The dependence of lipid monolayer lipolysis on surface pressure

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Brönsted–Bjerrum theory [Brönsted (1922) *Z. Phys. Chem.* **102**, 169–207; (1925) *Z. Phys. Chem.* **115**, 337–364; Bjerrum (1924) *Z. Phys. Chem.* **108**, 82–100] as applied to reactions at interfaces is used to interpret published data on the lipolysis of dinonanoyl phosphatidylcholine monolayers by pancreatic phospholipase A₂. Reasonable quantitative agreement between theoretical and experimental results occurs when the reported effects of surface pressure on the amount of adsorbed enzyme are used together with the assumption that the Langmuir–Szykowski [Lange (1967) *Nonionic Surfactants* (Schick, M., ed.), chap. 4, Marcel Dekker, London and New York] isotherm describes the adsorption of lipid. The equations of Mass Action kinetics are less successful. Equivalent data on the lipolysis of didodecanoyl phosphatidylglycerol by pancreatic lipase can also be interpreted by arguing that the adsorbed enzyme forms significant amounts of enzyme substrate complex which reacts to give products in accordance with the well-known Briggs–Haldane [Lehninger (1975) *Biochemistry*, 2nd edn., chap. 8, Worth, New York] mechanism. The positions of the observed maxima in rate versus surface pressure plots suggests that the adsorption of an enzyme molecule displaces less lipid than one expects from the pronounced differences in molecular sizes of the two adsorbed species.

INTRODUCTION

Some years ago Verger and co-workers reported a study of the lipolysis of dinonanoyl phosphatidylcholine monolayers by pancreatic phospholipase A₂ [1,2]. They found that, at constant bulk enzyme concentration, the rate of lipolysis, r , exhibited a maximum at a surface pressure, π , of 120 μN (12 dyn)/cm or thereabouts. Within experimental error they also found that the total amount of enzyme in the monolayer Γ_t decreased according to the equation:

$$\Gamma_t = \Gamma_0(1 - K\pi) \quad (1)$$

where Γ_0 is the value of Γ_t when $\pi = 0$ and the constant K is given by $K^{-1} = 210 \mu\text{N}$ (21 dyn)/cm.

Similar maxima in rates have also been observed in other systems [3,4] and by other workers [3–8]. The aim of the present paper is to reach quantitative conclusions concerning such maxima by applying the ideas about reactions at interfaces developed in [9].

Most quantitative investigations of enzyme reactions are based on the equations of Mass Action kinetics expressed in terms of concentrations and implicitly assume that these equations apply over the range of conditions involved. However, in many biological systems of practical interest this approach may not be valid. The present work is important, because it describes the development and application of methods which overcome this difficulty.

The philosophy of the paper, its structure and main conclusions are as follows. It is recognized that, at a molecular level, the reactions concerned are complex and that many of the details are inaccessible experimentally. Such details are allowed for implicitly by using empirical equations such as eqn. (1), together with other equations obtained from thermodynamic arguments. The strength of this type of approach is that it enables fairly complex behaviour to be interpreted at a phenomenological level. Such an approach cannot in itself say anything about the molecular details, which it deliberately avoids. Nevertheless, the values of parameters that emerge from the treatment give clues about interactions at a molecular level.

In the Theory section below an expression is derived (eqn. 9) which enables the dependence of r on π to be calculated given the dependence of Γ_t on π and the compression isotherm of the substrate monolayer. In the section entitled ‘Comparison of theory with experiment and with the Mass Action Law’ this expression is compared with the experimental data of [1]. Good quantitative agreement between theory and experiment is obtained when it is assumed that the compression isotherm conforms to eqn. (10). In contrast, a simple Mass Action approach (eqn. 16) cannot account for the data. In the section entitled ‘Other systems’ it is shown that the theory can also account quite well for the behaviour of other systems. In the Discussion section eqns. (22) and (23) are used to provide a framework for discussing the interactions which lead to a maximum in r and a linear decrease in Γ_t with increasing π . These equations are derived in Appendices 1 and 2. It is noteworthy that, unlike eqn. (9), eqn. (22) is free from assumptions concerning activity coefficients. An expression for r which allows explicitly for water as a substrate, presented in Appendix 1, is also discussed briefly in the Discussion section.

THEORY

We suppose that the lipolysis proceeds by the classical Briggs–Haldane mechanism [10]:

where E denotes uncomplexed enzyme, S denotes substrate and ES denotes enzyme–substrate complex. The reaction pathway I is assumed to include implicitly other pathways such as

where W denotes water. A justification of this simplification is provided in the Discussion section.

