Effects of domain connection and disconnection on the yields of in-plane bimolecular reactions in membranes

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ABSTRACT It has recently been shown (Vaz, W. L. C., E. C. C. Melo, and T. E. Thompson. 1989. *Biophys. J.* 56:869–875; 1990. *Biophys. J.* 58:273–275) that in lipid bilayer membranes in which ordered and disordered phases coexist, the ordered phase can form a two-dimensional reticular structure that subdivides the coexisting disordered phase into a disconnected domain structure. Here we consider theoretically the yields of bimolecular reactions between membrane-localized reactants, when both the reactants and products are confined to the disordered phase. It is shown that compartmentalization of reactants in disconnected domains can lead to significant reductions in reaction yields. The reduction in yield was calculated for classical bimolecular processes and for enzyme-catalyzed reactions. These ideas can be used to explain certain experimental observations.

INTRODUCTION

Biological membranes are made up of a lipid bilayer, and integral and surface-attached proteins. The lipid bilayer is viewed as a quasi-two-dimensional fluid-like sheet in which lateral diffusion and redistribution of components occurs (Singer and Nicholson, 1972). This lateral diffusion permits molecular reactions and interactions of physiological importance to occur in the plane of a membrane such as the mitochondrial inner membrane (Lenaz, 1988; Chazotte and Hackenbrock, 1989; Rajarathnam et al., 1989).

The lipid bilayer is a complex mixture of lipids that may vary significantly from each other in their chemical and physical properties. In such mixtures, phase separations leading to domain formation are possible (Wu and McConnell, 1975; Lee, 1977; Tocanne et al., 1989). Phase separation is dependent upon temperature, pressure, chemical composition, and for membranes with charged lipids, also upon the pH and ionic strength of the aqueous medium. Phase separation implies nonhomogeneous component distributions not only of the lipids but also of the membrane proteins, which may prefer one phase over another. For the case of reacting protein or lipid species, nonhomogeneous distributions can significantly affect reaction yields.

We have recently shown (Vaz et al., 1989, 1990; Bultmann et al., 1991) that membranes in which ordered and disordered phases coexist have a domain structure in which a small mass fraction of ordered phase forms a reticulum that subdivides the disordered phase into disconnected domains. It has been shown (Vaz et al., 1989; Bultmann et al., 1991) that the mass fraction of the ordered phase required to achieve this subdivision can be as low as 20% of the system.

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Here we consider specifically the effects of reticulation upon the reaction yield of bimolecular reactions involving membrane-bound reactants. From the standpoint of biological systems the effects of reticulation are twofold: (*a*) if the existence of reticulation is unknown to the outside observer, measurements of the reaction system can lead to incorrect thermodynamic parameters; (*b*) if the cell can by metabolic means move the membrane domain structure back and forth across the percolation threshold to connect or disconnect a particular phase, the cell can control the extent of membrane-localized reactions confined to that phase. The general problem concerns reactions occurring in a compartmentalized space and is, in principle, also applicable to reactions in systems with phase separations involving disordered phases only, but with an absolute solubility preference of the reactants for one phase over the other (Hatlee and Kozack, 1980).

Recently we have examined from a theoretical standpoint the effect of compartmentalization on a membrane-confined homodimerization reaction (Thompson et al., 1992). The ideas developed in this paper have been applied in an experimental analysis of the concentration dependence of electron spin resonance (ESR) line shapes of a phosphatidylcholine spin label in two-component, two-phase phosphatidylcholine bilayers (Sankaram et al., 1992). In the present manuscript, we extend the theoretical analysis to include more complex in-plane reaction systems.

THEORY

In general when reactants are randomly distributed over a compartmentalized system, the product yield will be lower than when the reactants are dispersed in a continuous system. The yield decreases as the average number of reactant molecules per compartment decreases. As a measure of the extent to which a reaction is hindered by reticulation of the membrane, let us define the expected relative yield,

$$\langle \varphi \rangle = \frac{\langle \Phi_{\mathrm{ret}} \rangle}{\langle \Phi_{\mathrm{cont}} \rangle}, \qquad (1)$$

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as the ratio of the yield expected to be achieved in the reticulated membrane, $\langle \Phi_{ret} \rangle$, to the yield expected to be achieved in a continuous one, $\langle \Phi_{cont} \rangle$. The yield or net yield, $\langle \Phi \rangle$ is defined as the final number of product molecules per compartment / the initial number of reactant molecules per compartment.

