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Enantioselectivity Enhancement in the Deacylation of *N*-Acyl Amino Acid Esters by Vesicular Systems of Long Chain Dipeptide Nucleophiles and a Cationic Double Chain Surfactant

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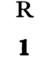
The enantioselective deacylation of short chain and long chain *N*-acyl amino acid *p*-nitrophenyl esters, $CH_3(CH_2)_nCONH\overset{*}{C}H(R)\text{-}CO_2C_6H_4NO_2\text{-}p$ ($R=C_6H_5CH_2$, $(CH_3)_2CHCH_2$, CH_3; $n=0$, 8, 12), was carried out at 4—25 °C (pH 7.68) with the vesicular systems composed of *N,N*-didodecyl-*N,N*-dimethylammonium bromide and long chain nucleophiles, N^{α}-dodecanoyl-L-histidine, N^{α}-(*N*-dodecanoyl-L-leucyl)-L-histidine (**3a**), N^{α}-(*N*-dodecanoyl-L-phenylalanyl)-L-histidine (**3b**), and *N*-(N^{α}-dodecanoyl-L-histidyl)-L-leucine (**3c**). Both the deacylation rate and the enantioselectivity were the largest in the deacylation of the substrates possessing a long *N*-acyl chain ($n=8$) with the efficient dipeptide nucleophiles (**3a** and **3b**) including the L-histidine moiety in their terminal position, and the neighboring amino acid binding to the L-histidine in **3a**, **3b**, and/or **3c** was found to play an important role in the enhancement of enantioselectivity. The lowering of the reaction temperature decreased the deacylation rate and increased the enantioselectivity. The maximum enantiomer rate ratio of 24.5 was obtained in the deacylation of *p*-nitrophenyl *N*-dodecanoylphenylalaninates with the dipeptide nucleophile **3b**. The characteristic features of the present reaction are discussed on the basis of kinetic parameters (binding constants and activation parameters) and isokinetic relationships.

The enantioselective hydrolysis of ester substrates by chiral bilayer membranes has recently received considerable attention in the biomimetic chemistry. There are four reports dealing with the following reactions: (a) the deacylation of *p*-nitrophenyl *N*-benzyloxycarbonylphenylalaninates by a synthetic functionalized membrane including L-histidine moiety with a maximum enantiomer rate ratio (k_L/k_D) of 4.4 (4 °C),[1] (b) the deacylation of *p*-nitrophenyl *N*-acylphenylalaninates by the vesicles including N^{α}-palmitoyl-L-histidine with a maximum rate ratio of 5.6 (25 °C),[2] (c) the deacylation of di- or tripeptide type amino acid *p*-nitrophenyl esters by the mixed vesicles of *N,N*-dihexadecyl-*N,N*-dimethylammonium bromide and 1-hexadecanethiol surfactant with a maximum ratio of 4.53 (25 °C),[3] and (d) the deacylation of *p*-nitrophenyl *N*-acylphenylalaninates by the vesicular systems of N^{α}-(*N*-benzyloxycarbonyl-L-leucyl)-L-histidine and a cationic double chain surfactant with maximum rate ratios of 29.6 (25 °C) and 83.6 (10 °C).[4] In this paper, we wish to report the enantioselective deacylation of *N*-acyl amino acid esters by vesicular systems composed of long chain dipeptide nucleophiles and *N,N*-didodecyl-*N,N*-dimethylammonium bromide.

Results and Discussion

When the ester substrate **1** (1×10^{-5} mol dm^{-3}) was hydrolyzed by the vesicles composed of the nucleophile **2** or **3** (5×10^{-5} mol dm^{-3}) and the surfactant **4** (1×10^{-3} mol dm^{-3}; cmc$\simeq5\times10^{-5}$ mol dm^{-3}) at 4—25 °C (pH 7.68) in Tris buffer (0.083 mol dm^{-3}) including 0.083 mol dm^{-3} KCl in 3% (v/v) CH_3CN-H_2O, the deacylation rate obeyed a good pseudo-first-order rate law. The apparent catalytic rate constant k was evaluated as an average value obtained from more than three reactions repeated under identical conditions.

