Biochimica et Biophysica Acta 814 (1985) 85–95
Elsevier

BBA 72499

Latency studies on rat liver microsomal glucose-6-phosphatase. Correlation of membrane modification and solubilization by Triton X-114 with the enzymatic activity *

Hans-Ulrich Schulze, Renate Kannler and Beate Junker

Biochemisches Institut der Justus Liebig-Universität, D6300 Giessen, Friedrichstrasse 24 (F.R.G.)

(Received September 3rd, 1984)

Key words: Glucose-6-phosphatase; Enzyme isolation; Latency; Detergent-membrane interaction; Membrane modification; Triton X-114; (Rat liver microsome)

Interrelationships between the catalytic properties of glucose-6-phosphatase and the membrane structure of rat liver microsomes were investigated. (1) Membrane modification and solubilization employing the nonionic surfactant Triton X-114 were standardized and analysed by ultracentrifugation, surface tension- and turbidity measurements. (2) The effect of Triton X-114 on the glucose-6-phosphatase activity was studied systematically and the whole magnitude of time- and temperature-dependent inactivation of this enzyme has been demonstrated. The results show that the activity measured is always a resultant of two processes, the beginning of inactivation and the release of latency. Maximal activation of about 600% (83% of apparent latency) was obtained at 0°C. (3) A correlation between membrane modification and solubilization and the conditions under preincubation and test incubation reveals that studies on detergent-disrupted microsomes are performed on structures reassembled from solubilizates and this implies a modified microenvironment in the reconstitutes. (4) Kinetic analyses suggest interrelationships between Triton X-114 and the permeability barrier of the glucose-6-phosphatase system. (5) At 0°C 2-propanol and ethanol are more potent tools for membrane modification than Triton X-114 and release 88% and 86% latent activity corresponding to an activation of the glucose-6-phosphatase of about 850% and 700%, respectively. These observations suggest that detergent treatment of microsomes could not preserve the functional integrity of the glucose-6-phosphate phosphohydrolase, which is one dogma of the substrate-transport hypothesis developed by Arion and his co-workers (Arion, W.J., et al. (1975) Mol. Cell. Biochem. 6, 75–83).

Introduction

The latency ** of rat liver glucose-6-phosphatase, an integral enzyme protein, localized mainly within the endoplasmic reticulum membranes, is very well documented and characterized both by an increase of V_{max} and a decrease of K_m [1,2]. Consequently, the activation of this enzyme observed in the presence of a broad variety of detergents is a function of the substrate concentration. At 30°C, it was estimated as approximately 150%–210%, respectively [3–10]. The latency has been rationalized with an activation of the glucose-6-phosphatase as an expression of changes in the conformational status of the enzyme protein [1,4,11] or with the involvement of a substrate

* Dedicated to Professor Dr. Hj. Staudinger on the occasion of his 70th birthday.

** Latency is calculated as 100 × (activity in disrupted microsomes − activity in untreated microsomes)/activity in disrupted microsomes [2].

carrier either penetrating the microsomal membrane as an independent protein (see, for example, Refs. 2 and 10) or being constitutive with the enzyme and situated on the cytoplasmic surface [9,12], limiting the hydrolysis on the luminal side [2,10] or within the membrane [9,12] and bypassed as a result of detergent treatment.

However, all tensides tested up to now were found to be able not only to release the latent activity of glucose-6-phosphatase but also to solubilize rat liver microsomal membranes which process is concomitant with inactivation of this enzyme in all cases [1,4,13].

In order to analyse the apparent biphasic effect of detergents and to optimize the conditions releasing maximal latency, we investigated the correlation between these two phenomena. Furthermore, this systematic study has become necessary, because previously there has been insufficient consideration of the fact that activation of glucose-6-phosphatase is superimposed by temperature and time-dependent inactivation, even if very low amounts of detergent are used. Therefore, the enzyme activity measured for the time being is always a resultant of both processes which implies that the real degree of latency has not yet been discovered.

An additional aim of the present communication was to point out the equally important and likewise inadequately appreciated fact that many meticulous kinetic measurements performed on detergent-disrupted microsomes reflect the properties of a partially inactivated enzyme protein in a substantially modified membrane microenvironment.