We model the reticulated membrane as a pattern of two-dimensional fluid domains that, regardless of shape, have the same area. Moreover, we consider that the barriers between domains cannot be crossed by the reactant molecules so that there is no exchange of reactants between reaction compartments. For the sake of simplicity, we assume point molecules. We make a further simplifying assumption that the reactions are not reversible so that the chemical processes within a compartment proceed until at least one of the reactants is fully consumed. It must be stressed that we do not consider the kinetics of the reactions but only their yield, so that neither the dimension nor the shape of the domains (two-dimensional vessels) needs to be taken into account. The lifetime of disconnected domains is assumed to be much longer than the time required to attain complete reaction.

In practice, we deal with dispersions of cells or lipid vesicles in which the reaction space is the lipid bilayer membrane. Both are *a priori* compartmentalized reaction systems. Reticulation of the membrane superimposes a secondary compartment pattern on this already discrete reaction space. We are concerned here only with the secondary compartmentalization that makes the single vesicle or cell, which we shall refer to as the "unit," our point of reference. We consider that encounters between all reactant molecules in a single nonreticulated unit are possible, whereas in the reticulated unit only reactions within single domains will take place. In both cases, the number, i, of product molecules, **P**, formed in a given reaction compartment is a function of the number of reactant molecules present in that compartment. It must be stressed that all the units are assumed equivalent and, in the absence of reticulation, reaction compartments (domains) and units are indistinguishable. Since, in our model, the reticulum divides each unit into domains of equal area, upon reticulation all reaction compartments remain equivalent. In both cases the available reactant molecules, **R** (**R** = **R1**, **R2**, $\cdots$), are distributed randomly in the reaction compartments. As a consequence, the observed yield is the mean value taken over the entire set of compartments.

In the homogeneous membrane, a concentration $[\mathbf{P}]_{cont}$ of product molecules results after completion of the reaction. For the reticulated case the final concentration of the product is $[\mathbf{P}]_{ret}$. Therefore, Eq. 1 may be rewritten

$$\langle \varphi \rangle = \frac{[\mathbf{P}]_{ret}}{[\mathbf{P}]_{cont}}. \tag{2}$$

Let us denote the unit $\mathcal{U}$ and the domain by $\mathcal{D}$. Hence, the expected concentration of product molecules is given by

$$[\mathbf{P}] = [\mathcal{D}] \sum_i i\mathcal{P}_P(i), \tag{3}$$

where $\mathcal{P}_P(i)$ stands for the probability of obtaining i product molecules in a domain. Recalling that in the nonreticulated situation the domain extends over the entire unit, the expected relative yield when each unit is divided into N_{dom} domains is

$$\langle \varphi \rangle = N_{dom} \frac{\sum_i i\mathcal{P}_P(i, N_{dom})}{\sum_i i\mathcal{P}_P(i, N_{dom} = 1)}. \tag{4}$$

The probability

$$\mathcal{P}_P(i, N_{dom}) = \sum_{n_{R1}} \cdots \sum_{n_{Rm}} \mathcal{P}(i|n_{R1}, \ldots, n_{Rm}) \times \mathcal{P}_{R1\ldots Rm}(n_{R1}, \ldots, n_{Rm}, N_{dom}) \tag{5}$$

depends on both the kind of reaction and the distribution of the reactant molecules **R** (**R** = **R1**, ..., **R**m) among the domains. In Eq. 5 the conditional probability $\mathcal{P}(i|n_{R1}, \ldots, n_{Rm})$ accounts for the mechanism of the reaction, while $\mathcal{P}_{R1\ldots Rm}(n_{R1}, \ldots, n_{Rm}, N_{dom})$ represents the joint probability of having respectively $n_{R1}, \ldots, n_{Rm}$ reactant molecules, **R1**, ..., **R**m, in a domain. For a bimolecular reaction, if the distributions of the reactant molecules (**R1** and **R2**), $\mathcal{P}_{R1}(n_{R1}, N_{dom})$ and $\mathcal{P}_{R2}(n_{R2}, N_{dom})$, are assumed to be mutually independent, Eq. 5 becomes

$$\mathcal{P}_P(i, N_{dom}) = \sum_{n_{R1}} \sum_{n_{R2}} \mathcal{P}(i|n_{R1}, n_{R2})\mathcal{P}_{R1}(n_{R1}, N_{dom})\mathcal{P}_{R2}(n_{R2}, N_{dom}). \tag{6}$$

