Formation of a three-stroke scheme for NO-synthase catalytic cycle model under conditions of hypoxia.

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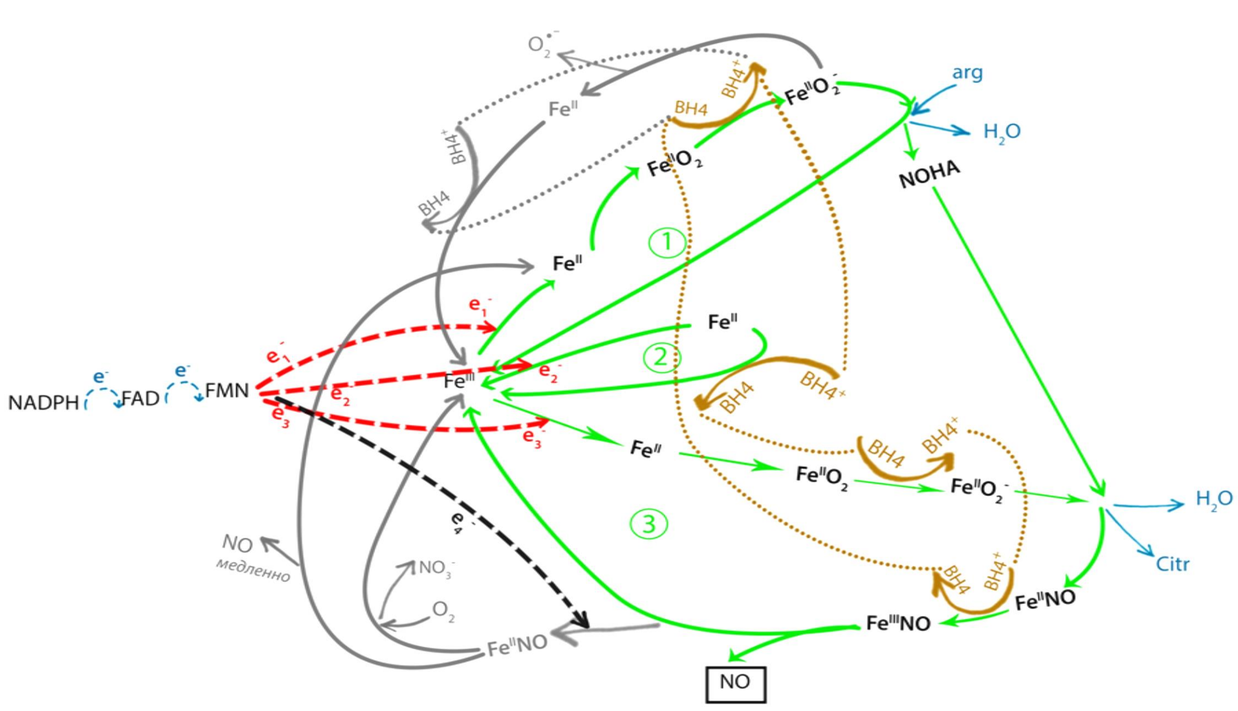
Introduction: In this work, we present a structural scheme for a universal NO-synthase catalytic cycle model, which can be used to describe the functions of all major enzyme isoforms.

The resulting three-stroke scheme allows us to fit together the available information on the interaction and algorithm of the enzyme domains and binding active centres both under normal physiological conditions and under conditions of hypoxia, substrate deficiency, or reduced rate of NO release from the enzyme pocket, which leads to the initiation of pathological variants of the cycle.

A three-stroke scheme for NO-synthase catalytic cycle model.



Depending on the degree of hypoxia, three options are possible:

1. **With severe hypoxia**: the cycle is blocked at the very beginning. There is no consumption of the substrate (Arg), the heme-FeII complex is stable;

2. **With moderate O2 deficiency**: heme-FeII-O2 complex is reduced by BH4 to heme-FeII-O2-, which reacts with Arg to form NOHA. The resulting heme-FeIII is reduced by the 2nd electron from the reductase domain and oxidized by the BH4+ radical. The 3rd electron from the reductase domain reduces heme-FeIII to heme-FeII. Then the cycle is blocked;

3. **With insignificant O2 deficiency**: BH4 activates the heme-FeII-O2 complex to heme-FeII-O2-, turning into the BH4+ radical; heme-FeII-O2- reacts with NOHA, forming heme-FeII-NO, the BH4+ radical oxidizes heme-FeII-NO to heme-FeIII-NO. In normal case heme-FeIII-NO releases NO, which leaves the enzyme pocket. If this does not happen, then NO is repeatedly bound and released from the heme-FeIII-NO complex.
In this case, the next electron coming from FMN will reduce either heme-FeIII or heme-FeIII-NO. In the first case, cycle is completed with a slight delay. In the second case, heme-FeIII-NO is reduced to heme-FeII-NO, which releases NO very slowly. the unproductive branch of the cycle is turned on.

The scheme also makes possible to explain a number of features of the behavior of NOS, such as the production of nitrate anions (NO3-) in case of delay of NO release from the enzyme pocket, the rapid generation of the superoxide anion radical (O2-) under conditions of lack of substrate (L-arginine), etc.

During the scheme formation, an assumption was made about the peculiarities of tetrahydrobiopterin (BH4) functioning, which allowed us to isolate the closed cycle of BH4 reactions within the catalytic cycle of NOS. This allows BH4 to provide and accelerate the cycle without wasting.

The presented structural scheme allows to create a simulation model of the enzymatic part of the NO cycle for predicting NOS behaviour in common pathological conditions.

Keywords: nitric oxide, respiratory tract, nitric oxide synthesis cycle, NO-synthase, NOS modelling.