According to the Brönsted–Bjerrum theory of reaction rates

[11,12] expressed in terms of transition-state theory [13,14] and applied to reactions at interfaces r_t , r_b and r are given by:

$$\begin{aligned} r_t &= k_t \frac{\Gamma_E \gamma_E \Gamma_s \gamma_s}{\gamma_t^\ddagger} \\ r_b &= \frac{k_b \Gamma_{ES} \gamma_{ES}}{\gamma_t^\ddagger} \\ r &= \frac{k \Gamma_{ES} \gamma_{ES}}{\gamma^\ddagger} \end{aligned} \quad (2a-2c)$$

where the dimensions of r_t , I_b and r are amounts per unit area per unit time, $\ddagger$ denotes a transition state, the Γ denote surface concentrations, the k values are rate constants, and the γ parameters denote activity coefficients and are defined so that they are unity in the limit that all $\Gamma \rightarrow 0$. An important feature of eqns. (2a)–(2c) is that they contain the activity coefficients of the transition states $\gamma_t^\ddagger$ and $\gamma^\ddagger$. We assume that:

$$\gamma_E = \gamma_{ES} = \gamma_t^\ddagger = \gamma^\ddagger \quad (3)$$

The physical significance of this assumption is apparent from eqns. (43) and (44) of [9]. In the present instance it is equivalent to assuming that, on adsorption, all four species displace the same amount of substrate from the monolayer.

Eqn. (3) is probably reasonable for the transition states and the enzyme–substrate complex, but may be less reasonable for the bare enzyme. However, it is necessary if we wish to make significant progress. Putting

$$\begin{aligned} \Gamma_{ES} &= r/k \\ \Gamma_E &= \Gamma_t - r/k \end{aligned} \quad (4a \text{ and } 4b)$$

and noting that, under steady-state conditions,

$$r = r_t - r_b \quad (5)$$

we find after some straightforward algebra that

$$r = \frac{\frac{k_t k}{k + k_b} \Gamma_t \Gamma_s \gamma_s}{1 + \frac{k_t}{k + k_b} \Gamma_s \gamma_s} \quad (6)$$

Since the amount of adsorbed enzyme is small, it is reasonable to argue that the dependence of γ_s and π on Γ_s are the same as for an enzyme free monolayer. From the Gibbs adsorption isotherm for such a monolayer we find that:

$$\left(\frac{d\mu_s}{d\pi} \right)_{T,p} = RT \left(\frac{d \ln \Gamma_s \gamma_s}{d\pi} \right)_{T,p} = \frac{1}{\Gamma_s} \quad (7)$$

which gives on integration:

$$\Gamma_s \gamma_s = B \exp \left[\frac{\int_0^\pi \frac{d\pi}{\Gamma_s}}{RT} \right] \quad (8)$$

where B depends only on Temperature (T) and pressure (p).

This equation enables us to write eqn. (6) in the general form:

$$r = \frac{k' \Gamma_t \exp \left[\frac{\int_0^\pi \frac{d\pi}{\Gamma_s}}{RT} \right]}{1 + l \exp \left[\frac{\int_0^\pi \frac{d\pi}{\Gamma_s}}{RT} \right]} \quad (9)$$

where k' and l are constants.

In the particular case that the adsorption isotherm of the lipid is of the Langmuir–Syskowski type [15] we have:

$$\pi = -RT \Gamma_\infty \ln [1 - \Gamma_s / \Gamma_\infty] \quad (10)$$

where Γ_∞ denotes the limiting adsorption of substrate and

$$\mu_s = \mu_s^\ominus + RT \ln \Gamma_s \gamma_s = \mu_s^0 + RT \ln \frac{\Gamma_s}{\Gamma_\infty - \Gamma_s} \quad (11)$$

where $\mu_s^\ominus$ and μ_s^0 depend only on T and p and where $\mu_s^\ominus$ is the standard chemical potential of adsorbed substrate. It follows from eqn. (10) that:

$$\Gamma_s \gamma_s = \frac{\Gamma_\infty \Gamma_s}{\Gamma_\infty - \Gamma_s} \quad (12)$$

which gives

$$r = \frac{k' \Gamma_t \frac{\Gamma_s}{\Gamma_\infty - \Gamma_s}}{1 + l \frac{\Gamma_s}{\Gamma_\infty - \Gamma_s}} = \frac{k' \Gamma_t \left[\exp \frac{\pi}{RT \Gamma_\infty} - 1 \right]}{1 + l \left[\exp \frac{\pi}{RT \Gamma_\infty} - 1 \right]} \quad (13)$$