Provided that a large number of reactant molecules are distributed over a large number of units without crowding effects, the distribution of reactant molecules over the different units follows a Poisson distribution law

$$\mathcal{P}_R(n) = \frac{\mu^n}{n!} e^{-\mu}. \tag{7}$$

The mean number of reactant molecules per reaction compartment, μ, is either $\mu = [\mathbf{R}]/[\mathcal{U}]$ for the nonreticulated case or $\mu = [\mathbf{R}]/([\mathcal{U}]N_{dom})$ when there is reticulation.

In the next section, the above formalism is applied to four important reaction mechanisms.

CASE STUDIES

We confine ourselves to the study of four cases: (*a*) a Michaelis–Menten type enzymatic reaction, (*b*) a conventional bimolecular reaction, (*c*) a dimerization reaction, and (*d*) consecutive reactions consisting of two sequential Michaelis–Menten type steps.

Enzymatic reaction

We consider a reaction in which an enzyme **E** catalyzes the transformation of a substrate **R** into a product **P**:

$$\mathbf{E} + \mathbf{R} \rightarrow \mathbf{E} + \mathbf{P}.$$

While in a homogeneous medium a minute amount of **E** is enough to cause a complete conversion of **R** to **P**, in a compartmentalized medium the yield will depend upon the probability of having at least one molecule of **E** in every compartment. Under certain conditions this can lead to a drastic decrease in the yield of **P**. The conditional probability that reaction takes place in a given domain with a net yield of i molecules of **P**, given that n_E molecules of enzyme and n_R molecules of reactant are present, is given by

$$\mathcal{P}(i|n_E, n_R) = \begin{cases} 1 & n_E = 0, \quad i = 0 \\ 1 & n_E \neq 0, \quad i = n_R. \\ 0 & \text{elsewhere} \end{cases} \tag{8}$$

Substitution of Eq. 7 and Eq. 8 in Eq. 6 leads to the relative yield

$$\langle \varphi \rangle = \frac{1 - \exp\left\{-\frac{[\mathbf{E}]}{[\mathcal{U}]N_{dom}}\right\}}{1 - \exp\left\{-\frac{[\mathbf{E}]}{[\mathcal{U}]}\right\}}. \tag{9}$$

It should be noted that, in this case, the relative yield, $\langle\varphi\rangle$, does not depend on the concentration of R, $[\mathbf{R}]$. In particular, it represents the ratio between the probabilities of finding at least one molecule of **E** in a given reaction compartment for both the reticulated and the nonreticulated situations. Since, in these compartments, all **R** molecules that can come in contact with an **E** molecule are converted, the expected net yield is, in both cases, proportional to the mean number of **R** molecules per compartment.

Fig. 1 presents a plot of $\langle\varphi\rangle$ versus N_{dom}. As expected, a sharp decrease in the relative yield of the reaction is observed upon reticulation when the number of enzyme molecules per reaction compartment is small. In fact, as a result of compartmentalization a large fraction of **R** molecules are not accessible to the enzyme, **E**.

In addition to the relative reaction yield, it is also important to evaluate how the net yield of **P** changes with $[\mathbf{E}]$ in the reticulated reaction space. In this case the net yield of **P** is given by (numerator of Eq. 9)

$$\langle\Phi_{\text{ret}}\rangle = 1 - \exp\left\{-\frac{[\mathbf{E}]}{[\mathcal{U}]N_{\text{dom}}}\right\}. \tag{10}$$

Fig. 2 presents a plot of $\langle\Phi_{\text{ret}}\rangle$ versus $[\mathbf{E}]/[\mathcal{U}]$ for several states of reticulation. The difference between the curves is simply a consequence of the change in the average value of $[\mathbf{E}]$ in the reaction compartment resulting from differences in the degree of compartmentalization. Hence the curves in Fig. 2 actually represent the same curve under different concentration scaling.

