Micellar catalysis for oxidation of nitric oxide (NO) in the multi-phase systems in vivo

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Abstract The equation of the dependence of the third-order reaction acceleration due to concentrating the reagents in a small volume of the hydrophobic phase on the partition coefficients of reagents (Q) and on the lipophilic phase fraction (x), $|k_{app}/k_2 = H(Q_{NO},Q_{O_2},x)|$ was analyzed. It was demonstrated that the numeric value of dH/dx at $x \rightarrow 0$ could not be used in order to calculate the efficiency of catalysis from the experimental data. It was shown that, unlike in two-phase systems (with an aqueous and a hydrophobic phase), the dependence of H on Q in multi-phase systems, that include all in vivo systems, is different. The multiple phase state of the systems has a determining role for a regulation of NO-dependent processes and in the realization of conditions of 'NO catastrophes'.

Key words: Nitric oxide; Nitrite; Partition coefficient; Competing pathway; NO catastrophe; Nitrosation

1. Introduction

Nitric oxide (NO) is the most important non-organic metabolite of all higher animals [1–5] which participates in the regulation of blood flow [3], septic shock [6], and the transmission and storage of information [4]. NO plays a role as a scavenger of free radicals [9,10] and is the precursor of peroxinitrite which kills pathogenic bacteria and tumor cells [7,8]. In mammalians, NO forms as a result of an O_2-dependent oxidation of arginine by NO-synthases [4,11]. NO is further oxidized by one of two competing pathways: it can be rapidly and irreversible transformed to nitrate by the Hb–O_2 complex (1) or oxidized to nitrite or nitroso compounds by free O_2 in the absence of transition elements (2) [1,5,12].

$$NO + Hb^{2+} - O_2 \rightarrow NO_3^- + Hb^{3+} \quad (1)$$

$$4NO + O_2 + 2H_2O \rightarrow 4NO_2^- + 4H^+;$$

$$4NO + O_2 + 2RSH \rightarrow 2RSNO + 2NO_2^- + 2H^+ \quad (2)$$

In fact, reaction (2) is reversible because in vivo nitrite and nitrosothiols can be reduced back to NO [5,13–15]. Thus, the rate ratio of processes (1) and (2) regulates the pool of NO and other reactive nitrogen intermediates (RNI), and ultimately affects the role of NO and RNI in various physiologic processes [5,10,16]. Two independent studies devoted to NO oxidation acceleration in heterogeneous media were recently reported [1,2]. Both studies discuss the same model but reach qualitatively different conclusions. In this study, we compare both studies and discuss the reasons underlying the different conclusions.

2. Materials and methods

The mathematical package Maple V has been used to make calculations to accomplish three-dimensional graphics.

3. Results and discussion

Studies [1] and [2] consider the model of NO oxidation in a heterogeneous medium of the water–lipid type, when the diffusion rates are much quicker than the rates of chemical reactions and the ratio of the concentrations of reagents is constant in each phase. Due to a larger solubility of NO and O_2 in the hydrophobic phase, the concentration of the reagents increases in this phase and decreases in the water phase. Since reaction (2) is a third-order reaction (the second order for NO and the first order for O_2):

$$-d[NO]/dt = 4k[NO]^2[O_2] \quad (3)$$

this concentrating considerably enhances the rate of the total reaction (for both phases). Therefore, the hydrophobic phase acts like an NO-lens [1] or an NO-sponge [2]. Fig. 1 shows a model of micellar catalysis for NO oxidation in vivo. The notation shown in Table 1 has been used both by the authors of [1,2] and by the authors of this study.

Liu et al. [1] tested the effects of various lipid additions on the rate of decreasing NO concentration during its oxidation in a water–lipid system. The reaction rate constant in a homogeneous aqueous buffer solution was 6.6×10^6 M^{-2} s^{-1}. However, upon addition of a suspension of liposomes (4%) the apparent rate constant increased 13 times (8.8×10^7 M^{-2} s^{-1}) as compared to a homogeneous solution. The acceleration had a weak dependence on the nature of the hydrophobic phase. The authors concluded that the NO oxidation rate in the lipid phase was approximately 300 times higher than in the surrounding aqueous phase and that it is the hydrophobic phases that are generating NOx.