The deacylation rate and the enantioselectivity (k_L/k_D) were considerably influenced by the structure difference in the substrates or the nucleophiles (Table 1). In the deacylation of **1a** ($n=0$, 8, 12) with the nucleophiles studied, the deacylation rate of the short chain **1a** ($n=0$) which would bind weakly to the vesicles was low, even though relatively high enantioselectivity was observed in the deacylation with the dipeptide nucleophiles (**3a** and **3b**). Both the deacylation rate and the enantioselectivity were the largest in the case of **1a** ($n=8$) possessing a long *N*-acyl



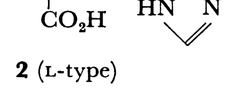
$$CH_3(CH_2)_nCONH\overset{*}{\underset{R}{C}}HCO_2C_6H_4NO_2\text{-}p$$

1

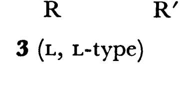
a: $R = C_6H_5CH_2$, $n = 0, 8, 12$
b: $R = (CH_3)_2CHCH_2$, $n = 8$
c: $R = CH_3$, $n = 8$

$$\begin{matrix}C_{12}H_{25}\\C_{12}H_{25}\end{matrix}{>}N(CH_3)_2Br$$

4



2 (L-type)



$$C_{11}H_{23}CONH\overset{*}{\underset{R}{C}}HCONH\overset{*}{\underset{R'}{C}}HCO_2H$$

3 (L, L-type)

a: $R = (CH_3)_2CHCH_2$, $R' = ImCH_2$
b: $R = C_6H_5CH_2$, $R' = ImCH_2$
c: $R = ImCH_2$, $R' = (CH_3)_2CHCH_2$
(Im = imidazolyl)

Table 1. Deacylation rate and enantioselectivity[a)]

Nucleophile		k/l mol^{-1}s^{-1}																
		1a (n=0)				**1a** (n=8)				**1a** (n=12)				**1b** (n=8)				**1c** (n=8)
		25 °C	15 °C	10 °C	4 °C	25 °C	15 °C	10 °C	4 °C	25 °C	15 °C	10 °C	4 °C	25 °C	15 °C	10 °C	4 °C	25 °C
2	L	392	262	200	49	1768	1398	1100	315	1550	738	598	188	1874	1332	1088	110	392
	D	240	146	114	12	380	256	200	26	398	176	144	16	426	254	190	9.0	230
	L/D	1.6	1.8	1.8	4.1	4.7	5.5	5.5	12.1	3.9	4.2	4.2	11.8	4.4	5.2	5.7	12.2	1.7
3a	L	1084	644	542	224	4318	3030	2148	1439	1654	1044	764	264	4118	2704	2126	620	
	D	156	74	56	13	654	386	270	128	538	254	190	52	620	330	260	53	
	L/D	6.9	8.7	9.8	17.2	6.6	7.8	8.0	11.2	3.0	4.1	4.0	5.1	6.6	8.1	8.2	11.7	
3b	L	764	556	436	128	3156	2254	2030	1520	1664	802	546	228	2040	1556	1172	471	
	D	118	54	38	5.4	340	226	172	62	352	154	106	21	360	226	158	37	
	L/D	6.5	10.3	11.5	23.7	9.3	10.0	11.8	24.5	4.7	5.2	5.2	10.9	5.7	6.9	7.4	12.7	
3c	L	122	68	58	17	844	536	456	229	576	326	210	56	722	526	420	37	
	D	120	66	56	6.0	136	68	56	20	90	52	32	2.4	112	72	60	2.8	
	L/D	1.0	1.0	1.0	2.8	6.4	7.9	8.1	11.5	6.4	6.3	6.6	23.3	6.4	6.9	7.0	13.2	

a) Tris buffer (0.083 mol dm^{-3}) containing 0.083 mol dm^{-3} KCl at 4—25 °C (pH 7.68) in 3% (v/v) CH_3CN-H_2O: [**1**]=1×10^{-5} mol dm^{-3}, [**2** or **3**]=5×10^{-5} mol dm^{-3}, and [**4**]=1×10^{-3} mol dm^{-3}.