In our study, we estimated the maximal latency of glucose-6-phosphatase at substrate saturation and at 0°C using Triton X-114 and organic solvents to 83% and 88% corresponding to an activation of up to about 600% and 850% of untreated microsomes, respectively. The nonionic surfactant Triton X-114 was chosen since Triton X-114 was found to be one of the most mildly acting tensides [14].

Materials and Methods

Glucose 6-phosphate, mannose 6-phosphate and reduced pyridine nucleotides are obtained from Boehringer Mannheim GmbH (F.R.G.), and Triton X-114 from Serva (Heidelberg, F.R.G.). Organic solvents and all the other reagents were of analytical and HPLC grade, respectively. Trypsin-solubilized cytochrome b_5 was isolated as an electrophoretically pure protein [15] from pig liver microsomes according to the technique of Strittmatter [16].

Preparation of microsomes. Male Wistar rats (AF/Han.) weighing about 250 g and fasted for 15 h were used. Microsomes were prepared by differential centrifugation in a solution containing 0.25 M sucrose, 1 mM EDTA buffered at pH 7.4 with 20 mM Tris-HCl, washed in 1.18% KCl, resuspended in 0.1 M Tris-HCl (pH 7.4) [17] and employed immediately for all experiments. 'Intactness' of the microsomal preparations was assessed by the latency of the 'low-K_m' mannose-6-phosphate phosphohydrolase activity [18] using 2 mM substrate.

Surface tension measurements. Surface tension measurements were performed at 0°C, 15°C and 30°C using a Lecomte du Noüy thermostatized interfacial tensiometer (Krüss, Hamburg, F.R.G.). All samples were suspended in deionized and double-distilled water (final volume of 20 ml), allowed to equilibrate with the tensiometer ring for 3 min after gentle stirring as soon as the temperature chosen was reached. Prolonged equilibration has no significant effect on the surface tension measured throughout the range of the surfactant concentrations employed.

Enzyme assays. The activity of the NADH : ferricytochrome-b_5 oxidoreductase was measured at 30°C as described previously [19]. Glucose-6-phosphatase activities were determined in 175 mM imidazole-HCl buffer (pH 6.5, measured at 20°C) [20] containing 12.5 mM Tris-HCl, 0.2 mg microsomal protein, and, if not otherwise stated, 20 mM glucose 6-phosphate in a final volume of 0.8 ml. Triton X-114 concentrations are specified in the figures. Product production was a linear function of incubation time under all the conditions reported. The specific enzyme activities ($nmol \cdot min^{-1} \cdot (mg\ protein)^{-1}$) are expressed as oxidized NADH and released inorganic phosphorus, respectively.

Analytical procedures. Protein was assayed by the biuret method of Bode et al. [21]. The phos-

pholipids were extracted with chloroform/methanol (2:1, v/v) [22] and determined by inorganic phosphate analysis [23]. Experimental details are described in Ref. 24. High performance liquid chromatography was carried out with a Pye Unicam Liquid Chromatograph (Serie PU 4000) equipped with a computing integrator CD P4 from Philips. The column used was a 4 mm i.d. × 25 cm LiChrospher Si 100 (10 μm) from Merck, Darmstadt, F.R.G. The solvents were filtered and degassed through Schleicher and Schüll filters (RC 55, 0.45 μm). The water was deionized and double-distilled before use.

Reproducibility. All experiments were repeated at least six times. The results presented in Fig. 1B, in Figs. 4 and 6 and in the individual ones of Figs. 5, 7, 8 were always obtained from a single microsomal preparation and are therefore directly comparable. The lines of Fig. 1A are strongly reproducible with one charge of Triton X-114, but small differences of the critical micelle concentration (CMC) estimated [25] were observed in comparison with other charges of the same tenside [14] and with different microsomal preparations. Fig. 3 contains typical chromatograms obtained with the ring film material of the experiments presented in Fig. 1B. Figs. 1B, 4 and 6 represent typical results and the curves of Figs. 5, 7, 8 are obtained from data of that experiment of a series performed with microsomes more than 97% intact, which released the greatest latency. In principle, experiments with microsomes less than 97% intact under our conditions were omitted.