Gordin and Nedospasov [2] deduced the general equation for reaction acceleration in micellar catalysis:

$$U = \frac{\sum_{i=1}^{i=n-1} k_i Q_{N,i}^p Q_{M,i}^q x_i + k_n\left(1-\sum_{i=1}^{i=n-1} x_i\right)}{\left(\sum_{i=1}^{i=n-1} Q_{N,i}x_i + 1-\sum_{i=1}^{i=n-1} x_i\right)^p \left(\sum_{i=1}^{i=n-1} Q_{M,i}x_i + 1-\sum_{i=1}^{i=n-1} x_i\right)^q} \quad (4)$$

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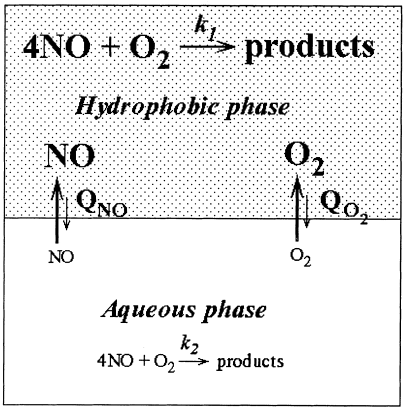

Fig. 1. Micellar catalysis of NO oxidation.

where U is regarded as k_{app} and is expressed in reaction rate constant units; $Q_{N,i}$, $Q_{M,i}$ are the partition coefficients of the reagents; $x_i = V_i/\Sigma_{j=1}^{j=n} V_j$ is the relative volume of the ith phase; p and q denote the reaction orders for each reagent. NO oxidation in a two-phase system illustrates that the dependence of catalysis efficiency on the relative volume of the hydrophobic phase has a resonance-like maximum at small ($\sim$1%) ratios of the lipid phase. Acceleration dependencies on the reaction order and the lipid phase fraction are presented graphically. Emergence of bifurcations and catastrophes due to minor changes of micellar catalysis parameters are discussed. The effect of salts in the aqueous phase on parameter Q changes is stated. The study provides no experimental proof of the theory.

Liu et al. [1] introduced and experimentally calculated the value of the accelerating factor λ as a coefficient in the equation $(1+C_h v_h \lambda) = C_h$ (the function in brackets was presumed to be 'an apparent rate constant' equivalent to k_{app} in study [2]). Thus the parameter λ was regarded to be the limit of increment in the observed reaction rate at small increments of the lipophilic phase fraction (i.e. dH/dx for $x \to 0$ according to the terminology accepted in [2], see Table 2, column 3).

The derivative dH/dx was discussed earlier [2], but the reported equation is incorrect (see Table 2). In fact, for reaction (2) in the two-phase system, Eq. 4 can be transformed, if divided by k_2:

$$(k_{app}/k_2) = H = \frac{k_1 Q_{NO}^2 Q_{O_2} x + k_2(1-x)}{k_2(Q_{NO}x + 1-x)^2(Q_{O_2}x + 1-x)} =$$

$$\frac{(k_1/k_2) Q_{NO}^2 Q_{O_2} x + (1-x)^2}{(Q_{NO}x + 1-x)^2(Q_{O_2}x + 1-x)} \quad (5)^1$$

H appears to be a non-dimensional value showing how many times the observed reaction rate in the two-phase system will increase in comparison with the rate in homogeneous water solution. The first member of the numerator describes the reaction in the hydrophobic phase and is equivalent to $\lambda C_h v_h$ in [1]. The second member of the numerator describes the reaction in the water phase, and it was substituted by 1 in [1] because $X = C_h v_h$ had been considered very small. The denominator describes changes in concentrations of reagents in the water phase, due to their translocation to the hydrophobic phase in accordance with Q parameters. Only at very small fractions of the hydrophobic phase ($x \ll 1/Q$), each to the denominator's brackets is ≈ 1. In [1] this condition was violated initially ($x < 0.1$, $Q > 9$). Therefore, the equation, used by the authors for the calculation of parameters of the reaction acceleration caused by micellar catalysis [1], does not take into account the losses of the reagent concentrations in the aqueous phase due to the translocation and, thus, cannot be righteously applied.

The value of $H'(x)$ will be calculated according to the conventional equation for the quotient derivative $(u/v)' = (u'v - v'u)/v^2$:

$$(dH/dx) = \frac{(k_1/k_2) Q_{NO}^2 Q_{O_2} - 1}{(Q_{NO}x + 1-x)^2(Q_{O_2}x + 1-x)}$$

$$-2\frac{((k_1/k_2) Q_{NO}^2 Q_{O_2} x + 1-x)(Q_{NO}-1)}{(Q_{NO}x + 1-x)^3(Q_{O_2}x + 1-x)}$$

$$-\frac{((k_1/k_2) Q_{NO}^2 Q_{O_2} x + 1-x)(Q_{O_2}-1)}{(Q_{NO}x + 1-x)^2(Q_{O_2}x + 1-x)^2} \quad (6)$$

When $Q_{NO} = Q_{O_2} = Q$, $k_1 = k_2$

$$(dH/dx) = -\frac{(Q-1)^2(2xQ^2 - Q + 2Qx - 2 + 2x)}{(Qx + 1-x)^4} \quad (7)$$

For $x \to 0$ we obtain from Eq. 6

$$(k_1/k_2) Q_{NO}^2 Q_{O_2} - 2Q_{NO} - Q_{O_2} + 2 \quad (8)$$

We can ignore members 2–4 at larger Q and obtain the formula suggested in [1]. However, at small Q values the difference becomes significant. For instance, at $Q_{NO} = 9$, $Q_{O_2} = 3$ (which are the values accepted in [1]) the exact value of H (by Eq. 8) makes 224 and the approximate value 243, the error amounting to 8.5%.