CONVENTIONAL BIMOLECULAR REACTION

In the case of the bimolecular reaction

$$\mathbf{R1} + \mathbf{R2} \rightarrow \mathbf{P},$$

the conditional probability $\mathcal{P}(i|n_{R1}, n_{R2})$ that describes the reaction mechanism is now given by

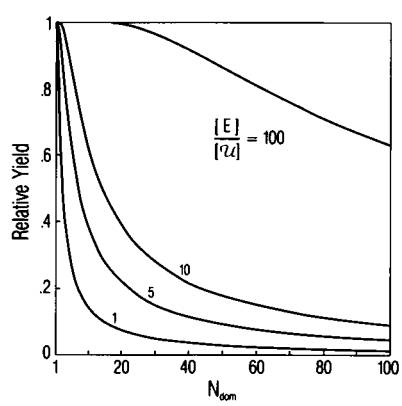

FIGURE 1 Relative yield of product formation for a catalyzed reaction as a function of the number of domains per reaction unit. The different curves correspond to varying catalyst concentrations, $[\mathbf{E}]/[\mathcal{U}] = 1, 5, 10$, and 100.

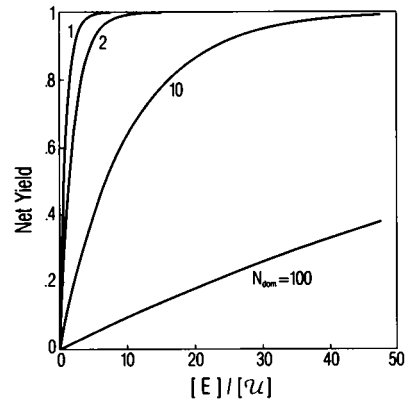

FIGURE 2 Net yield of a catalyzed reaction as a function of the catalyst concentration for values of $N_{\text{dom}} = 1, 2, 10$, and 100.

$$\mathcal{P}(i|n_{R1}, n_{R2}) = \begin{cases} 1 & n_{R1} \le n_{R2}, \quad n_{R1} = i \\ 1 & n_{R1} > n_{R2}, \quad n_{R2} = i. \\ 0 & \text{elsewhere} \end{cases} \tag{11}$$

As in the previous case, the relative yield is obtained as the ratio between two probabilities, i.e.,

$$\langle\varphi\rangle = \frac{1 - f\left(\frac{[\mathbf{R1}]}{[\mathcal{U}]N_{\text{dom}}}, \frac{[\mathbf{R2}]}{[\mathcal{U}]N_{\text{dom}}}\right)}{1 - f\left(\frac{[\mathbf{R1}]}{[\mathcal{U}]}, \frac{[\mathbf{R2}]}{[\mathcal{U}]}\right)} \tag{12}$$

where

$$f(\mu_{R1}, \mu_{R2}) = \exp[-(\mu_{R1} + \mu_{R2})] \times \sum_{n_{R1}=0}^{\infty} \sum_{n_{R2}=0}^{n_{R1}-1} (n_{R1} - n_{R2}) \frac{\mu_{R2}^{n_{R2}}}{n_{R2}!} \frac{\mu_{R1}^{n_{R1}-1}}{n_{R1}!}. \tag{13}$$

In Eq. 13, $f(\mu_{R1}, \mu_{R2})$ stands for the probability that no reaction takes place in the whole system.

Fig. 3 shows that for a fixed value of $[\mathbf{R2}]/[\mathcal{U}]$, the

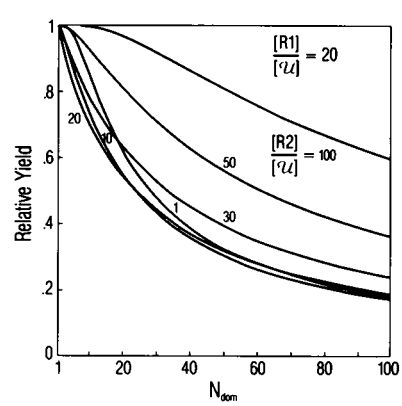

FIGURE 3 Relative yield of a conventional bimolecular reaction as a function of compartmentalization for a fixed concentration of one of the reactants. $[\mathbf{R1}]/[\mathcal{U}]$ is set as 20, while $[\mathbf{R2}]/[\mathcal{U}] = 1, 10, 20, 30, 50$, and 100.