COMPARISON OF THEORY WITH EXPERIMENT AND WITH THE MASS ACTION LAW

Eqn. (9) shows that the dependence of r on π at constant bulk enzyme concentration can be predicted from the dependence of Γ_t on π and the experimental surface equation of state of lipid. Unfortunately Verger and co-workers [1,2] do not give the latter information. If the dependence of Γ_t on π is given by the empirical eqn. (1), then it is fairly straightforward to show from eqns. (4), (6) and (9) that a maximum in Γ occurs when

$$RT \Gamma_s = \frac{\Gamma_E}{\Gamma_t} \left(\frac{1 - K\pi}{K} \right) \quad (14)$$

The physical reasons for the occurrence of a maximum are best understood in terms of eqn. (22) below. Given that $\pi = 120 \mu\text{N}$ (12 dyn)/cm and that $K^{-1} = 210 \mu\text{N}$ (21 dyn)/cm, it follows from eqn. (14) that, at the maximum, $RT \Gamma_s \leq 90 \mu\text{N}$ (9 dyn)/cm. For dialkyl phospholipids one expects the limiting area per molecule $1/\Gamma_\infty$ to be of the order of 0.4 nm^2 ($40 \text{ \AA}^2$) per molecule, which is twice the limiting area of close-packed alkyl chains. This corresponds to a value of $RT \Gamma_\infty = 103 \mu\text{N}$ (10.3 dyn)/cm. Thus if Γ_E/Γ_t is close to unity, the maximum in rate occurs under conditions close to saturation adsorption of the lipid.

To be more explicit, we suppose that the lipid conforms to eqn. (10). This implies that the lipid monolayer does not exhibit a two-dimensional phase transition on compression. Such a transition does occur for longer-chain-length dialkyl phosphatidylcholine but is unlikely for the di- C_9 material. From eqns. (4), (6) and (14) it now follows that:

$$\frac{\Gamma_E}{\Gamma_t} = \frac{1}{1 + \frac{l \Gamma_s}{\Gamma_\infty - \Gamma_s}} \quad (15)$$

which, together with eqn. (13), enables us to conclude that if r has a maximum when $\pi = 120 \mu\text{N}$ (12 dyn)/cm, then $l = 0.122$. The appropriate graph of r versus π as given by eqn. (13) is shown in Fig. 1. The line is theoretical, the points are experimental and the value of K is chosen so that the curve passes through the experimental point for $\pi = 120 \mu\text{N}$ (12 dyn)/cm.

The graph of r versus π for which $l = 0$ is given in Fig. 2. In this

Fig. 1. Lipolysis of dinonanoyl phosphatidylcholine by pancreatic phospholipase A at 298 K

$10^{-13} \times \text{Rate}$ (molecules/min per cm^2) was plotted against surface pressure ($\mu\text{N}/\text{cm}$). $\odot$, Data from [1]; —, calculated from eqn. (13) with $l = 0.122$.

Fig. 2. Lipolysis of dinonanoyl phosphatidylcholine by pancreatic phospholipase A at 298 K

A plot of $10^{-13} \times \text{rate}$ (molecules/min per cm^2) versus surface pressure ($\mu\text{N}/\text{cm}$) is shown. $\odot$, Data from [1]; —, calculated from eqn. (13) with $l = 0$.

case the value of k' was chosen such that the maximum rate at $135 \mu\text{N}$ ($13.5 \text{ dyn}/\text{cm}$) is equal to the experimental rate at $\pi = 120 \mu\text{N}$ ($12 \text{ dyn}/\text{cm}$).

There is little to choose between the two graphs, and in both cases the agreement between theory and experiment is quite good.

According to the simple Mass Action approach in terms of surface concentrations $\gamma_s = 1$ and

$$r = \frac{k''\Gamma_t\Gamma_s}{1 + l\Gamma_s} \quad (16)$$

where k'' is a constant.

It follows from this equation that at the maximum in r we should have

$$\frac{d \ln \Gamma_s}{d \ln \pi} \geq \frac{K\pi}{1 - K\pi} = \frac{4}{3} \quad (17)$$

This value seems unreasonably large. At the maximum in r we know that

$$\pi > RT\Gamma_s \quad (18)$$

because $RT\Gamma_s \leq RT\Gamma_\infty \approx 103 \mu\text{N}$ ($10.3 \text{ dyn}/\text{cm}$).

Although it is not impossible for eqns. (17) and (18) to be compatible, the dependence of π on Γ_s required to achieve this is rather unlikely. Also, if eqn. (10) applies, then it follows from eqn. (16) that the greatest value of π for which r can have a maximum occurs when $l = 0$ and turns out to be $83 \mu\text{N}$ ($8.3 \text{ dyn}/\text{cm}$). We conclude that the simple Mass Action approach is unable to account for the position of the maximum in r versus π .

