NO catastrophes in vivo as a result of micellar catalysis

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Abstract Micellar catalysis plays a crucial role in NO metabolism because media in vivo are heterogeneous and the concentration of NO in different phases at different levels of solubility differs by degrees of magnitude. The relative volumes of the hydrophobic phases are usually small. At small volumes (which are calculated) of these phases the reaction rates of NO metabolism change. The dependence on the relative volumes is resonance-like. Not only regulation, but bifurcations and catastrophes are possible in vivo as a result of this changing due to the small change of effectiveness of micellar catalysis.

Key words: Nitric oxide; Micellar catalysis; Redox signalling; Nitrosothiol; Free radical; Lipid membrane

1. Introduction

Biogenous nitric oxide (NO) has emerged as a wide-ranging mediator in higher animal physiology [1]. It participates in the regulation of blood flow [2–4], pulmonary circulation [5]; modulates apoptosis [6] and differentiation [7]; plays a key role in deactivation of free radicals [8], in bactericidal and tumoricidal actions of macrophages [1] and acts as a neuronal messenger [9], including memory formation [10]. But even 10 years after the discovery of NO in vivo [3,4] many details of its biochemistry remain unknown and appear paradoxical [11]. Measured rates of reactions differ greatly in vivo and in vitro. One of the difficulties is the ability of NO to convert into NO equivalents [12,13]. Media in vivo are heterogeneous and the concentration of NO in different phases at different levels of solubility differs by degrees of magnitude. The relative volumes of the hydrophobic phases are usually small. This represents the conventional argument in favour of neglecting their impact on the kinetics of NO reactions. Here we show that this is not true. At small volumes (which we calculate) of these phases the reaction rates of NO metabolism change. Moreover, the dependence on the relative volumes is resonance-like. Not only regulation, but bifurcations and catastrophes are possible in vivo as a result of this changing due to the small change of effectiveness of micellar catalysis.

2. Materials and methods

Let us consider a model reaction (e.g. NO with targets): M+N → products (M and N are reagents). In a heterogeneous medium (e.g. lipids-water) with volumes V_i, $i=1,...,n$, if the reaction is much faster than diffusion, the concentrations are spatially constant in every phase. Let $Q_{N,i}$, $Q_{M,i}$ be partition coefficients, i.e. $[N]_i = Q_{N,i}[N]_n$, $[M]_i = Q_{M,i}[M]_n$, $i=1,...,n-1$.

Let us consider the reaction with orders p and q, correspondingly: $d_t[N]_i = -k_i[N]_i^p[M]_i^q - P_{Ni}/V_i$, $d_t[M]_i = -k_i,[N]_i^p,[M]_i^q - P_{Mi}/V_i$, $i=1,...,n$. $\Sigma_{i=1}^n P_{Ni} = \Sigma_{i=1}^n P_{Mi} = 0$. Then the coefficient $k_{app} = U$ in the equation $dN/dt = dM/dt = -U(N^pM^q)/V^{p+q-1}$ may be calculated by the following formula:

$$U = \frac{\sum_{i=1}^{i=n-1} k_i Q_{Ni}^p Q_{Mi}^q x_i + k_n\left(1-\sum_{i=1}^{i=n-1} x_i\right)}{\left(\sum_{i=1}^{i=n-1} Q_{Ni}x_i + 1-\sum_{i=1}^{i=n-1} x_i\right)^p \left(\sum_{i=1}^{i=n-1} Q_{Mi}x_i + 1-\sum_{i=1}^{i=n-1} x_i\right)^q} \quad (1)$$

where $x_i = V_i/\Sigma_{j=1}^{j=n}$ and V_j is the relative volume of the i-th phase. We can generalise the formula onto the case of an infinite number of phases. Then we should use integrals instead of sums and introduce the distribution $V = V(i)$, $i\in\Omega$, of the volumes by a parameter i. In that case the coefficient U may be calculated by the following formula:

$$U = \frac{\left[\int_\Omega k(i)Q_N^p(i)Q_M^q(i)V(i)di\right]\left[\int_\Omega V(i)di\right]^{p+q-1}}{\left[\int_\Omega Q_N(i)V(i)di\right]^p\left[\int_\Omega Q_M(i)V(i)di\right]^q}$$

If the functions $Q_N(i)$, $Q_M(i)$, $V(i)$, $k(i)$ depend on a parameter ε (scalar, vector or function), and we can consider some functional K[U] as a criterion of quality, we can optimise it with respect to the parameter. Two important partial cases of Eq. 1 at $n=2$ were investigated [14]: (1) p is arbitrary, $q=0$; (2) $p=q=1$.