decrease in reaction yield of the simple bimolecular reaction considered here is somewhat less than that observed for the enzyme catalyzed reaction considered earlier (Figs. 1 and 2). Another result of the calculation is that the relative reaction yield is not a monotonic function of [**R1**]. This effect is more clearly visible when we analyze the evolution of the net reaction yield

$$\langle \Phi_{\text{ret}} \rangle = \begin{cases} 1 - f\left(\dfrac{[\mathbf{R1}]}{[\mathcal{U}]N_{\text{dom}}}, \dfrac{[\mathbf{R2}]}{[\mathcal{U}]N_{\text{dom}}}\right); & \text{if } \dfrac{[\mathbf{R1}]}{[\mathcal{U}]} \le \dfrac{[\mathbf{R2}]}{\mathcal{U}]} \\ 1 - f\left(\dfrac{[\mathbf{R2}]}{[\mathcal{U}]N_{\text{dom}}}, \dfrac{[\mathbf{R1}]}{[\mathcal{U}]N_{\text{dom}}}\right); & \text{if } \dfrac{[\mathbf{R1}]}{[\mathcal{U}]} > \dfrac{[\mathbf{R2}]}{[\mathcal{U}]} \end{cases}, \tag{14}$$

with $f(\cdot, \cdot)$ given in Eq. 13. In Fig. 4 we plot the net yield, $\langle \Phi_{\text{ret}} \rangle$, with respect to [**R1**]/[$\mathcal{U}$] for a given value of [**R2**]/[$\mathcal{U}$]. We see that the net yield exhibits a minimum at [**R1**] = [**R2**]. The meaning of this minimum is easy to understand if extreme cases, and a simplified scenario, are considered. If the number of **R1** molecules in a unit is much smaller than the number of **R2** molecules in it, the probability that upon reticulation an **R1** molecule is in the presence of at least one **R2** molecule in the same compartment is relatively high. Conversely, if the number of **R1** molecules per unit is much larger than the number of **R2** molecules, upon reticulation a large fraction of the **R2** molecules are expected to react. If the unit is populated with an equal number of **R1** and **R2** molecules, in the absence of reticulation reaction is expected to go to completion. However, reticulation will result in a certain fraction of reaction compartments (domains) which have unequal number of **R1** and **R2** molecules so that the net result will be a significant fraction of unreacted reactants.

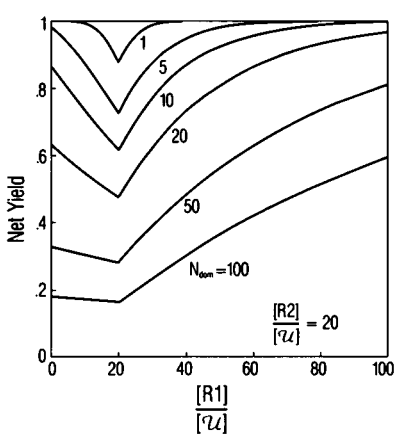

FIGURE 4 Net reaction yield for a conventional bimolecular reaction as a function of [**R1**]/[$\mathcal{U}$], for [**R2**]/[$\mathcal{U}$] = 20, and N_{dom} = 1, 5, 10, 20, 50, and 100.

Dimerization reaction

Here we deal not only with the simple bimolecular reaction

$$2\mathbf{R} \rightarrow \mathbf{R}_2$$

but we also compare it to the more general case of polymer formation reactions for the cases of aggregation of 3 and 4 monomers

$$k\mathbf{R} \rightarrow \mathbf{R}_k \quad (k = 2, 3, \text{and } 4).$$

If, in homogeneous media, a dimerization reaction proceeds until total consumption of the monomer, upon reticulation every unpaired molecule will be lost for the process, with a concomitant decrease in yield. This drop in yield is still more marked in the case of the formation of trimers or tetramers.

The conditional probability that the reaction takes place in a given domain with a net yield of i molecules of $\mathbf{R}_k$, given that n_R molecules are present, is given by

$$\mathcal{P}(i|n_R) = \begin{cases} 1 \;\; i = n_R/k, & n_R \text{ multiple of } k \\ 1 \;\; i = (n_R - 1)/k, & (n_R - 1) \text{ multiple of } k \\ \vdots \qquad \vdots & \qquad \vdots \\ 1 \;\; i = (n_R - k + 1)/k, & (n_R - k + 1) \text{ multiple of } k \\ 0 \;\; \text{elsewhere} \end{cases} \tag{15}$$