chain. The suitably long hydrophobic chain of **1a** ($n=8$), **1b** ($n=8$), or **1c** ($n=8$) contributes to the incorporation of **1a** ($n=8$), **1b** ($n=8$), or **1c** ($n=8$) *per se* into the vesicles and intensifies the approximation between the nucleophile and the substrate. However, the substrate **1a** ($n=12$) has an excessively long *N*-acyl chain which might refuse the close approach of the susceptible carbonyl group in **1a** ($n=12$) to the imidazolyl moiety in the nucleophile; thus **1a** showed a decreased rate and lower enantioselectivity. It is unlikely that all parts of the substrate and the nucleophile are incorporated into the vesicle layer out of the aqueous phase. Thus such an interaction between the hydrophobic benzyl group (substrate) and imidazolyl moiety (nucleophile) seems important for making the reacting positions of the nucleophile and the substrate come closer together. In this sense, the order of the deacylation rate and of the enantioselectivity in the deacylation of **1a** ($n=8$), **1b** ($n=8$), and **1c** ($n=8$) with **2**, **1a** ($n=8$)>**1b** ($n=8$)>**1c** ($n=8$), might reflect the extent of the magnitude of the interaction between the hydrophobic imidazolyl part (nucleophile) and the apolar R (R=$C_6H_5CH_2$, $(CH_3)_2CHCH_2$, or CH_3) substituent (substrate).

We studied the structural effects of the nucleophiles. The dipeptide nucleophiles (**3a** and **3b**) including the L-histidine moiety in their terminal position resulted in the large deacylation rate and the high enantioselectivity as compared with those of the nucleophile (**2**). The L-leucine or L-phenylalanine moiety, which is adjacent to the L-histidine part, might intensify the hydrophobic interaction between **3a** or **3b** and the substrate so as to enhance the deacylation rate and the enantioselectivity, even in the deacylation of the short chain substrate **1a** ($n=0$) which is condensed or partly incorporated by the vesicles. The chirality of the neighboring group of L-leucine or L-phenylalanine in **3a** or **3b** is also important, because the use of the D-isomer instead of the above neighboring group decreases both the deacylation rate and the enantioselectivity considerably.

The dipeptide nucleophile **3c**, which includes a L-leucine unit in its terminal position, showed low hydrolyzing activity in the deacylation of the present substrates and resulted in little or no enantioselectivity for the short chain substrate **1a** ($n=0$). Presumably the steric hindrance of the terminal L-leucine part in **3c** against the reaction of **3c** itself with the substrates decreased the deacylation rate (and/or enantioselectivity), especially in the case of the deacylation of the weakly hydrophobic short-chain substrate **1a** ($n=0$). However, the relatively high enantioselection of the hydrophobic, long-chain substrates (**1a** ($n=8$, 12) and **1b** ($n=8$)) by **3c** suggested the contribution of the L-leucine part to the enhancement of enantioselectivity for the long chain substrates which bind strongly to the vesicles. The deacylation rate and enantioselectivity were also influenced by the reaction temperature, which affects the mobility of the substrate and the nucleophile and the liquid crystal-gel equilibrium of the vesicles. As can be seen from Table 1, the lowering of the reaction temperature decreased the deacylation rate and increased the enantioselectivity. These reaction circumstances were well reflected in the binding

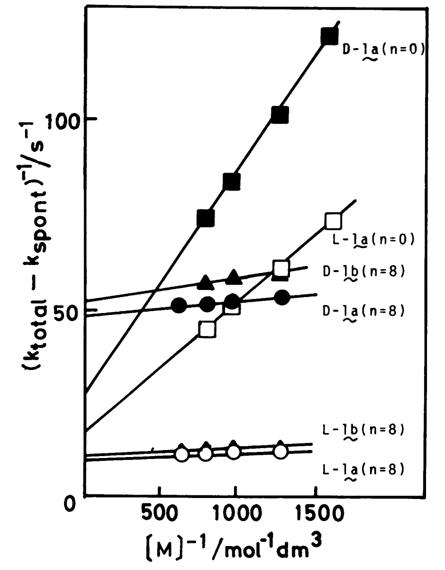


Fig. 1. Typical examples of the linear relationships between the reciprocal values of (k_{total}−k_{spont}) and [M] ([M]=[surfactant]+[nucleophile]−[surfactant]$_{cmc}$). The deacylation of **1** (1×10^{-3} mol dm^{-3}) with **2** ((3.0—7.5×10^{-5} mol dm^{-3}) and **4** ((0.6—1.5)×10^{-3} mol dm^{-3}; [**4**]/[**2**]=20) at 25 °C (pH 7.68).