The factor U does not depend on time and it is a natural generalisation of k for reactions in heterogeneous media.

The deviation of the reaction rate as a result of the heterogeneity may be significant. Certainly, $U = k_2$ as $x = V_1/(V_1+V_2) \to 0$. However, if x is small and Q_N, Q_M are large, the result depends on some details. For instance, at $x \approx Q_N^{-1} \approx Q_M^{-1} \gg 1$ the reaction rate increases proportionally $1/x$. Similar asymptotics may be obtained as $x \to 1$.

The balance of the concentration ratio (1) is possible as a result of reagents flows among the phases

$$P_{Ni} = d_t[N]_iV_i + k_i[N]_i^p[M]_i^qV_i,\ P_{Mi} = d_t[M]_iV_i + k_i[N]_i^p[M]_i^qV_i$$

3. Results and discussion

In micellar catalysis [15] reaction rates increase due to the concentration of reagents in a small volume of the second phase. For the kinetic-controlled reaction (e.g. NO with targets): M+N → products (M and N are reagents) the apparent rate constant depends on the partition coefficients of the reagents (Q_M, Q_N), on the order of the reaction for both reagents (p, q), on the rate constants in each phase (k_1, k_2) and on the volume ratio of the second (hydrophobic) phase and the total volume $x = V_1/(V_1+V_2)$ (see Section 2). For effectiveness of the catalysis the order of reaction is the crucial factor: for reactions of the null or first order ($p+q \le 1$) the acceleration is possible due to $k_2 > k_1$ only. But for reactions of the second and, especially, third order, even if $k_1 = k_2$ the rate of growth of the $(k_{app}/k_1 = H = (Q_M^pQ_N^qx+1+x)/\{(Q_Mx+1+x)^p(Q_Nx+1+x)^q\}$ may be very large (see Fig. 1).

Since the coefficient H determines the dynamics of the metabolites, three kinds of bifurcations (catastrophes) are possible here.

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1. A homogeneous medium transforms into a heterogeneous one, when through the birth or the disappearance of a new phase, a small deviation of x or Q, the H value jumps;
2. A sign change of the derivative dH/dx of the reaction rate (intersections of the solid line in Fig. 2);
3. A catastrophe in the external system as a result of a strong deviation of H, which in turn is a result of a small deviation of some parameter ε: $x = x(\varepsilon)$, $Q_N = Q_N(\varepsilon)$, $Q_M = Q_M(\varepsilon)$; this is more probable on the abrupt slopes of the graph, see Fig. 2.

It is widely accepted that the impact of the lipophilic phase in the chemistry of NO is not significant because the ratio of the volume phases is small. A comparison of Figs. 1a,c and 1b,d reflecting the differences shows that in reality the dependence is almost opposite: the contribution of a heterogeneity is especially great when the volume ratio is small ($x \approx 1\%$). So, for almost ($0.01 < x < 1$) always the smaller x is, the greater the effect.

In Figs. 1 and 2 H was calculated when the rate constants for both phases are equal ($k_1 = k_2$). In reality, such an equality usually is violated: for ion reactions in many cases the differences are as high as several orders of magnitude, but for radical reactions the dependence of k on the solvent is not large [16]. So, for competitive substrates or reactions a change from homo- to heterogeneous systems, or a change in Q and x can change not only the rates, but the main mechanism as well as the major direction of the process [17].

Besides lipid membranes there are many hydrophobic phases, e.g. cholesterol and low-density lipoprotein for its transport, fatty acids, and hydrophobic core of protein molecules with different x and Q.

Partition coefficients Q depend on the composition of each phase. In general, gases and hydrophobic non-polar compounds are salted out of an aqueous solution by neutral salts. So, Q is enhanced by the increase of the content of electrolytes in the mixture as well as by a fraction of more hydrophobic compounds in the lipid phase.