Taking into consideration that, for these reactions, all the involved molecules pertain to the same reactant, the relative yield is

$$\langle \varphi \rangle = \frac{1 - f\left(\dfrac{[\mathbf{R}]}{[\mathcal{U}]N_{\text{dom}}}\right)}{1 - f\left(\dfrac{[\mathbf{R}]}{[\mathcal{U}]}\right)} \tag{16}$$

where

$$f(\mu_R) = \exp(-\mu_R) \sum_{j_1=0}^{\infty} \sum_{j_2=0}^{k-1} j_2 \frac{\mu_R^{(kj_1+j_2-1)}}{(kj_1 + j_2)!}. \tag{17}$$

In Fig. 5 we present plots of the relative yield of formation of dimer, trimer and tetramer for a single concentration of **R**, [**R**]/[$\mathcal{U}$] = 20, as a function of the number of domains. The differences in yield for dimers and higher aggregation numbers are clearly observed.

From the study of the relative yield we can find the extent of hindrance resulting from membrane reticulation. The primary reticulation effect, which is always present in vesicles or cell suspensions, is better dealt with through examination of the net yield of the reaction

$$\langle \Phi_{\text{ret}} \rangle = 1 - f\left(\frac{[\mathbf{R}]}{[\mathcal{U}]N_{\text{dom}}}\right), \tag{18}$$

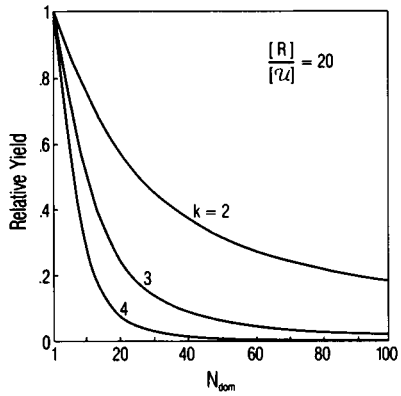

FIGURE 5 Relative yield of a dimerization reaction ($k = 2$) for $[\mathbf{R}]/[\mathcal{U}] = 20$ as a function of the number of domains per unit, N_{dom}. Plots are also presented for the case of trimer ($k = 3$) and tetramer ($k = 4$) formation.

with $f(\cdot)$ given in Eq. 17. The net yield as function of $[\mathbf{R}]/[\mathcal{U}]$ for $k = 2$, and 4, is plotted in Figs. 6, *A* and *B*.

Consecutive reactions

We consider two cases of sequential enzyme-catalyzed reactions: (*a*) the action of an enzyme on a pro-enzyme to produce a second enzyme that catalyzes the subsequent reaction in the sequence, and (*b*) the product of the first enzymatic reaction is the substrate of the second enzymatic reaction in the sequence. We consider the former case first:

$$\mathbf{E1} + \mathbf{R1} \rightarrow \mathbf{E1} + \mathbf{E2}$$
$$\mathbf{E2} + \mathbf{R2} \rightarrow \mathbf{E2} + \mathbf{P}$$

where **E1** and **E2** are the catalysts. It can be shown that, whether the system is reticulated or not, the net yield of **P** is the joint probability of having at least one **E1** and one **R1** molecule in the smallest reaction compartment. Therefore, if the distributions of all reactants are assumed to be independent, from Eq. 1, the relative yield is

$$\langle\varphi\rangle = \frac{\left(1 - \exp\left\{-\frac{[\mathbf{E1}]}{[\mathcal{U}]N_{dom}}\right\}\right)\left(1 - \exp\left\{-\frac{[\mathbf{R1}]}{[\mathcal{U}]N_{dom}}\right\}\right)}{\left(1 - \exp\left\{-\frac{[\mathbf{E1}]}{[\mathcal{U}]}\right\}\right)\left(1 - \exp\left\{-\frac{[\mathbf{R1}]}{[\mathcal{U}]}\right\}\right)}. \tag{19}$$

Noting that the concentrations of **R1** and **E2** are equal when the first reaction step is performed with probability one, the above expression is the product of the relative yields for both reactions.

In Fig. 7, we compare the single step catalyzed reaction with the consecutive mechanism, for the case of $[\mathbf{E1}]/[\mathcal{U}] = 10$. The consecutive reaction is performed at three different mean occupancy numbers for **R1**. As expected, when the concentrations of the two starting reactants are very different, one of the steps becomes the bottleneck of the global mechanism. But when the concentrations of **E1** and **R1** are comparable, the decrease in yield depends on the concentration of both the enzyme and the reactant.