Results and Discussion

Treatment of rat liver microsomes with Triton X-114

Modification and solubilization of rat liver microsomal membranes by the nonionic surfactant Triton X-114 is dependent on temperature, the concentration of detergent, and the Triton/protein ratio used. A significant increase of the CMC with decreasing temperature, as obtained by surface tension measurements, is illustrated in Fig. 1A and in agreement with results reported for other tensides and measured by other methods [25,26]. Fig. 1B shows the influence of microsomes, expressed as the protein concentration, on the surface tension and the actual CMC of Triton X-114

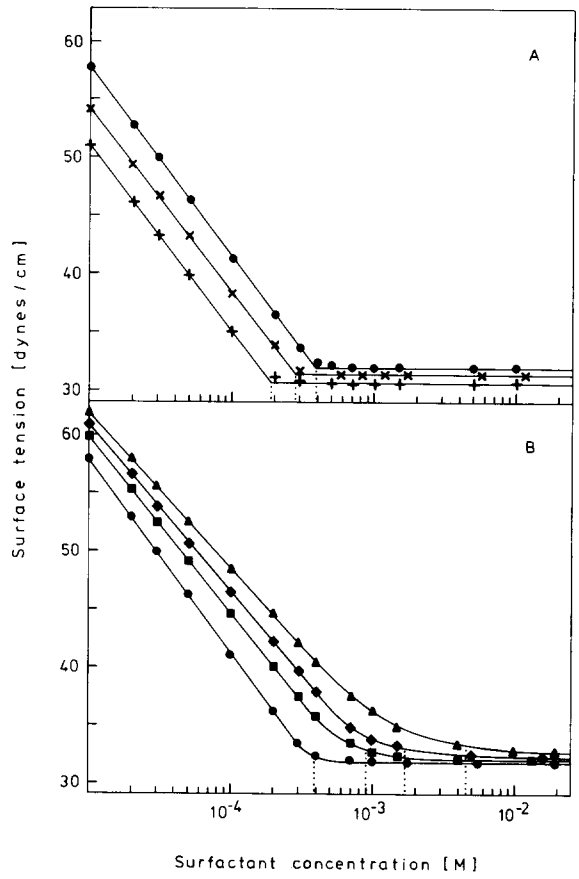

Fig. 1. Critical micelle concentrations (CMC) of Triton X-114 solutions. Semilog plot of the surface tension in aqueous solutions as a function of surfactant concentration. The CMC values are established where the change of surface tension with surfactant concentration approaches zero [25] and indicated by vertical dotted lines. (A) At different temperatures (°C); ●, 0; × 15; +, 30. (B) At 0°C in the presence of various amounts of microsomes (mg protein/ml) employing the supernatant after centrifugation (60 min; $106000 \times g_{max}$): ●, 0; ■, 0.15; ◆, 0.25; ▲, 0.5.

solutions at 0°C. The data permit at least rough estimation of the amount of tenside incorporated into the membranes and bound to proteins and lipids already released from them and the proportion remaining free in solution at every partition equilibrium below CMC of the detergent (Fig. 2). The admissibility of this approach is demonstrated by high performance liquid chromatograms (Fig. 3) of the ring film material which verify the decrease of the free detergent concentration in the presence of microsomes up to values of the corre-