The algorithm for determining the maximum for H at fixed Q values is given in [2]. In fact, by equating dH/dx (Eq. 5) to zero we obtain the value x_{max} at which H is a maximum. For instance, we find from Eq. 7:

$$x_{max} = \frac{1}{2}\frac{(Q+2)}{(Q^2 + Q + 1)} \quad (9)$$

We substitute the value obtained into Eq. 4 and calculate

$$H_{max} = \frac{4}{27}\frac{(Q^2 + Q + 1)^3}{Q^2(Q+1)^2} \quad (10)$$

The approximated value of H_{max}, presented in [2], can be obtained at very large Q values:

$$H_{max} \approx 4Q^2/27 \quad (11)$$

For instance, if $Q = 60$, H_{max} is 542.7 according to Eq. 10 and H_{max} is 533 according to Eq. 11, the error making only 2%. Study [2] shows an approximate equation for calculating H_{max} for Q_{NO} not equal to Q_{O_2}.

Thus, even at $x \to 0$ changes in the content of the aqueous phase can be ignored only at large Q. This grows more im-

[1] There is a misprint in Eq. 5 in [2]; in [5] this formula is correct.

Table 1
A list of symbols of [1], [2] and this article

	Liu et al. [1]	Gordin and Nedospasov [2]	This article[b]
Partition coefficient for NO	k_1/k_{-1}	Q_{NO}	Q_{NO}
Partition coefficient for O_2	k_3/k_{-3}	Q_{O_2}	Q_{O_2}
Rate constant for oxidation of NO in the aqueous phase	k_2	k_1, k_2^a	k_2
Rate constant for oxidation of NO in the hydrophobic phase	k_2'	k_1, k_2^a	$k_1^{(n)c}$
Concentration of the hydrophobic phase	C_h (in g/ml)	not used	C_h (in g/ml)
Partial specific volume of the hydrophobic phase	v_h (in ml/g)	not used	v_h (in ml/g)
Volume of the hydrophobic phase	not used	V_1	V_2, V_3,...
Volume of the water phase	not used	V_2	V_1, for an aqueous phase with the minimal Q_{NO}
Relative volume of the hydrophobic phase (the fraction of total volume that is in the hydrophobic phase)	$C_h v_h$	$x = V_1/(V_1+V_2)$	$x = V_2/(V_1+V_2)$ for two-phase media; $x = (\Sigma V_n - V_1)/(\Sigma V_n)$ is the relative volume of the sum of all hydrophobic phases for multi-phase media; $x' = V_2'/(\Sigma V_n - V_1)$ is the relative volume of the one of the hydrophobic phase for multi-phase media; $\Sigma(x',x'',...) = x$
Acceleration factor (this is a ratio of the reaction rates in the hydrophobic phase and in the aqueous phase of the same system	$\lambda = (k_1/k_{-1})^2 (k_3/k_{-3})(k_2'/k_2)$ (independent of x)	not used; in terms of (2) it will be $\lambda = (Q_{NO})^2 Q_{O_2} (k_1/k_2)$	$\lambda = (Q_{NO})^2 Q_{O_2} (k_1/k_2)$ for two-phase media; λ is not dH/dx ($x \to 0$)
Acceleration coefficient (this is a ratio of the total reaction rate in both phases and in homogenous medium (aqueous phase)	not used	$H = k_{app}/k_2$ is the function of Q_{NO}, Q_{O_2}, k_1, k_2 and x (see Table 2)	for two-phase media $H = k_{app}/k_2$ is the function of Q_{NO}, Q_{O_2}, k_1, k_2 and x; for multi-phase media H is the function of Q_{NO}, Q_{O_2}, k and V for each phase

[a]There are some misprints in symbols in [2].
[b]When it is improbable, we use the symbols of [2].
[c]The n is the total amount of phases.

possible if x considerably differs from zero. Due to the fast growth of the influence of the denominator in Eq. 5, the real H function assumes a maximum even at small x and starts diminishing at further growth of x.