For the second case of the consecutive reactions mentioned above, we consider the following sequence

$$\mathbf{E1} + \mathbf{R1} \rightarrow \mathbf{E1} + \mathbf{P1}$$
$$\mathbf{E2} + \mathbf{P1} \rightarrow \mathbf{E2} + \mathbf{P2}.$$

In this case the net yield of **P2** is the joint probability of having at least one **E1** and one **E2** molecule in the smallest reaction compartment. Again, recalling that the distributions of all reactants are independent, from Eq. 1, the relative yield is given by

$$\langle\varphi\rangle = \frac{\left(1 - \exp\left\{-\frac{[\mathbf{E1}]}{[\mathcal{U}]N_{dom}}\right\}\right)\left(1 - \exp\left\{-\frac{[\mathbf{E2}]}{[\mathcal{U}]N_{dom}}\right\}\right)}{\left(1 - \exp\left\{-\frac{[\mathbf{E1}]}{[\mathcal{U}]}\right\}\right)\left(1 - \exp\left\{-\frac{[\mathbf{E2}]}{[\mathcal{U}]}\right\}\right)}. \tag{20}$$

Both Eqs. 19 and 20 are formally identical. The relative yield of the final product is, for both cases, the product of

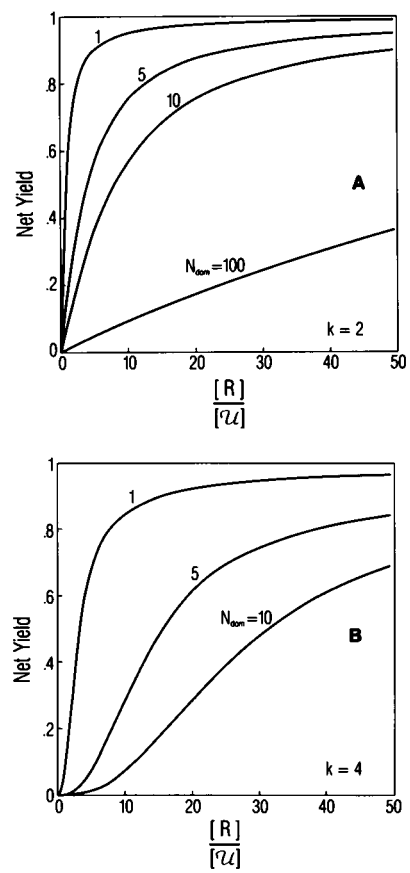

FIGURE 6 Representation of the net yield as a function of the mean number of **R** molecules per unit for different number of compartments per unit. (*A*) the case of dimer formation. (*B*) the case of tetramer formation.

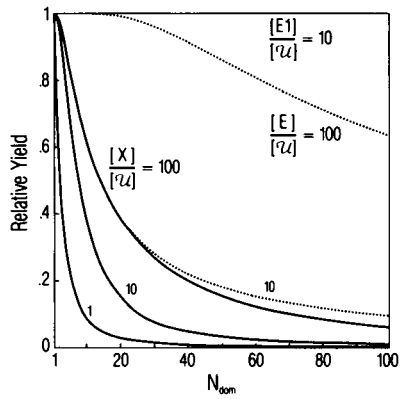

FIGURE 7 Relative yields for the final product of two consecutive catalyzed reactions as a function of N_{dom} with $[\mathbf{E1}]/[\mathcal{U}] = 10$. The yields shown are identical for two different types of reaction sequences (see text of Eqs. 14 and 15). **X** represents **R1** in the first case (Eq. 14) and **E2** in the second case (Eq. 15). Curves are given for $[\mathbf{X}]/[\mathcal{U}] = 1$, 10, and 100. For reference, the corresponding curves for a single step catalyzed reaction with $[\mathbf{E}]/[\mathcal{U}] = 10$ and 100 are also shown (*broken lines*).

two relative yields. However, they represent two physically distinct situations. The relative yield of the final product as a function of number of domains for a fixed mean value of **E1** per domain is identical for Eqs. 19 and 20, and given in Fig. 7.

DISCUSSION

We have demonstrated that the reaction yield of four types of bimolecular reactions confined to a membrane surface is decreased by compartmentalization of the surface for a random distribution of reactants over the compartments. The yield is highly dependent upon both the reaction mechanism and the reactant concentrations.