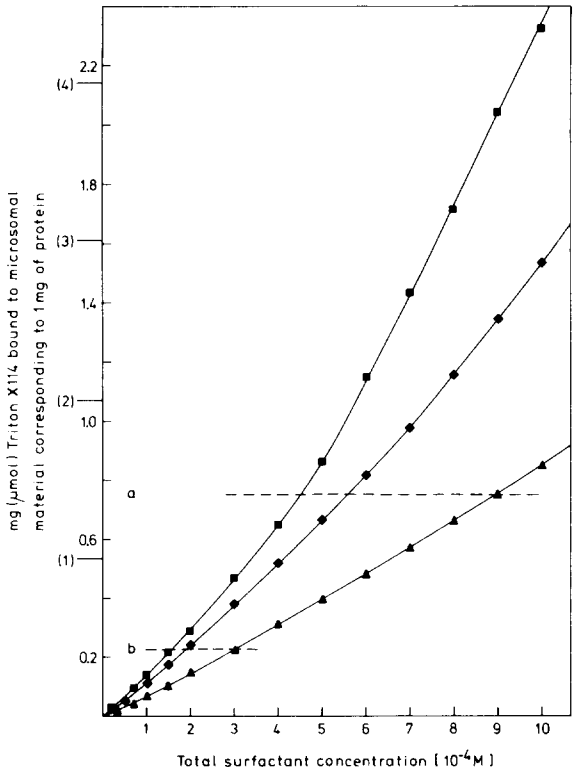

Fig. 2. Binding of Triton X-114 to microsomal components at 0°C. Isotherms at various amounts of microsomes corresponding to 0.15 (■), 0.25 (◆) and 0.5 (▲) mg microsomal protein/ml. The calculations were performed from the data of Fig. 1B assuming that the surface tension measured below the discontinuity reflect, at least approximately, the free monomer equilibrium concentration of surfactant in the presence of microsomes. According to this approach, at each surface tension the difference of the Triton X-114 concentrations with and without microsomes corresponds to the amount of detergent incorporated into the membranes and used for the formation of mixed micelles of the microsomal material already released. The dashed lines, reflecting the same degree of membrane modification, intersect the isotherms at decreasing Triton/protein ratios; line a at 1.6, 1.2, 0.95 and line b at 0.55, 0.4, 0.32, respectively. The Triton/protein ratio was determined to standardized the Triton/membrane ratio.

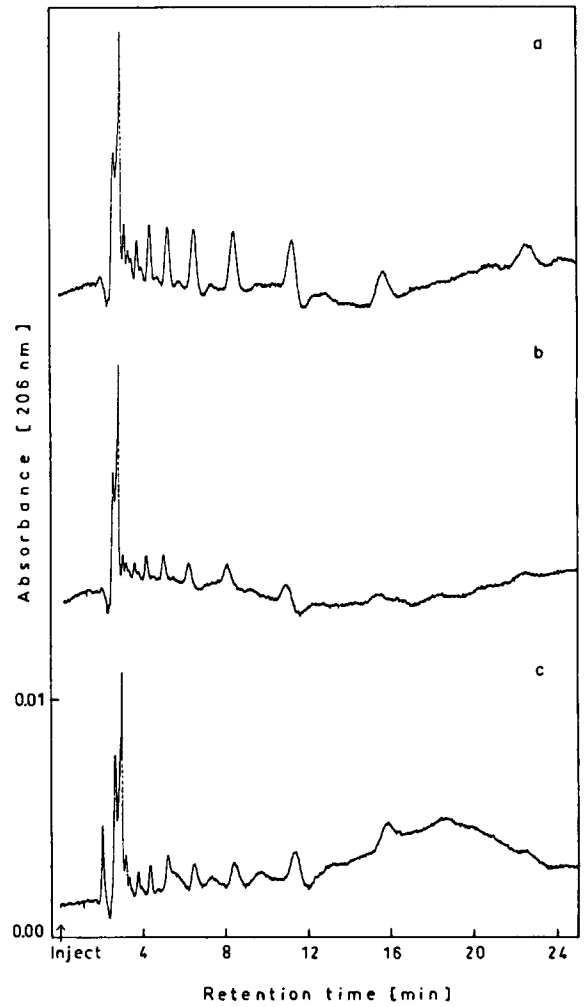

Fig. 3. High performance liquid chromatograms of Triton X-114. Eluent, hexane/2-propanol/water (7:2.5:0.25, v/v); flow rate, 1 ml/min; detection, ultraviolet-absorption at 206 nm at 0.03 units full scale; loaded volume, 7 μl; calibration, 10^{-9}–10^{-8} mol Triton X-114 in water; samples, ring film material of the experiments presented in Fig. 1B, supernatant of Triton X-114 suspensions: a, $4 \cdot 10^{-4}$ M; b, $1.6 \cdot 10^{-4}$ M without and c, $4 \cdot 10^{-4}$ M with microsomes (0.25 mg/ml) after centrifugation (60 min, 106000 × g_{max}).

sponding surface tension of the pure tenside. As expected, the microsomal components change the course of the trace progressively with increasing solubilization and the rather narrow concentration range of the CMC of Triton X-114 becomes wider, the higher the proportion of solubilized material in the surface phase.