Fig. 2 shows examples of surfaces, specified by Eq. 5. Since the number of independent variables in Eq. 5 is more than three, the common parameter Q is used for the partition coefficients, NO and O_2, with (k_1/k_2) presumed to be equal to 1. $Q_{NO} = Q_{O_2}$ in Fig. 2A, $Q = Q_{NO} = 4Q_{O_2} - 3$ in Fig. 2B (the authors of [1] counted Q_{NO} as 9 and Q_{O_2} as 3, and these parameters correspond to the upper bound of the green zone in Fig. 2B). In all cases, Q_{NO} values are plotted along the Q axis. A comparison of Fig. 2A and B shows that the general picture of the dependence changes very little at changed Q_{NO}/Q_{O_2}, but in the second case H values appear to be lower, as was expected from Eqs. 5 and 10. The red line in the figures denotes the top of the crest, where $dH/dx = 0$ at preset Q: at growing x, H values grow on the left of the line and decrease on the right of it. Fig. 2C shows on a larger scale the area of small Q which was studied by the authors of [1], the blue line marking the dependence $H = f(x)$ at $Q_{NO} = 9$, $Q_{O_2} = 3$.

As shown in Fig. 2C and D, this dependence has a maximum at $x < 0.1$ (the intersection point of the red and the blue lines in Fig. 2C ($Q_{NO} = 9$, $Q_{O_2} = 3$), $H_{max} = 6.532$ at $x = 0.0848$. The $H(x)$ dependence curve (fold increase in the total reaction) shown by the authors in Fig. 2D was borrowed from Fig. 4 in [1]. The method of plotting the curve had not been described in [1].

The description of the experimental results of study [1] allows no real values of x_{max} and H_{max} to be established for the studied systems. Only data for $0 < x < 0.003$ applied to eight investigated lipid phases and to one point at $x = 0.04$ for liposomes (as is evident from the text, H_{max} is at least ≥ 13 or $= 13$ in the latter case) are presented. As it can be seen from our analysis (see Fig. 2D), the maximum acceleration of the reaction at $Q = 9$ must be observed at $x_{max} < 0.1$, which contradicts the authors' conclusions (Fig. 2D shows that curve 2 grows at $x = 0.1$).

In study [1], the points of the area $0 < x < 0.003$ virtually specify the value of the derivative dH/dx. As is clear from Eq. 6, the decrease of Q values may be compensated by an increase of (k_1/k_2) (the λ parameter in [1] also included the product $(k_1/k_2)Q_{NO}^2 Q_{O_2}$). However, for the curve $H(x)$ to assume the maximum at $x \gg 0.1$ and to pass across the point $x = 0.04$, $H = 13.3$ quoted by the authors of [1], Q_{NO} and/or Q_{O_2} must be extremely low according to Eq. 6. For instance, the set $(k_1/k_2) = 18.5$, $Q_{NO} = 3$, $Q_{O_2} = 2.3$ meets the condition, but these values disagree with the well-known experimental data and with the statements of the authors of [1].

Thus, the experiment described in study [1] is not in agreement either with the formula with the λ parameter from [1] or with Eq. 5 and, in other words, with the model of micellar catalysis in a two-phase system.

It is, however, unreasonable to state that dependencies of the curve 2 type cannot be realized at $Q > 9$. In fact, Eq. 5 can be applied to two-phase systems only, and the authors of [2] also considered them in the formation of the concept of NO catastrophes. In the case of multi-phase system, Eq. 4 is operative (the authors of study [2] stressed that the substitution of Q by an average value is not correct for a multi-phase system). If a system includes a number of hydrophobic phases that differ by Q value for NO and/or O_2, the initial portion of H growth at small x will be to a larger extent determined by the phases with higher Q values because these phases' contribution will be the most significant in dH/dx at $x \to 0$. On the contrary, the location of the maximum point of x_{max} ($dH/dx = 0$) is determined by all phases, including those with lower

Table 2
Used formulas and parameters in [1], [2] and this article

	Liu et al. [1]	Gordin and Nedospasov [2]	This article[a]
Number of the phases	2	≥ 2	≥ 2
H (fold increase in total reaction); formula	unknown; for small x used $H = xQ_{NO}^2 Q_{O_2} k_1/k_2 + 1$[b]	$H = (k_{app}/k_2) = \dfrac{k_1 Q_{NO}^2 Q_{O_2} x + k_2(1+x)}{(Q_{NO}x + 1 + x)^2 (Q_{O_2}x + 1 + x)}$[c]	$(k_{app}/k_2) = \dfrac{k_1 Q_{NO}^2 Q_{O_2} x + k_2(1-x)}{(Q_{NO}x + 1 - x)^2 (Q_{O_2}x + 1 - x)}$
dH/dx; formula	not used	not used	Eq. 6
dH/dx ($x \to 0$); formula	$Q_{NO}^2 Q_{O_2} k_1/k_2$	$Q_{NO}^2 Q_{O_2}(p + q + 1) = 4Q_{NO}^2 Q_{O_2}$[d]	$(k_1/k_2) Q_{NO}^2 Q_{O_2} - 2Q_{NO} - Q_{O_2} + 2$
Q_{NO}	9	≤ 70	may be as high as 70 in concentrated salt solutions
Q_{O_2}	3	? ($Q_{NO} \approx Q_{O_2}$)	may be high in concentrated salt solutions
H	≈ 16?	≤ 1000	for multi-phase media H is a relative parameter (see text)