It is interesting to examine the explicit dependence of r on Γ_s

as predicted by eqns. (13) and (16) with $l = 0$. From eqn. (13) we obtain

$$\frac{r}{k'\Gamma_0} = [1 + KRT\Gamma_\infty \ln(1 - \Gamma_s/\Gamma_\infty)] \frac{\Gamma_s}{\Gamma_\infty - \Gamma_s} \quad (19)$$

whereas eqn. (16) gives

$$\frac{r}{k''\Gamma_0\Gamma_\infty} = [1 + KRT\Gamma_\infty \ln(1 - \Gamma_s/\Gamma_\infty)] \frac{\Gamma_s}{\Gamma_\infty} \quad (20)$$

Plots of the right-hand side of eqns. (19) and (20) versus Γ_s/Γ_∞ are given in Fig. 3. It is notable that eqn. (19) gives a graph which is close to linear over a substantial range of Γ_s/Γ_∞ .

Fig. 3. Comparison of eqns. (19) and (20)

Upper curve, right-hand side of eqn. (19) plotted against Γ_s/Γ_∞ ; lower curve, right-hand side of eqn. (20) plotted against Γ_s/Γ_∞ .

Table 1. Values of Γ_t/Γ_0 versus π according to eqn. (21) for $l = 0.5$

π ($\mu\text{N}/\text{cm}$)	Γ_t/Γ_0	π ($\mu\text{N}/\text{cm}$)	Γ_t/Γ_0
0	1	140	0.956
20	1.015	160	0.956
40	1.031	180	0.846
60	1.047	200	0.666
80	1.056	220	0.396
100	1.063	220	0.395
120	1.053	240	0.216

OTHER SYSTEMS

A similar study in which the enzyme was pancreatic lipase and the substrate was didodecanoyl phosphatidylglycerol has been reported briefly by Verger and co-workers in [3]. The lipid is stated to have no discontinuity or phase transition in its compression isotherm. Although experiments both in the presence and absence of colipase are reported, we will consider only the latter. The dependence of rate on surface pressure is similar to that described above, with the maximum at a slightly higher surface pressure of about $160 \mu\text{N}$ ($16 \text{ dyn}/\text{cm}$). However, the dependence of the amount of adsorbed enzyme Γ_t on surface pressure is markedly different. Γ_t is approximately constant in the range $100 \mu\text{N}$ ($10 \text{ dyn}/\text{cm}$) $\leq \pi \leq 220 \mu\text{N}$ ($22 \text{ dyn}/\text{cm}$) and then falls rapidly to zero over the range $220\text{--}240 \mu\text{N}$ ($22\text{--}24 \text{ dyn}/\text{cm}$). This difference can be understood by arguing that the dependence arises from the difference in l values. As we have already shown, l for the system discussed above is certainly small. Hence it may well be reasonable to suppose that eqn. (1) describes the dependence of Γ_E on π and to write

$$\Gamma_t = \Gamma_0(1 - K\pi)[1 + l[\exp \pi/RT\Gamma_\infty - 1]] \quad (21)$$

Values of Γ_t/Γ_0 versus π according to eqn. (21) for $l = 0.5$ are given in Table 1. K^{-1} was taken to be $240 \mu\text{N}$ ($24 \text{ dyn}/\text{cm}$).

Evidently Γ_t is almost independent of π up to about $170 \mu\text{N}$ ($17 \text{ dyn}/\text{cm}$) and then declines quite rapidly. Also if Γ_E is given by eqn. (1), the predicted π at the maximum in r , $\sim 160 \mu\text{N}$ ($16 \text{ dyn}/\text{cm}$), is in reasonable agreement with experiment. Less well predicted is the dependence of r on π before the maximum. Nevertheless the overall agreement between theory and experiment is quite good bearing in mind the assumptions made.

Studies of dipalmitoyl phosphatidylcholine lipolysis as a function of lipid surface excess have also been reported [16]. The agreement with eqn. (18) is better than with eqn. (19). Since this lipid may well exhibit a two-dimensional phase transition at the temperature of interest the significance of such agreement is perhaps not great.

Some of the systems discussed in [4] show no maximum in rate with increase in surface pressure. Two features of these systems are that, in the absence of lipid, the enzyme exerts a significant surface pressure in its own right and that most of it is adsorbed. The contributions to π from the enzyme decline rapidly as the amount of adsorbed lipid is increased. This shows that the adsorbed enzyme is displaced from the surface so that μ_E in solution increases. As the equations in Appendix show that this in turn leads to a greater rate than would be observed if μ_E was constant.

