules as free-radical compounds. This disproportionation represents mutual single-electron oxidation-reduction of two NO molecules to NO^+

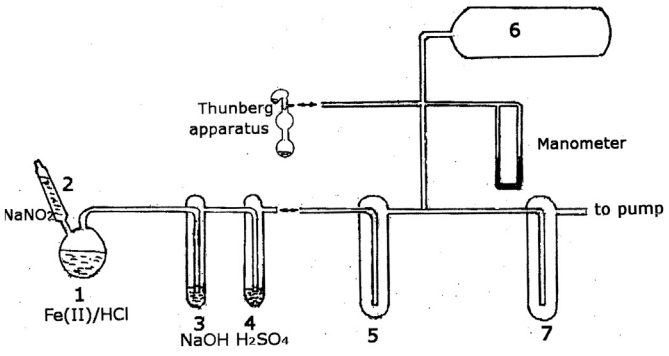

Fig. 1. A schematic representation of a vacuum unit for generation and accumulation of gaseous NO purified from the NO_2 admixture and its application for obtaining aqueous solutions of DNIC in a Thunberg apparatus. Vessel 1 is a reactor for generating gaseous NO containing an NO_2 admixture by a reaction of Fe^{2+} ions with nitrous acid in aqueous HCl. Vessel 2 contains a sodium nitrite solution loaded dropwise into Vessel 1. Vessels 3 and 4 contain concentrated solutions of NaOH and sulfuric acid for eliminating NO_2 and water from the NO-NO_2 mixture. Vessel 5 is cooled with liquid nitrogen to enable the transition of NO and NO_2 to the liquid state. Vessel 6 accumulates gaseous NO purified from the NO_2 admixture. Vessel 7 is a low-temperature trap for capturing H_2O before evacuation. The unit design also includes a Thunberg apparatus where DNIC synthesis in aqueous solutions is performed and a gauge for measuring NO pressure.