TABLE 2. KINETIC PARAMETERS[a)]

Nucleophile	parameters[b)]	**1a** (n=0) L	D	**1a** (n=8) L	D	**1b** (n=8) L	D
2	K_b/N	634	538	10245	9448	8031	7878
	$10^2 k_v$	6.1	4.4	11.6	4.0	10.7	2.8
	k_v^L/k_v^D	1.4		2.9		3.8	
3a	K_b/N	1109	766				
	$10^2 k_v$	10.5	2.2				
	k_v^L/k_v^D	4.8					
3c	K_b/N	399	389	4721	2024	4352	3300
	$10^2 k_v$	3.1	3.1	7.0	2.9	5.4	1.6
	k_v^L/k_v^D	1.0		2.4		3.4	

a) The reaction conditions are the same as in Table 1 except [**2** or **3**]=(3—7.5)$\times 10^{-5}$ mol dm^{-3}, [**4**]=(0.6—1.5)$\times$ 10^{-3} mol dm^{-3}, and [**4**]/[**2** or **3**]=20. b) The binding parameters (K_b/N and k_v) are given for the reactions at 25 °C, and the units of parameters are K_b/N (dm^3 mol^{-1}), and k_v (s^{-1}).

constant K_b/N (N is the aggregation number) and the rate constant k_v; these kinetic parameters were obtained in the usual way,[5)] as indicated in Fig. 1, by taking the deacylation of **1a** (n=0, 8) and **1b** (n=8) with **2** as an example. They are listed in Table 2. The fast deacylation rate and high selectivity of the long-chain substrates are well reflected in the large values of K_b/N and k_v^L/k_v^D obtained in the deacylation of **1a** (n=8) and **1b** (n=8) with **2** or **3c**. The dipeptide nucleophile **3a** which possesses a higher enantioselective activity than that of **2** or **3c**, showed large values of K_b/N and k_v^L/k_v^D in the deacylation of short chain substrate **1a** (n=0). Furthermore, the relatively small K_b/N values of the dipeptide nucleophile **3c**, as compared with those of **2**, are in harmony with the low deacylation ability of **3c**, and the negligibly small enantioselection of the short chain substrate **1a** (n=0) by **3c** is also reflected in the markedly small K_b/N and k_v values.

The change of deacylation rate due to the temperature decrease from 25 to 4°C was expressed by closely linear Arrhenius plots in which all the plots obtained at 4°C deviated from linear Arrhenius plots (Fig. 2). Such a deviation of the plots at 4°C from the Arrhenius relation might be related to the liquid crystal-gel phase transition.[6)] The temperature decrease from 10 to 4°C remarkably increased the enantioselectivity with the maximum enantiomer rate ratio 24.5 in the hydrolysis of **1a** (n=8) by the efficient dipeptide nucleophile **3b**. The characteristic features of the present reaction were also reflected in the activation parameters (ΔH^* and ΔS^*) for the reaction at 10—25 °C (Table 3); that is, the smaller (or negatively larger) ΔH^* (or ΔS^*) values of the L-substrates, as compared with those of the D-substrates, indicate the preferential attack of the nucleophile on the L-substrate, and the small ΔH^* (or ΔS^*) values for the deacylation of **1a** (n=8) or **1b** (n=8) supported the intensified interaction between the efficient dipeptide nucleophile and the substrates possessing the N-acyl chain of appropriate length (n= 8). In this respect, the relatively low deacylation rate of **1a** (n=12) was reflected in the relatively large ΔH^* or ΔS^* values of **1a** (n=12). These aspects of the present reaction were also deduced from the isokinetic relation between the ΔH^* and ΔS^* values (Fig. 3). The extent of enantioselectivity can be seen in the difference of the

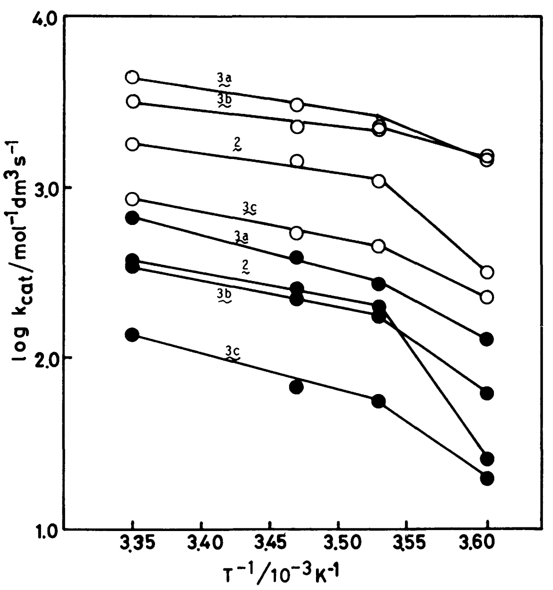


Fig. 2. Typical examples of Arrhenius plots (the deacylation of **1a** (n=8) with **2** or **3**).
○: L ●: D