DISCUSSION

As the above analysis shows, formulating the theory in terms of rate constants and activities provides a convenient math-

ematical framework for interpreting the cited experimental data. On the other hand, the approach used in Appendix 1 provides a different, and in some ways better, insight into the interactions responsible for the observed behaviour. Consider for instance eqn. (A7), which may be written as:

$$RT \frac{d \ln r}{d\pi} = \frac{1}{\Gamma_s} \left[1 + \frac{r_b}{r_t} \left(\frac{\partial \Gamma_s}{\partial \Gamma^*} \right)_{\mu_s} + \frac{r}{r_t} \left(\frac{\partial \Gamma_s}{\partial \Gamma^*} \right)_{\mu_s} \right] \quad (22)$$

This equation shows that the effect of surface pressure on the rate is governed by the terms $(\partial \Gamma_s / \partial \Gamma^*)_{\mu_s}$ and $(\partial \Gamma_s / \partial \Gamma^*)_{\mu_s}$. Both quantities can be expected to be negative. In magnitude they are the amounts of substrate displaced from the surface by the two transition states. As might be expected, the transition state which forms products dominates when the complex ES is almost in equilibrium with E and S during the reaction. It is noteworthy that, apart from the usual steady-state assumption, the only approximations in eqn. (22) are those inherent in transition-state theory itself, namely (a) that the breakdown characteristics of a transition state are not greatly influenced by its environment and (b) the equilibrium hypothesis. In particular it is clear from eqn. (22) that the maximum in rate occurs when the right-hand side of eqn. (22) is zero. This in turn implies that $(\partial \Gamma_s / \partial \Gamma^*)_{\mu_s}$ and $(\partial \Gamma_s / \partial \Gamma^*)_{\mu_s}$ are of the order of unity.

In general one expects a large molecule such as an enzyme to occupy a large surface area and that the number of lipid molecules displaced will be of similar order to the number occupying the area taken up by the enzyme. In the present example the Γ_s at which the maximum occurs is approx. 90% of the saturation value. The above reasoning suggests that, at the maximum in r , the enzyme occupies an area of about 0.45 nm^2 ($45 \text{ \AA}^2$), which is clearly very small. A possible explanation of this is that the enzyme threads through the interface so that only a small fraction of the adsorbed enzyme molecule competes with the lipid by occupying its adsorption plane. Alternatively, if parts of the enzyme which do not occupy the adsorption plane of the lipid bind lipid, this will contribute positively to $(\partial \Gamma_s / \partial \Gamma^*)_{\mu_s}$ and will help to offset any negative contribution due to excluded area effects. Clearly the competitive effect is less pronounced than one expects on grounds of molecular size.

To obtain further insight into the competitive adsorption of enzyme and substrate, we consider the following result, derived in Appendix 2.

$$\left(\frac{\partial \ln \Gamma_t}{\partial \pi} \right)_{\mu_s} = \frac{1}{RT\Gamma_s} \left[\frac{\Gamma_{ES}}{\Gamma_t} + \frac{\Gamma_E}{\Gamma_t} \left(\frac{\partial \Gamma_s}{\partial \Gamma_E} \right)_{\mu_s} + \frac{\Gamma_{ES}}{\Gamma_t} \left(\frac{\partial \Gamma_s}{\partial \Gamma_{ES}} \right)_{\mu_s} \right] \quad (23)$$

This equation applies if $d\mu_{ES} = d\mu_E + d\mu_s$, which in turn follows if $r_t \gg r$ or if $\gamma_t^* = \gamma^*$.

Like the derivatives on the right-hand side of eqn. (22) $-(\partial \Gamma_s / \partial \Gamma_E)_{\mu_s}$ and $-(\partial \Gamma_s / \partial \Gamma_{ES})_{\mu_s}$ respectively are the amounts of substrate displaced from the surface by an adsorbed enzyme molecule and an adsorbed enzyme-substrate complex.

When the dependence of Γ_t on π is given by eqn. (1)

$$\left(\frac{\partial \ln \Gamma_t}{\partial \pi} \right)_{\mu_s} = \frac{-K}{1 - K\pi} \quad (24)$$

It follows from eqns. (23) and (24) that

$$\left[\frac{\Gamma_E}{\Gamma_t} \left(\frac{\partial \Gamma_s}{\partial \Gamma_E} \right)_{\mu_s} + \frac{\Gamma_{ES}}{\Gamma_t} \left(\frac{\partial \Gamma_s}{\partial \Gamma_{ES}} \right)_{\mu_s} \right]$$

becomes increasingly negative with increasing π , even though Γ_s is not changing by very much. There is no obvious simple interpretation of this behaviour in terms of excluded area effects. However, there is considerable scope for alternative kinds of

explanation. For example, if the adsorbed enzyme occupies a large area but binds substrate, a small change in Γ_s at constant amount of bound substrate could make a large contribution to $(\partial\Gamma_s/\partial\Gamma_{ES})_{\mu_s}$. Obviously eqn. (1) cannot be expected to apply strictly throughout the entire range $0 \leq \pi \leq 210 \mu\text{N}$ (21 dyn)/cm. Nevertheless, it does fit the experimental data reasonably well over most of this range.