It can be seen from Fig. 2 that the same degree of membrane modification induced by any given amount of detergent incorporated is achieved at different Triton/protein ratios and the values decrease with increasing concentration of microsomes (dashed lines). Hence it follows that for evaluation and reproduction of experiments resulting in changes of the molecular organization within the membrane structure, such as latency studies on

glucose-6-phosphatase, the detergent/protein ratio should be taken into consideration and stated in additional to the detergent concentration employed.

An operational criterion for the complex process of membrane solubilization is the gradual dissociation of lipids and proteins, if the Triton/protein ratio is continuously increased. Fig. 4A shows the separation of microsomal membrane components by ultracentrifugation as estimated by analysing the sedimentable material. Whereas low

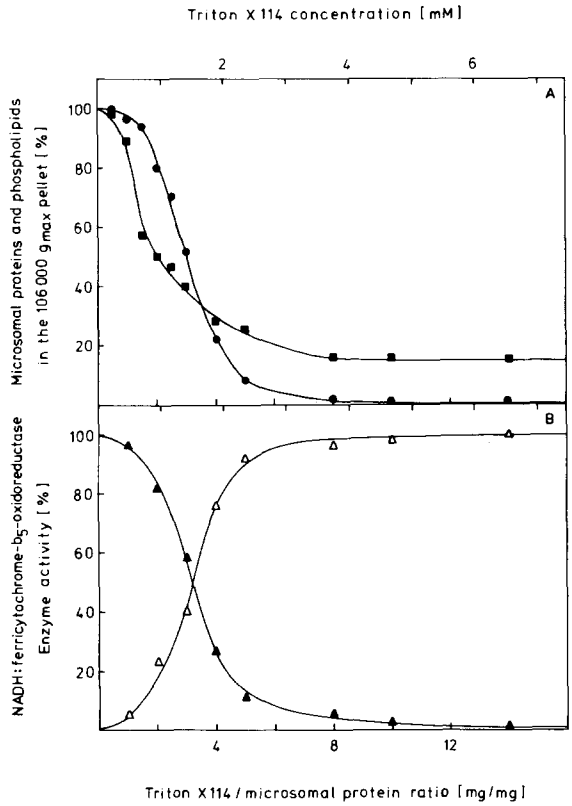

Fig. 4. Modification and solubilization of microsomal membranes by Triton X-114. Microsomes (30 mg protein) were preincubated for 10 min at 0°C in 0.1 M Tris-HCl (pH 7.4) (final volume of 120 ml) containing amounts of Triton X-114 to give the surfactant concentrations and the corresponding Triton/protein ratios as indicated. Thereafter, the samples were centrifuged at $106000 \times g_{max}$ for 60 min in a Spinco ultracentrifuge (rotor 30). The supernatants were collected, the pellets carefully rinsed and resuspended in 0.1 M Tris-HCl (pH 7.4). (A) Dissociation of proteins (■) and phospholipids (●) was determined by analysing the pellets. (B) Solubilization of NADH: ferricytochrome-b_5 oxidoreductase activity in the pellet (▲) and the supernatant (△).

concentrations of detergent solubilize relatively more proteins, higher concentrations of detergent solubilize relatively more phospholipids. The point of intersection of both curves (Triton/protein ratio of approx. 3.7 for 0.25 mg protein/ml) coincides with the range of the CMC (approx. 1.7 mM, Fig. 1B) for Triton X-114 at 0°C in the presence of the same amount of microsomes and is interpreted to indicate that the complete disintegration of the membrane structures is just taking place. The end point of solubilization is obtained at a Triton/protein ratio of 10; almost all of the phospholipids are in the supernatant and no significant additional release of membrane proteins is observed upon further addition of detergent. About 15% of the microsomal membrane proteins are not to be solubilized by Triton X-114, presumably because isoelectric precipitation is unaffected by the non-ionic detergent under the pH and ionic strength used [27].

Fig. 4B shows the solubilization of NADH: ferricytochrome-b_5 oxidoreductase which is a typical integral membrane protein of the endoplasmic reticulum [16]. The results are plotted as percentage of the total enzyme activity and a correlation with the solubilization of the membrane itself is stringent.