TABLE 3. ACTIVATION PARAMETERS[a)]

No.	Nucleophile	Activation parameter	**1a** (n=0) L	D	**1a** (n=8) L	D	**1a** (n=12) L	D	**1b** (n=8) L	D
1	**2**	ΔH^*	6.9	7.8	4.6	6.5	10.3	11.1	4.7	5.9
		ΔS^*	−43.3	−41.3	−48.0	−44.4	−28.9	−29.2	−47.7	−46.7
2	**3a**	ΔH^*	7.3	11.0	7.0	9.2	7.9	11.2	6.8	9.3
		ΔS^*	−39.8	−31.2	−38.1	−34.4	−36.2	−28.2	−39.0	−34.4
3	**3b**	ΔH^*	5.6	12.2	4.4	6.9	11.9	12.9	5.4	8.5
		ΔS^*	−46.2	−27.9	−47.3	−43.3	−23.7	−23.2	−44.9	−38.1
4	**3c**	ΔH^*	8.0	8.1	6.9	9.6	10.9	19.4	5.8	7.5
		ΔS^*	−41.7	−41.4	−41.6	−36.2	−28.9	−2.9	−45.6	−43.5

a) Obtained for the reaction performed under the conditions in Table 1 at 10—25 °C. The units are kcal/mol for ΔH^* and e.u. for ΔS^* (1 cal=4.184 J).

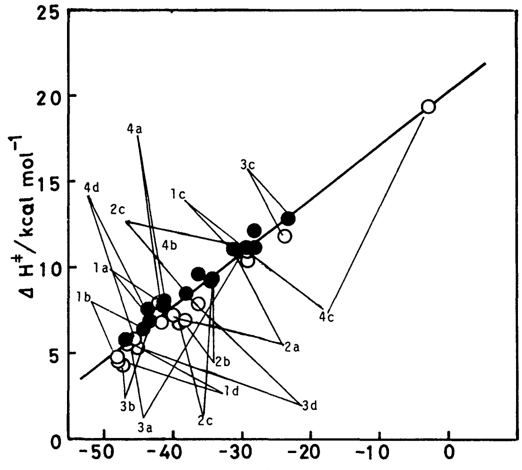


Fig. 3. A isokinetic relation obtained for the deacylation of **1a** (n=0—12) and **1b** (n=8) with **2** and **3**.
○: L, ●: D; a. **1a** (n=0), b. **1a** (n=8), c. **1a** (n=12), d. **1b** (n=8); 1. **2**, 2. **3a**, 3. **3b**, 4. **3c**.

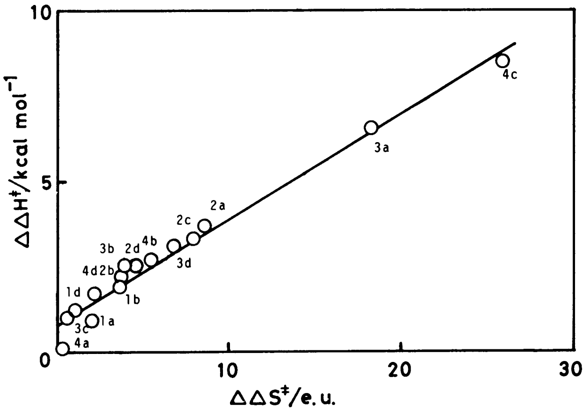


Fig. 4. A linear correlation between $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values.
(Notations are the same in Fig. 3).

activation parameters between the enantiomers ($\Delta\Delta H^{\ddagger} = \Delta H^{\ddagger}_{\mathrm{D}} - \Delta H^{\ddagger}_{\mathrm{L}}$ or $\Delta\Delta S^{\ddagger} = \Delta S^{\ddagger}_{\mathrm{D}} - \Delta S^{\ddagger}_{\mathrm{L}}$). The high enantioselectivity was well reflected in the large $\Delta\Delta H^{\ddagger}$ (or $\Delta\Delta S^{\ddagger}$) values, and there is an approximately linear correlation between the $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values (Fig. 4).