As eqn. (A10) in Appendix 1 shows, the explicit inclusion of water as a substrate complicates matters considerably. When the first step of pathway II is effectively in equilibrium, eqn. (22) is in effect an average over the two pathways and is entirely adequate if one pathway dominates the overall rate or if the breakdown of ES to products is rate-determining. Eqn. (22) is also adequate if the transition states which lead to the formation of ES have similar breakdown characteristics or displace similar amounts of adsorbed substrate.

Concluding remarks

The agreement between theory and experiment illustrates the value of the new formulation of transition-state theory for reactions at interfaces [9] and suggests that the additional approximations inherent in eqn. (3) are also reasonable. In comparison, applying the Law of Mass Action in terms of surface concentrations fails quite badly.

As Verger and co-workers have shown [1,2], other aspects of lipolysis kinetics are well explained in terms of Mass Action kinetic arguments. For example, doubling the bulk concentration of enzyme doubles the rate. However, none of these findings conflicts with the approach described above.

Eqn. (22) shows clearly that a maximum in r with increasing π is to be expected. What is surprising is that the maxima do not occur at lower surface coverages of lipid.

The results discussed provide good evidence for the validity of the Briggs-Haldane mechanism at interfaces. The results for dinonanoyl phosphatidylcholine support the view that most of

the adsorbed enzyme is free, whereas the results for didodecanoyl phosphatidylglycerol suggest that most of the adsorbed enzyme is present as enzyme-lipid complex, especially at higher surface pressures.

REFERENCES

1. Verger, R., Rietsch, J., van Dam-Mieras, M. C. E. & de Haas, G. H. (1976) *J. Biochem. (Tokyo)* **251**, 3128–3133
2. Verger, R. & de Haas, G. H. (1976) *Annu. Rev. Biophys. Bioeng.* **5**, 77–117
3. Verger, R., Rietsch, J. & Desnuelle, P. (1977) *J. Biochem. (Tokyo)* **252**, 4319–4325
- 3a. Verger, R., Mieras, M. C. E. & de Haas, G. H. (1973) *J. Biochem. (Tokyo)* **248**, 4023–4034
4. Sugihara, A., Gargouri, Y., Pieroni, G., Riviere, C., Sarda, L. & Verger, R. (1986) *Biochemistry* **25**, 3430–3434
5. Colacicco, G. & Rapport, M. (1966) *J. Lipid Res.* **7**, 258–263
6. Zografi, G., Verger, R. & de Haas, G. H. (1971) *Chem. Phys. Lipids* **7**, 185–206
7. Shah, D. O. & Schulman, J. H. (1967) *J. Colloid Interface Sci.* **25**, 107–119
8. Olive, J. & Dervichian, D. G. (1971) *Biochimie* **53**, 207–213
9. Hall, D. G. (1989) *J. Chem. Soc. Faraday Trans. I* **85**, 1881–1895
10. Lehninger, A. L. (1975) *Biochemistry*, 2nd edn., chapter 8, Worth Publishers, New York
11. Brönsted, J. N. (1922) *Z. Phys. Chem.* **102**, 169–207
- 11a. Brönsted, J. N. (1925) *Z. Phys. Chem.* **115**, 337–364
12. Bjerrum, N. (1924) *Z. Phys. Chem.* **108**, 82–100
13. Glasstone, S., Laidler, K. J. & Eyring, H. (1941) *The Theory of Rate processes*, McGraw-Hill, New York
14. Eyring, H. & Urry, E. W. (1963) *Ber. Bunsenges Phys. Chem.* **67**, 731–740
15. Lange, H. (1967) *Nonionic Surfactants* (Schick, M., ed.), chap. 4, Marcel Dekker, London and New York
16. Miller, I. R. & Ruyschaert, J. M. (1971) *J. Colloid Interface Sci.* **35**, 340–345
17. Hall, D. G. (1986) *J. Chem. Soc. Faraday. Trans. 2*, **82**, 1305–1314

APPENDIX 1

Derivation of eqn. (22)