Effects of Triton X-114 on the glucose-6-phosphatase activity

The results of this series of experiments are presented in Fig. 5. It can be seen that latency of glucose-6-phosphatase at optimal Triton/protein ratio varied from 0 to 83% depending on the temperature and time of preincubation. In principle, prolonged preincubation continuously decreased the activation achieved with Triton X-114 for any given temperature. On the other hand, at the same time of preincubation, the activation increased with decreasing temperature. Thus, a maximal latency of 83% corresponding an activation of 600% compared to the untreated controls is released at 0°C * and short-time preincubation.

* It should be mentioned here that Arion and Walls [28] recently published similar results. However, these were obtained indirectly correcting the low degree of intactness of the guinea pig liver microsomes used. The apparent latency directly measured was only approx. 66%.

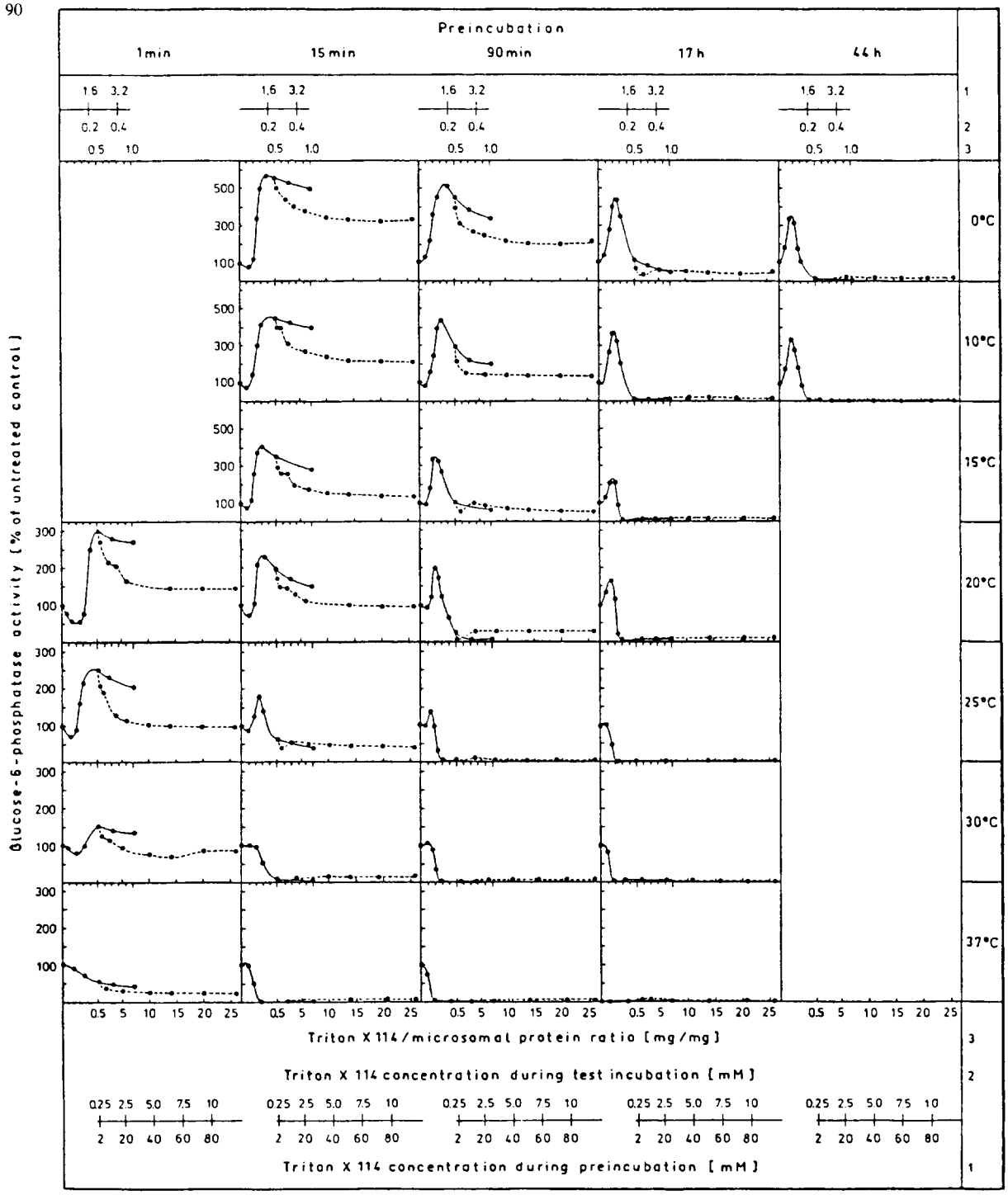

Fig. 5. Effects of Triton X-114 on the glucose-6-phosphatase activity. Microsomes (2 mg protein/ml) were preincubated at 0°C and (for times indicated) in 0.1 M Tris-HCl (pH 7.4) containing varying concentrations of Triton X-114 corresponding to Triton/protein ratios given in the abscissae 1 and 3, respectively. Aliquots (0.1 ml) were transferred to assay tubes containing 0.7 ml of a medium composed to 16 μmol of glucose 6-phosphate and 0.2 M imidazole-HCl (pH 6.5). (Triton concentrations during test incubation in the final volume of 0.8 ml are given in abscissa 2.) Thereafter, the enzyme activity was measured at temperatures indicated for 15 min (at 0°C for 60 min). Upper abscissae for solid line curves and lower abscissae for dashed line curves reflecting the Triton/protein ratios up to 1.0 and from 0.5 to 25, respectively. Ordinates: note the different scale. Untreated controls are microsomes without Triton supplementation but treated in the same way as the samples. 100% activity corresponds to 7 (0°C), 25 (10°C), 48 (15°C), 90 (20°C), 162 (25°C), 240 (30°C), 490 (37°C) mU/mg protein at 15 min of preincubation.

Furthermore, the Triton/protein ratio releasing maximal latency at any temperature measured, decreased progressively with increasing time of preincubation. The same tendency was observed with increasing temperature at any time of preincubation.