[a] When it is improbable, we use the symbols of [2].
[b] Acceleration relative to the rate in the aqueous phase in the same heterogeneous system.
[c] Acceleration relative to the rate in the homogeneous system.
[d] Misprint.

values of Q, since the translocation of the reagents from phase to phase (as well as the corresponding growth of the denominator of Eq. 4) with increasing x proceeds with no interruption, although its contribution becomes visible only at x exceeding $1/Q$ (see above).

Correspondingly, the deduction of [2] about the existence of a narrow resonance-like maximum of the function $H(Q,x)$ is strictly applicable to only two-phase systems. Fig. 3 shows $H(x)$ dependencies for a two-phase system (curves 1–3) and a three-phase system (curves 4–6) with two hydrophobic phases at $Q_{NO} = Q_{O_2} = Q$. In the case of two-phase systems ($Q = 20$, 40, 60), the x_{max} value shifts to 0 with Q growth, H_{max} grows approximately like the square of Q does in accordance with Eq. 11, and the area of x with high H values (the bulk of the peak in Fig. 2A) decreases more and more. For a three-phase system, however, when the fractions of the phases with $Q = 20$ and $Q = 60$ are equal and the total fraction of the hydrophobic phases equals to the fraction of the hydrophobic phase in the two-phase system ($x_1^{(3)} + x_1'^{(3)} = x_1^{(2)}$ where the figures in brackets are the total amount of phases): curve 5, the position of x_{max} considerably shifts to the right and H_{max} is much higher than in a two-phase system with the same average values of Q (curve 2). Accordingly, the width of the ridge considerably grows.

Curves 6 and 4 show the $H(x)$ dependence to change upon substituting a small portion of the hydrophobic phase in the two-phase system by a third hydrophobic phase with a different value of Q (the initial sum total value of x being retained). Substitution of 10% of the phase with $Q = 60$ for the third phase with $Q = 20$ (curve 6) changes weakly the type of dependence in the area of low x shown in the figure (compare curves 3 and 6). In the contrary, basic changes were observed upon substitution of 10% of the hydrophobic phase with $Q = 20$ by a new phase with $Q = 60$ (curve 4) especially at low x typical for systems in vivo. Namely, the H_{max} value grew three times (from 62.7 at $x = 0.0262$ to 185.8 at $x = 0.0217$).

In two-phase systems, Q values always determine not only H but also dH/dx for any x value. If the number of phases is more than two, systems with equal values of H and x but different dH/dx values can be formed by combining the phases (for instance, M and N are the intersection points of curve 6 with curves 5 and 4 in Fig. 3).

The authors of [2] mention three types of possible NO catastrophes: (1) transformation of a homogeneous medium into a heterogeneous one when through small deviations of Q or x the dH/dx value changes abruptly, (2) a sign change of dH/dx (intersection of the red line in Fig. 2A, B), (3) catastrophes in NO-dependent systems of regulation when dramatic H changes take place as a result of small changes of Q or x. In the light of the above discussed, two more types emerge: (4) change of the number of phases and/or (5) change of their relative fractions in multi-phase systems. It is clear that changing the dH/dx value provides a method to modify the system's resistance to NO catastrophes. Multiple phase state allows its modification without changes in H.

In study [1], experimental examples were observed that corresponded to the first type catastrophes, involving soap-like substances with the critical concentration of the micellar formation (CCM) considerably differing from 0; at small concentrations H is practically concentration independent (at $C_h < CCM$, $x = 0$). Raising CCM values leads to a jump-like