In the case of the enzyme catalyzed reaction it is easy to understand that, since only very small amounts of the enzyme need be present, compartmentalization can lead to a very low average enzyme concentration per compartment, even if the size of the compartments is relatively large. This is the situation depicted on the right-hand side of the curves in Fig. 1. Thus, the consequence of compartmentalization for an enzymatically catalyzed reaction in a biological membrane is a marked inhibition of the process regardless of compartment size. When the average number of enzyme molecules per compartment is less than five the reaction is essentially shut off. Similar but more dramatic effects are seen for consecutive enzyme catalyzed reactions shown in Fig. 7.

For a bimolecular reaction involving two different reactants the effect of compartmentalization on the relative yield, as shown in Fig. 3, is not as great as it is for an enzyme catalyzed reaction. The yield is, however, a rather complex function of the concentrations of the two reactants, as shown in Fig. 4. In the special case of a single homodimerization reaction, the decrease in the relative yield observed upon compartmentalization originates only in an increase of the number of molecules left over in domains with an odd number of molecules. When the formation of either trimers or tetramers is considered, the mean number of molecules left over per domain increases, leading to a further decrease in the relative yield (see Fig. 5). A similar argument can also be applied to the analysis of the net yield, as depicted in Fig. 6. The fraction of unreacted molecules decreases as a consequence of the increase in the $[\mathbf{R}]/[\mathcal{U}]$. This becomes more evident in the sigmoidal shape of curves in Fig. 6 *B*. In fact, for very small values of $[\mathbf{R}]/[\mathcal{U}]$, the probability of having at least k molecules in a single domain is very low, being much smaller for $\mathbf{k} = 4$ than for $\mathbf{k} = 2$. Therefore, a nearly zero net yield is observed when $[\mathbf{R}]/[\mathcal{U}]$ is very low.

The arguments presented in this paper have been given in the context of compartmentalization as a result of changes of the phase structure of a lipid bilayer membrane in which the reactants are confined. It is clear, however, that the conclusions are independent of the means of compartmentalization, which in biological membranes could include compartmentalization by means of protein components (Freire and Snyder, 1982) and cytoskeletal structures (Saxton, 1990) as well as for hypothetical situations in which proteins are separated from each other by preferential solubilization in phase-separated domains in the same membrane. We have also limited our considerations to reactions confined to two-dimensional systems. The same treatment also applies to compartmentalized three-dimensional systems and the conclusions are, in every aspect, similar.

Recently the basic ideas developed in this paper have been used to understand experimental results obtained in two unrelated studies. In the first of these, the line broadening of a spin labeled phosphatidylcholine due to spin–spin interactions between labels was examined by ESR spectroscopy in bilayers formed from mixtures of dimyristoyl phosphatidylcholine and distearoyl phosphatidylcholine as a function of temperature and composition (Sankaram et al., 1992). The detailed nature of the line broadening in the region of coexistence of fluid and gel phases can be understood quantitatively only by taking into account the compartmentalization of the spin-labeled phospholipid in certain portions of the two-phase region. This study provides a direct experimental test of the basic ideas outlined above. In the second, the difference between the apparent equilibrium poise of a reaction in disconnected, as compared to connected systems, has provided an explanation for an anomalous situation observed in photosynthetic systems (Lavergne and Joliot, 1991). The anomaly concerns the difference in equilibrium constant determined under low light intensity and the constants determined using artificial redox carriers. Lavergne and co-workers explain the difference as being due to the attainment of global equilibrium among the subsystems of each chloroplast through the

use of artificial redox mediators, whereas at low illumination intensity each global equilibrium subsystem attains its own equilibrium. The ideas that lead to the successful resolution of the anomalous behavior of photosystems are conceptually analogous to the ideas developed in this paper concerning the effects of in-plane connection and disconnection on membrane-localized reactions.

Although not directly relevant to the problem of domain structures in biological membranes, much very interesting work on the structure of phases has been done on two-component monolayers at an air/water interface (for a review, see McConnell, 1991). The large dipole moment associated with these monolayers gives rise to relatively large domains, which are usually arranged in a regular two-dimensional super lattice. Frequently, domain shapes exhibit symmetry properties arising from the chirality of component molecules. Structures of this type and size are absent in bilayers, presumably because there is almost complete compensation of the dipole moment of one of the monolayers by the dipole of the other monolayer comprising the bilayer. The discussion of the effects of connection and disconnection on bilayer-confined reactions outlined above can, of course, be readily applied to reactions confined to multiphasic monolayers at the air/water interface.

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