Taken together, the results clearly indicate the whole magnitude of time- and temperature-dependent inactivation of glucose-6-phosphatase by Triton X-114 as a function of the detergent/protein ratio; some details have been known for other amphiphiles since the work of Stetten and Burnett [4] and differently evaluated in the subsequent literature (e.g., Refs. 1, 5, 9). The activity of glucose-6-phosphatase measured in the presence of detergent is always a resultant of two processes, the beginning of inactivation and the release of latency. These result in a modest activation under the conditions usually used (pH 6.0–6.5, 30°C, 5–20 min). Therefore, studies on detergent-treated microsomes are at all events performed on a more or less altered enzyme protein.

In order to minimize the significant detergent-dependent inactivation of glucose-6-phosphatase during prolonged preincubation (Fig. 5) at pH 6.5, the microsomes are pretreated throughout with Triton X-114 (concentrations given in Fig. 5) at pH 7.4. For the enzyme test, the detergent-supplemented microsomes are added to the assay medium (pH 6.5) with the result that both microsomes and detergent are diluted 8-fold. After dilution, a large part of detergent required to reach the equilibrium monomer concentration in the test medium releases the co-micelles of already solubilized material and, if still existent, the membranes themselves, resulting necessarily in reconstitution of intermediate structures corresponding to the drastically lowered detergent concentration. The line b in Fig. 2 reveals that at a Triton/protein ratio of 0.4, releasing maximal latency at 0°C in the test medium (0.19 mM Triton X-114, 0.25 mg protein/ml, Fig. 5), about 0.2 mg Triton/mg protein are incorporated into the microsomes whilst under preincubation (1.5 mM Triton X-114, 2 mg protein/ml, Fig. 5) it should be significantly more. This is a compelling consequence of the 'membrane-modification isotherm' (dashed line b in Fig. 2) which convincingly demonstrates that the Triton/protein ratio decreases if the protein concentration increases and a result of turbidity measurements presented in Fig. 6. The coincidence of the curves of Fig. 4A and 6A can be taken as evidence for the correlation between turbidity of microsomal suspensions and membrane solubilization. The data therefore corroborate the low degree of modification of the microsomes during test incubation (0.19 mM Triton X-114, Fig. 6A). On the other hand, they disclose the advanced disintegration process under the conditions of preincubation (1.5 mM Triton X-114, Fig. 6B) which corresponds with a situation near the breakdown of the membrane (Fig. 4A). When, however, at a dilution of 10-times starting with a sample containing 1.8 mg protein/ml and 0.4% taurocholate (indicated in Fig. 6C) 10 mg defatted albumin per ml test solution are additionally present (e.g. Ref. 18) and the detergent/protein ratio is decreased, the loss of detergent from the microsomal components must be much greater and finally a more complete reconstitution occurs probably including the insertion of mixed detergent-albumin micelles.