As regards micellar catalysis, solutions of proteins must be considered two-phase mixtures. The hydrophobic residues of the protein core play the role of the second phase. That is why the solubility of non-polar compounds is higher in protein solutions (solubilisation) [15]. For instance, the solubility of

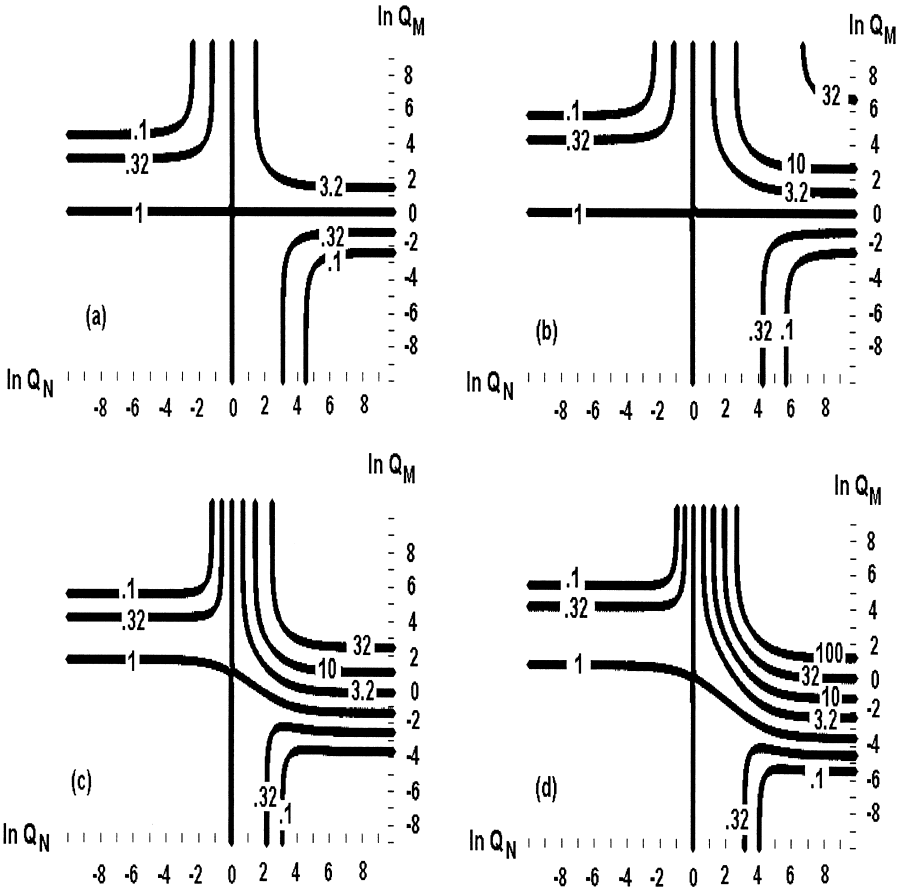

Fig. 1. In a two-phase medium the reaction rate is different from the analogous rate in a homogeneous one and depends on the partition coefficients Q_N, Q_M and relative volume $x = V_1/(V_1+V_2)$. When $k_1 = k_2$, for reactions with orders $p = q = 1$ (a, b) the acceleration factor $k_{app}/k_1 = H > 1$ at $Q_N > 1$, $Q_M > 1$ and at $Q_N < 1$, $Q_M < 1$ and $H < 1$ if the signs of the inequalities are opposite. The isolines of H for $x = 0.1$ (a) and x=0.03 (b) are plotted in logarithmic co-ordinates. At Q_N,Q_M the function $H = f(x)$ has a unique extremum at $x_{extr} = 1/(1+\sqrt{Q_N Q_M})$; $H_{extr} = k(1+\sqrt{Q_N Q_M})^2/(\sqrt{Q_N}+\sqrt{Q_M})^2$). It is a minimum if the signs of the inequalities are opposite and a maximum if they are identical. In (c) and (d) the similar isolines for the reactions of the third order ($p = 2$, $q = 1$) are shown. In these cases in the quadrant $Q_N < 1$, $Q_M < 1$, $1 < 1+x$. One can see that the acceleration here is more effective, as well as at the smaller ($x = 0.03$) volume of the second phase. The derivatives in the ends of the segment [0, 1] may be calculated. $dH/dx(0) = Q_N^p Q_M^q$ $(1+p+q)$; $dH/dx(1) = [Q_N^p Q_M^q - pQ_N^{p-1} - qQ_M^{q-1}]/Q_N^p Q_M^q$. At large values of the partition coefficients the derivatives may be huge: $dH/dx(0) \approx Q_N^p Q_M^q$, against $dH/dx(1) \approx 1$, see Fig. 2.