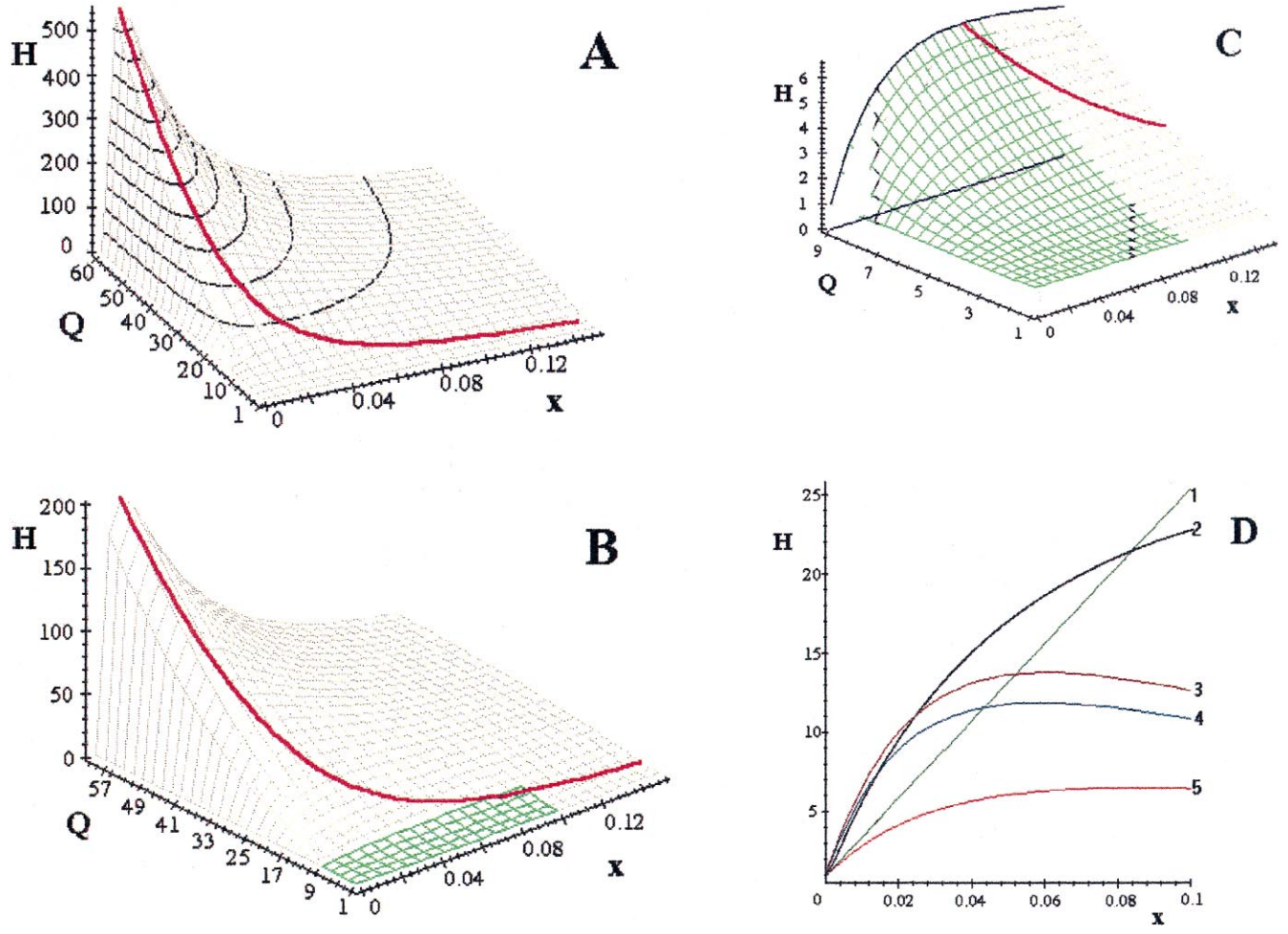

Fig. 2. The dependence of reaction acceleration

$$\left(H = k_{app}/k_2 = \frac{Q_{NO}^2 Q_{O_2}\, x + 1 - x}{(Q_{NO}x + 1 - x)^2\,(Q_{O_2}x + 1 - x)},\ k_1 = k_2\right)$$