The present vesicular system including the dipeptide nucleophile exhibited a relatively high enantioselectivity through the efficient interaction between the L-phenylalanyl(or L-leucyl)-L-histidine moiety in the nucleophile and the hydrophobic substrates incorporated (or condensed) by the vesicles.

Experimental

Materials. N^{α}-dodecanoyl-L-histidine (**2**) and N-(N^{α}-dodecanoyl-L-histidyl)-L-leucine (**3c**) were obtained by the acylation of commercially available L-histidine and L-histidyl-L-leucine with dodecanoyl chloride.[7,8] N^{α}-(N-dodecanoyl-L-leucyl)-L-histidine (**3a**) and N^{α}-(N-dodecanoyl-L-phenylalanyl)-L-histidine (**3b**) were prepared from N-hydroxysuccinimide ester of N-benzyloxycarbonyl-L-leucine and N-benzyloxycarbonyl-L-phenylalanine by the coupling with L-histidine, the hydrobromination of the N-benzyloxycarbonyl group, and then the acylation of the NH_2HBr group with dodecanoic anhydride. Satisfactory elemental analyses were obtained for the nucleophiles, and those for new compounds are shown below. **3a**: Found: C, 63.18; H, 9.39; N, 12.30%. Calcd for $C_{24}H_{42}N_4O_4$: C, 63.97; H, 9.39; N, 12.43%. **3b**: Found: C, 66.32; H, 8.74; N, 10.50%. Calcd for $C_{27}H_{40}N_4O_4$: C, 66.91; H, 8.32; N, 11.56%. **3c**: Found: C, 63.81; H, 9.17; N, 12.33%. Calcd for $C_{24}H_{42}N_4O_4$: C, 63.97; H, 9.39; N, 12.43%.

The ester substrates (**1**) were prepared by the method described previously,[9] and the double chain surfactant **4** was obtained by the reaction of N,N-dimethyldodecylamine and dodecyl bromide.[9] Satisfactory elemental analyses were also obtained for **1** and **4** (C, H, N).

Hydrolysis and Kinetic Measurements. The hydrolysis of 1×10^{-5} mol dm^{-3} substrates (**1**) by the vesicles of 5×10^{-5} mol dm^{-3} nucleophile (**2** or **3**) and 1×10^{-3} mol dm^{-3} surfactant (**4**) were carried out at 4—25 °C, pH 7.68 in 0.083 mol dm^{-3} tris(hydroxymethyl)aminomethane buffer involving 0.083 mol dm^{-3} KCl ($\mu = 0.083$) in H_2O including 3 vol% CH_3CN, where the stock solutions were prepared by dissolving **2** (or **3**) and **4** in the buffer by sonication at 45 °C for 1 h.

The hydrolysis rate, which obeyed a good pseudo-first-order rate law (up to *ca.* 80% conversion), was followed by the spectrophotometric determination of p-nitrophenolate (400 nm). The second-order catalytic rate constant k was evaluated from the pseudo-first-order rate constants k_{total} (obtained with the nucleophile) and k_{spont} (obtained without the nucleophile) in the usual way ($k = (k_{\mathrm{total}} - k_{\mathrm{spont}})/[\text{nucleophile}]$). The binding constant K_b/N and the rate constant k_v were obtained (correlation coefficient>0.990) in the deacylation of 1×10^{-5} mol dm^{-3} substrate (**1**) by the vesicles of $(3-7.5) \times 10^{-5}$ mol dm^{-3} nucleophile (**2** or **3**) and $(0.6-1.5) \times 10^{-3}$ mol dm^{-3} surfactant (**4**) ([**4**]/[**2** or **3**] = 20) at 25 °C (pH 7.68) in the usual way with the following simplified reactions.[5]

$$\mathrm{S} + \mathrm{V} \overset{K_b}{\rightleftharpoons} \mathrm{SV} \xrightarrow{k_v} \mathrm{V} + \mathrm{P}$$

$$\mathrm{S} \xrightarrow{k'} \mathrm{P},$$

where S = substrate, V = vesicles, SV = substrate-vesicle complex, and P = p-nitrophenolate.

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