Let μ_E and μ_S denote chemical potentials of E and S and let μ_{ES} denote the chemical potential of the complex ES. Also let Γ_E , Γ_S and Γ_{ES} denote the respective amounts adsorbed. For the reaction mechanism 1 above the theory described in [9] enables us to write:

$$RT d \ln r_t = \left[1 + \left(\frac{\partial \Gamma_E}{\partial \Gamma_t^*} \right)_{\mu_E, \mu_S} \right] d\mu_E + \left[1 + \left(\frac{\partial \Gamma_S}{\partial \Gamma_t^*} \right)_{\mu_S} \right] d\mu_S + \left(\frac{\partial \Gamma_{ES}}{\partial \Gamma_t^*} \right)_{\mu_{ES}, \mu_S} d\mu_{ES} \quad (\text{A1})$$

$$RT d \ln r = \left[1 + \left(\frac{\partial \Gamma_{ES}}{\partial \Gamma_t^*} \right)_{\mu_{ES}, \mu_S} \right] d\mu_{ES} + \left(\frac{\partial \Gamma_S}{\partial \Gamma_t^*} \right)_{\mu_S} d\mu_S + \left(\frac{\partial \Gamma_E}{\partial \Gamma_t^*} \right)_{\mu_E, \mu_S} d\mu_E \quad (\text{A2})$$

where Γ_t^* and Γ^* respectively denote amounts adsorbed of activated complexes for the formation of ES and the formation of products. The derivatives may be regarded as amounts of the various species concerned associated with an activated complex.

Under steady-state conditions we also have:

$$r_t - r_b = r \quad (\text{A3})$$

and [17]

$$RT [d \ln r_t / r_b] = d\mu_E + d\mu_S - d\mu_{ES} \quad (\text{A4})$$

Under the conditions of the experiments μ_E is constant. Also, since there is very little enzyme in the interface, it is reasonable to put all derivatives of Γ_E and Γ_{ES} equal to zero.

Hence eqns. (A1) and (A2) simplify to give:

$$RT d \ln r_t = \left[1 + \left(\frac{\partial \Gamma_S}{\partial \Gamma_t^*} \right)_{\mu_S} \right] d\mu_S \quad (\text{A5})$$

$$RT d \ln r = d\mu_{ES} + \left(\frac{\partial \Gamma_S}{\partial \Gamma_t^*} \right)_{\mu_S} d\mu_S \quad (\text{A6})$$

Our next task is to eliminate $d\mu_{ES}$ from eqn. (A6). This is done by substituting for $d\mu_{ES}$ as given by eqn. (A4), eliminating $d \ln r_b$ by use of eqn. (A3) and then substituting for $d \ln r_t$ as given by eqn. (A5). After some straightforward manipulation we obtain the result:

$$RT d \ln r = \left[1 + \frac{r_b}{r_t} \left(\frac{\partial \Gamma_S}{\partial \Gamma_t^*} \right)_{\mu_S} + \frac{r}{r_t} \left(\frac{\partial \Gamma_S}{\partial \Gamma_t^*} \right)_{\mu_S} \right] d\mu_S \quad (\text{A7})$$

We note that $-(\partial \Gamma_S / \partial \Gamma_t^*)_{\mu_S}$ may be regarded as the amount of substrate displaced by an activated complex, which forms

products and can be expected to be very similar to $-(\partial\Gamma_s/\partial\Gamma_t^\#)_{\mu_s}$. If we suppose further that

$$\left(\frac{\partial\Gamma_s}{\partial\Gamma_t^\#}\right)_{\mu_s} = \left(\frac{\partial\Gamma_s}{\partial\Gamma_t^\#}\right)_{\mu_s} = \left(\frac{\partial\Gamma_s}{\partial\Gamma_{ES}^\#}\right)_{\mu_s} = \left(\frac{\partial\Gamma_s}{\partial\Gamma_E^\#}\right)_{\mu_s} \quad (\text{A8})$$

We note that eqn. (A8) is equivalent to eqn. (3).

If we wish to allow explicitly for water as a substrate, we may obtain the following result by methods similar to those used to derive eqn. (A7):

$$RT \ln r = \frac{r_t + (r_t - r_b)(\partial\Gamma_s/\partial\Gamma_t^\#)_{\mu_s} + \left(r_b + \frac{r_1^b r_2^b}{r_1^b + r_2^b}\right)(\partial\Gamma_s/\partial\Gamma^\#)_{\mu_s} + \frac{r_2^t r_1^b}{r_1^b + r_2^t} + \left(\frac{r_1^t - r_1^b}{r_1^b + r_2^t}\right)[r_2^t(\partial\Gamma_s/\partial\Gamma_1^\#)_{\mu_s} + r_1^b(\partial\Gamma_s/\partial\Gamma_2^\#)_{\mu_s}]}{r + r_b + \frac{r_1^b r_2^b}{r_1^b + r_2^t}} \quad (\text{A10})$$

we obtain

$$RT \ln r = \left[1 + \left(\frac{\partial\Gamma_s}{\partial\Gamma_E^\#}\right)_{\mu_s}\right] d\mu_s \quad (\text{A9})$$

where $\Gamma_1^\#$ and $\Gamma_2^\#$ refer to transition states which lead to the formation of ES by pathway II and $\Gamma_t^\#$ refers to the corresponding transition state via pathway I.