on the partition coefficients (Q) and on the lipophilic phase fraction in the total volume ($x = V_{lip}/(V_{lip}+V_{aq})$) as a result of micellar catalysis. The solid red line in A–C is the locus of $H(x)$ maxima at fixed Q. A: $Q = Q_{NO} = Q_{O_2}$. H intersections (black) pass across 50. For instance, at $Q = 60$ the maximum H value 542.5 (the upper point on the curve) is obtained at $x = 0.00847$ (see the text for calculations). The efficiency of the catalysis diminishes with further x growth. B: $Q = Q_{NO} = 4Q_{O_2} - 3$. At $Q_{NO} = 60$, $Q_{O_2} = 15.75$ and $H_{max} = 198.5$. Thus, a considerable decrease of Q_{O_2} as compared with Q_{NO} does not practically change the overall view of the surface, but the H_{max} value decreases. C shows the green region on a larger scale. C: The dependence of the $H(Q,x)$ reaction acceleration at minor Q and x. $Q = Q_{NO} = 4Q_{O_2} - 3$. The blue line on the $H(Q,x)$ surface at $Q_{NO} = 9$, $Q_{O_2} = 3$ (the parameters used in [1]) is numbered 5 in D. The line of H_{max} (red) intersects the blue line at $x < 0.1$. The H value diminishes with further rise of x. D: The dependence of H on the lipophilic phase fraction in the total volume (x). Straight line 1 corresponds to the dependence $1 + C_h V_h \lambda = x Q_{NO}^2 Q_{O_2} + 1$, at $k_2 = k_2'$, $Q_{NO} = 9$, $Q_{O_2} = 3$ (compare it with Fig. 3B in [1]). Curve 2 marks the fold increase in the total reaction (Fig. 4 in [1]). The manner of curve construction was not identified in [1], and the curve was plotted by dots. Curves 3–5 are the graphs of $H(x)$ functions at different Q. Curve 3 ($Q_{NO} = Q_{O_2} = 9$) corresponds to the intersection at $Q = 9$ in A. Curve 4 ($Q_{NO} = 13$, $Q_{O_2} = 4$) corresponds to the intersection at $Q = 13$ in B. For these Q values, the initial portion is similar to curve 2. Curve 5 was plotted for $Q_{NO} = 9$, $Q_{O_2} = 3$, and even at small Q accepted in [1] the maximum falls within the 0–0.1 interval.

change of dH/dx, and further increase of C_h results in a fast growth of H ($C_h >$ CCM, $dH/dx \gg 0$).

It is clear that the number of liberty degrees grows owing to the growth of the number of independent variables during a transition from a two-phase model to multi-phase ones. For instance, in the experiment of study [1], which is difficult for description even in terms of a two-phase model, even a three-phase model (two hydrophobic phases with different Q) yielded a great deal of solutions, Fig. 4 showing four of them.

The four systems were formed by way of varying Q_{NO} values and selecting suitable ratios of second and third phase fractions (Q_{O_2} remained unchanged in all cases in the second and third phases). In real in vivo systems, the number of both hydrophobic and hydrophilic phases is much more than 1. In fact, salt concentrations in aqueous phases on both sides of membranes in vivo are different and, hence, the solubility of reagents is different there. The radius of curvature participates in the free energy equation for colloid size particles, and, therefore, the aqueous phase within and outside the spherical membrane shows different properties even in the absence of salts. The formation of the third phase was experimentally observed in various artificial systems [17–19], including systems similar to those studied in [1]. Membranes' compositions are different; not only membranes' but lipoproteins' micelle

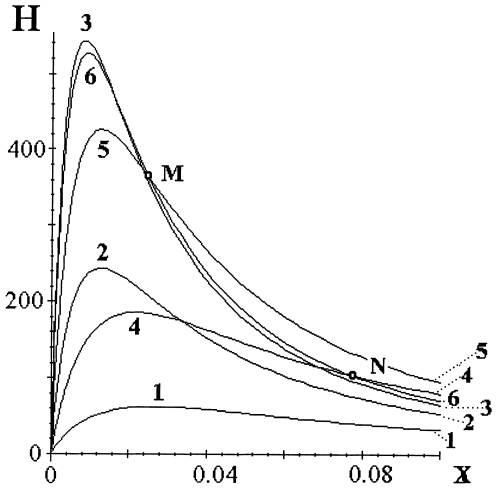

Fig. 3. The dependence of reaction acceleration (H) on the volume fraction of hydrophobic phases (x) for two-phase (curves 1, 2, 3) and three-phase (curves 4, 5, 6) systems. $Q_{NO} = Q_{O_2} = Q$. Curve 1: $Q = 20$. Curve 2: $Q = 40$. Curve 3: $Q = 60$. Curves 4–6: In phase 2, $Q = 20$, in phase 3, $Q = 60$. Curve 4: The phase volume ratio $V_2/V_3 = 9$. Curve 5: The same as in 4, $V_2/V_3 = 1.6$. Curve 6: The same as in 5. $V_2/V_3 = 1/9$. The curve numbers on the left of the figure show the positions of H_{max}. M is the intersection point of curves 5 and 6. N is the intersection point of curves 4 and 6.

and interior globules of proteins etc. form hydrophobic phases. Values of the partition coefficients Q, that we used, had been initially taken from two-phase systems, showing how many times better the reagent is dissolved in the hydrophobic phase than in the aqueous phase. And how to deal with a situation when both phases are not one but several? It seems reasonable to agree upon using a certain first phase as a 'reference one', like in study [2], and to express the concentrations of reagents in other phases (both hydrophobic and hydrophilic) relative to it. If we want Q to be always more than 1, this aqueous phase must be a phase in which the reagents are less soluble, i.e. it should not be water but an aqueous phase with a maximal content of salts (because of the salting out the solubility of gases in water will drop).