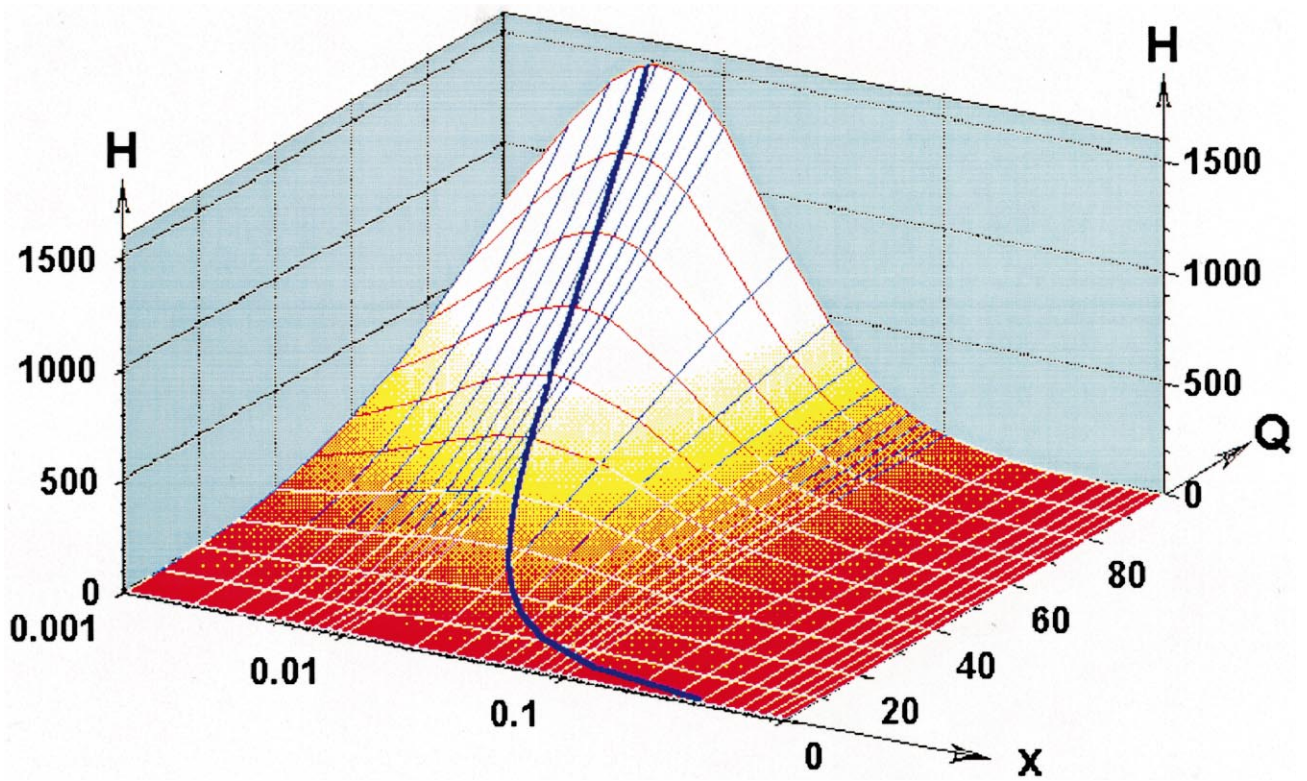

Fig. 2. The graph of the acceleration coefficient H(Q,x) for a third-order reaction (p = 2, q = 1) at equal partition coefficients ($Q_N = Q_M = Q$). The scale along the variable x (relative volume of the second phase) is logarithmic since the dependence at small x is very strong. The restrictions at Q = const (red), and the restriction at x = const (blue) are shown. For the coefficient H(x) maximisation at given Q_N, Q_M we solve the equation $dH/dx = 0$ analytically (the solid line indicates the graph in the points where H(x) takes its maximum). At large values of the partition coefficients we obtain the asymptotics: $(x_{extr} \approx (\sqrt{1+8\eta}-1)/4Q_M$, where $\eta = Q_M/Q_N$ (at $Q_N = Q_M \Rightarrow x_{extr} \approx 1/2Q$. $H(x_{extr}) \approx 16Q_N^2(\sqrt{1+8\eta}-1)/(\sqrt{1+8\eta}-1+4\eta)^2(\sqrt{1+8\eta}+3)$ (at $Q_N = Q_M \Rightarrow H_{extr} \approx 4Q^2/27$). Thus, at a given Q the graph of H(x) may be divided into three zones: (i) a linear growth at very small x; (ii) a zone of the maximum; (iii) a decreasing zone, the 'slope' of the graph after the maximum is like a quadratic hyperbola. The width (x_1-x_2) of the zone, where $H \geq h$, with growth of h diminishes. The isoline $H(Q_N, Q_M, x) = h$ at large $Q_N = Q_M$ has two branches: x_1 (Q, h) $\approx (h-1)Q^{-3}$, $x_2 \approx h^{-0.5}$ $[1+1.5Q^{-1}(1-h^{0.5})]$.

oxygen in water and in blood plasma is similar: the decrease of solubility in the water phase because of salts can be compensated by solubilisation. The protein concentration in the blood plasma is approximately 7%, and lipid content about 0.4–0.7%.

NO is fairly non-polar, and in lipid-water mixtures $Q \gg 1$. For octanol-water mixture $Q_{NO} \approx 8$, and for natural mixtures in vivo the upper boundary of Q_{NO} was evaluated to be 70 [18,19]. Often octanol-1 is considered a model solvent with average hydrophobicity of membranes. But we have shown (see Section 2) that H for multi-phase media is not equal to H for a two-phase medium with average Q_{NO}. So, NO in vivo is oxidised faster than in water.