APPENDIX 2

Derivation of eqn. (23)

According to the Gibbs adsorption equation:

$$d\pi = \Gamma_s d\mu_s + \Gamma_E d\mu_E + \Gamma_{ES} d\mu_{ES} \quad (\text{A11})$$

When $\gamma_t^\# = \gamma^\#$ or when $r_t \gg r$, $d \ln r_t = d \ln r_b = d \ln r$ and we have

$$d\mu_{ES} = d\mu_E + d\mu_s \quad (\text{A12})$$

This enables us to rewrite eqn. (A11) as:

$$d\pi = (\Gamma_s + \Gamma_{ES}) d\mu_s + (\Gamma_E + \Gamma_{ES}) d\mu_E \quad (\text{A13})$$

which gives, on cross-differentiation:

$$\left(\frac{\partial\Gamma_t}{\partial\mu_s}\right)_{\mu_E} = \left(\frac{\partial(\Gamma_s + \Gamma_{ES})}{\partial\mu_E}\right)_{\mu_s} \quad (\text{A14})$$

The right-hand side of eqn. (A14) may be written as $[\partial(\Gamma_s + \Gamma_{ES})/\partial\Gamma_t]_{\mu_s} \left(\frac{\partial\Gamma_t}{\partial\mu_E}\right)_{\mu_s}$, and when Γ_E and Γ_{ES} are both small, we may put:

$$\left(\frac{\partial\Gamma_t}{\partial\mu_E}\right)_{\mu_s} = \frac{\Gamma_t}{RT} \quad (\text{A15})$$

Eqn. (A14) now becomes:

$$RT \left(\frac{\partial \ln \Gamma_t}{\partial \mu_s}\right)_{\mu_E} = \left[\frac{\partial(\Gamma_s + \Gamma_{ES})}{\partial(\Gamma_E + \Gamma_s)}\right]_{\mu_s} \quad (\text{A16})$$

When eqn. (A12) holds and Γ_t is small we have:

$$\left(\frac{\partial\Gamma_{ES}}{\partial(\Gamma_E + \Gamma_s)}\right)_{\mu_s} = \frac{\Gamma_{ES}}{\Gamma_E + \Gamma_s} \quad (\text{A17})$$

At constant μ_s we may regard Γ_s as a function of Γ_E and Γ_{ES} and write:

$$d\Gamma_s = \left(\frac{\partial\Gamma_s}{\partial\Gamma_E}\right)_{\mu_s} d\Gamma_E + \left(\frac{\partial\Gamma_s}{\partial\Gamma_{ES}}\right)_{\mu_s} d\Gamma_{ES} \quad (\text{A18})$$

where the derivatives depend only on μ_s because Γ_t is small. It follows from eqns. (A18) and (A17) that:

$$\left(\frac{\partial\Gamma_s}{\partial(\Gamma_E + \Gamma_{ES})}\right)_{\mu_s} = \left(\frac{\partial\Gamma_s}{\partial\Gamma_E}\right)_{\mu_s} \frac{\Gamma_E}{\Gamma_E + \Gamma_{ES}} + \left(\frac{\partial\Gamma_s}{\partial\Gamma_{ES}}\right)_{\mu_s} \frac{\Gamma_{ES}}{\Gamma_E + \Gamma_{ES}} \quad (\text{A19})$$

Hence:

$$RT \left(\frac{\partial \ln \Gamma_t}{\partial \mu_s}\right)_{\mu_E} = \Gamma_s RT \left(\frac{\partial \ln \Gamma_s}{\partial \pi}\right)_{\mu_E} = \left[\frac{\Gamma_{ES}}{\Gamma_t} + \left(\frac{\partial\Gamma_s}{\partial\Gamma_E}\right)_{\mu_s} \frac{\Gamma_E}{\Gamma_t} + \left(\frac{\partial\Gamma_s}{\partial\Gamma_{ES}}\right)_{\mu_s} \frac{\Gamma_{ES}}{\Gamma_t}\right] \quad (\text{A20})$$

which is the required result.