The experimental data hitherto published are insufficient to account for the origin of 'multi-phaseness' in [1], and investigating the $H(x)$ dependence at large x, when the contribution of phases with low Q values, H changes depending on the composition of buffer solution and the degree of dispersion and homogeneity of the studied colloidal systems are manifested, seems expedient.

Table 2 shows that the considerable differences between [1] and [2] are also due to the values of accepted Q parameters. The authors quote Shaw and Vosper's experiments on NO solubility in aqueous and non-aqueous media [20], data of [21] by Malinski et al. who determined the value $Q_{NO} = 8$ in an octanol–water system and a study by Wood and Gartwaite [22]. The authors of [2] maintain that enhancement of Q can be achieved by increasing the concentration of electrolytes in the aqueous phase. Our calculations, however, show that the salt concentration needed for a substantial increase of Q should be rather high. And in fact, the solubility of gases in aqueous solutions of electrolytes can be approximately described by law: $\log(C_o/C) = K_s C_s$ where C_o and C are the solubility of gas in water and in water solution of electrolytes, respectively, C_s is concentration of the electrolyte. When the added salt only dissolves in the aqueous phase, it virtually exerts no effect on the solubility of gas in lipid. Thus, Q grows exponentially with C_s growth. For instance, K_s for an oxygen/NaCl aqueous solution system was calculated from data on gas solubility in water and NaCl water solution. According to these estimates, a ten-fold growth of Q values can be expected in 2 M NaCl solution. Note that the added salt not only decreases gas solubility but also the solubility in water of the lipophilic phase compound. Consequently, the composition of the aqueous phase becomes more 'hydrophilic' and, at least for some compounds, the dependence may be steeper.

Thus, the carried out analysis showed that micellar catalysis is really able to considerably accelerate the oxidation of NO by oxygen, thus regulating the NO pool and NO-dependent processes both in norm and in pathologies. The contradictions observed between theory and experimentation are associated with the fact that the theoretical two-phase system differs essentially from the artificial systems studied in [1] and from

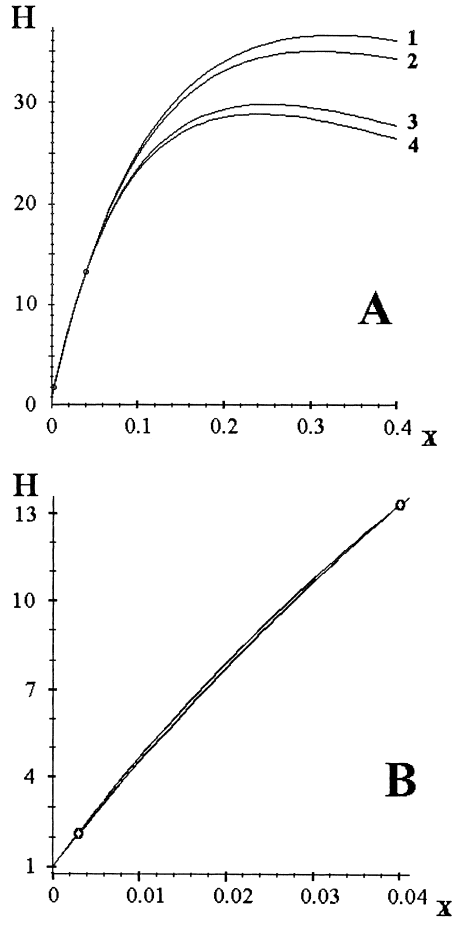

Fig. 4. A majority of curves can be plotted in a three-phase model through the experimental points $x = 0.003$ and $x = 0.04$, marked by circles [1]. In B, the initial portion is given on a large scale. The composition of hydrophobic phases for curves 1–4:

	Phase 2		Phase 3		$V_2/(V_2+V_3)$
	Q_{NO}	Q_{O_2}	Q_{NO}	Q_{O_2}	
1	2.3	2.3	60	20.5	0.995115
2	2.3	2.3	45	20.5	0.991221
3	3.0	2.3	60	20.5	0.994920
4	3.0	2.3	45	20.5	0.990910

all realistic systems in vivo, in which the number of different phases involved in the rearrangement of concentrations of NO and oxygen is much more than two. Within the boundaries of micellar catalysis in multi-phase systems, it is not only possible to explain available experimental data but also to predict the paths of directed influence on NO metabolism, either creating opportunities for realization of conditions for NO catastrophes or blocking them. With this in view, multi-phase state of a medium in vivo is the most important factor of the stability of living systems.

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