NO is the sole stable odd (free radical, 15 electrons) molecule. The probability of reaction of an NO molecule with an odd metabolite is much higher than with an even one. For the majority of reactions with the second order by NO, ambient temperature, and low concentrations of NO, the reaction rates are low. For instance, in 10 ppm NO-air mixtures will be half gone in 7 h [20], so slowly that the mixture can be used for inhalation by lung disorder patients [21]. So, these reactions have usually been ignored in NO biochemistry. The principal targets of NO in vivo are believed to be the following: (1) free radicals and (2) derivatives of d-elements [20]. Hydrophobic superoxides of lipids or water-solved superoxide anion (O_2^-) correspond to (1), and haemoglobin, other haem-containing proteins, metalloenzymes, and low molecular metal complexes (that are usually water-solved) to (2). Hence, heterogeneity is the strongest regulator of NO activity. In reactions with hydrophilic targets ($Q < 1$) it acts as an inhibitor, for hydrophobic compounds it acts as a catalyst. In any case lipids or protein core molecules play the role of an NO sponge.

We conclude from Figs. 1 and 2 that even for a relative volume of the lipid phase of 10^{-2}–10^{-3} the reaction rate (and, therefore, diffusion distance) of NO may be changed 10^0–10^3 times. If here $Q_M \gg 1$ (e.g. reactions with peroxolipids (right top part of Fig. 1b) or a reaction with oxygen (right top part of Fig. 1d and also Fig. 2)), $H \gg 1$, i.e. the rates greatly increase and the distances decrease. In the opposite case, $Q_M < 1$ (a majority of the metal-contained targets and O_2^- (right lower parts in Fig. 1a–d)), $H < 1$, the rates decrease and the distances increase.

With regard to micellar catalysis the oxidation of NO by O_2 will be significant. Since the acceleration of the reaction in comparison with homogeneous medium is essential near the extreme values of x (solid line in Fig. 2), the impact of the reaction on the total balance of NO is strongly dependent not only on the average [NO] and [O_2], but on Q and x. This oxidation could lead not only to a fall of [NO], but also to an increase of concentrations of NO equivalents.

The majority of the metabolites arise and disappear as a result of the enzymatic reactions, null-order by [S] at $[S] \gg K_M$

(with growth [S] the rate has a limit V_{max} when the enzyme is saturated with substrate). In some cases excessively large [S] can lead to inhibition. Hence, an acceleration by the micellar catalysis is visible when $[S] < K_M$. But for the non-enzymatic oxidation of NO there is no upper boundary for V_{max}: the kinetic equations are not changed at high concentrations of NO including unachievable in vivo ones [22].

Besides NO there are other metabolites for which micellar catalysis is essential. Nitrosothiols (RSNO) are important forms of NO equivalents [12]. The impact of the reaction of NO with RSH is essential only when oxidising agents or catalysts are present [23,24].

Let us consider these two versions. For homogeneous aerobic solutions the formation of RSNO is described by the same kinetic equation as for the oxidation of NO (N = NO, M = O_2, p = 2, q = 1, $k = (6 \pm 2) \times 10^6$ M^{-2} s^{-1}) [22–24] because both reactions have the same intermediate NO_X (dinitrosoperoxide ONOONO; [25]) and the same rate-limiting step. Since both NO and O_2 are hydrophobic ($Q \gg 1$), the H value for both $-d[NO]/dt$ and $d[RSNO]/dt$ can be as high as 100 and more (see Fig. 2). For instance, at $Q_N = Q_M = 50$ we obtain $x_{extr} \approx 0.010$, $H_{extr} \approx 370$.

In contrast, the reaction of the d-metal catalytic synthesis of RSNO could be the first order to [NO]: the ion of metal can play the role of the second odd reagent. So, the value of H for hydrophilic d-metal complex catalysts will be < 1. Thus, the relative impact of the first reaction increases hundreds of times as a result of the sponge concentration.

For hydrophobic thiols ($Q > 1$) NO_X can be the principal nitrosating agent (in the lipid phase), for hydrophilic ones NO-Me complexes could be the agents. Thus, the differences in rates for the two competing mechanisms in homo- and heterogeneous media could be several orders of magnitude.

When the redox potential falls, RSNO can be reduced back to NO, e.g. by thiols and ascorbate [26].

We can see the unique combination of the seven chemical peculiarities of NO: (1) a large coefficient Q (as a result of its lipophilicity) leads to its large concentration in lipids; (2) the high reactivity, since non-enzymatic reactions run at ambient temperature with large rates; (3) the high (third for the oxidation by the oxygen) order of reactions, due to the very strong dependence of acceleration coefficient H on Q; (4) the lack of an upper boundary for V_{max}; (5) the rate (k) of the NO reaction with oxygen is roughly the same in water and in lipids; (6) the reversibility of redox reactions of NO; (7) there is a wide choice of NO targets with different lipophilicity in any phase. This leads to the exceptional effectiveness of micellar catalysis in the biochemistry of NO.

Though fast diffusion was assumed above, some processes dependent on NO are diffusion-controlled. The corresponding reaction-diffusion equation should take into account the heterogeneity (especially in vivo). There are many versions with different values of lipid dispersions, diffusion coefficients and reaction rates. For instance, even if the Q values are the same for the cholesterol in a lipoprotein micelle and for a cholesterol plaque on a blood vessel (or for tar microdrops in a smoker's lung), the resulting impact on NO metabolism will be quite different, because the dimensions of the lipid phases and the impacts of the diffusion of NO and its targets will be very different as well. We evaluated the dynamics and limit solutions for the natural geometry and coefficients of the reaction-diffusion equation. The result in brief is: in many cases the impact of the small lipid phase is predominant. If the dispersion of the phase is very small, the average coefficients may be evaluated.

So, NO-dependent processes may be controlled in vivo and in vitro, and we can control them optimally if we know the parameters of the equations. The micellar sponge concentrating in lipids and protein cores is typical for many metabolites, but for NO it is the most significant. The obtained formulae may be used for the description of the dynamics of metabolites as well as in chemical industry.

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