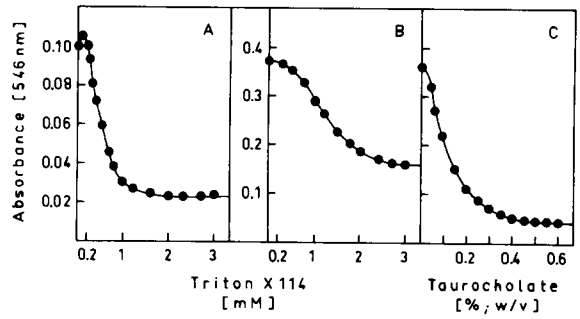

Fig. 6. Turbidity measurements. The turbidity of microsomal suspensions (0.1 M Tris-HCl, pH 7.4) was measured (15°C, 5 min equilibration, 10 mm (A) and 5 mm (B, C) cuvettes) as a function of surfactant concentration at 546 nm. Triton X-114: 0.25 mg protein/ml (A) and 2 mg protein/ml (B). Taurocholate: 1.8 mg protein/ml (C).

This analysis demonstrates that studies on glucose-6-phosphatase measured in this way (as is mostly the case) are performed on structures randomly reassembled from solubilizates without necessarily regaining the original orientation of the molecules. In addition to earlier considerations of Nordlie [1] and Gunderson and Nordlie [11], it suggests that the results might indeed reflect changed properties of the enzyme in such a virtually modified microenvironment.

Kinetic analyses of Triton-treated microsomes

Fig. 7 represents the results of kinetic experiments in which the apparent Michaelis constants, K_m, and the values for maximal velocity, V_{max}, of glucose-6-phosphatase are compared as a function of Triton/protein ratio over the whole range of detergent concentrations used in solubilization and latency studies and at different temperatures. In contrast to Fig. 5, the data have been obtained without preincubation; the assay was started by addition of microsomes to the test medium containing different levels of Triton X-114 which immediately [26] modifies the membranes devoid of reconstitution. The rapid process of modification was controlled by means of turbidity measurements (data not shown).

The relevant data are summarized: (1) Low concentrations of Triton X-114 around 0.2 mM (Triton/protein ratio of approx. 0.4) increase the K_m to maximal values while V_{max} is almost simultaneously depressed to a minimum (at approx. 0.14 mM), which is also recognizable in Fig. 5 and was partly observed previously for other tensides [4,29]. (2) Increasing concentrations up to approx. 0.32 mM (Triton/protein ratio of approx. 0.7) result in very fast acceleration of V_{max} concomitant with retarded slope of K_m to reach minimal values close to 0.5 mM (Triton/protein ratio of approx. 1). (3) Between approx. 0.5 mM and 2.5 mM (including the Triton/protein ratio of 3.7, ≈ CMC, Fig. 1B) V_{max} is quite quickly lowered by about 30% while K_m increases slightly. Finally, (4) the interval up to 12 mM (Triton/protein ratio of approx. 25) is characterized by slow but continuous decrease of V_{max} and nearly constant K_m. The results suggest the following sequence of events as the equilibrium concentration of Triton X-114 is increased. (1) The detergent influences the permeability barrier (carrier moiety) and diminishes the substrate transport as the rate-limiting step of microsomal glucose 6-phosphate hydrolysis and the affinity for glucose 6-phosphate as well. (2) The obstruction of the barrier (inhibited carrier) is progressively eliminated (bypassed), the latency released up to the catalytic rate of the substrate-saturated hydrolase and its higher or changed substrate affinity are measured. (3) Along with increasing micellization (Figs. 1B, 4, 6) of membrane components, the hydrolase is (mixed) inhibited.

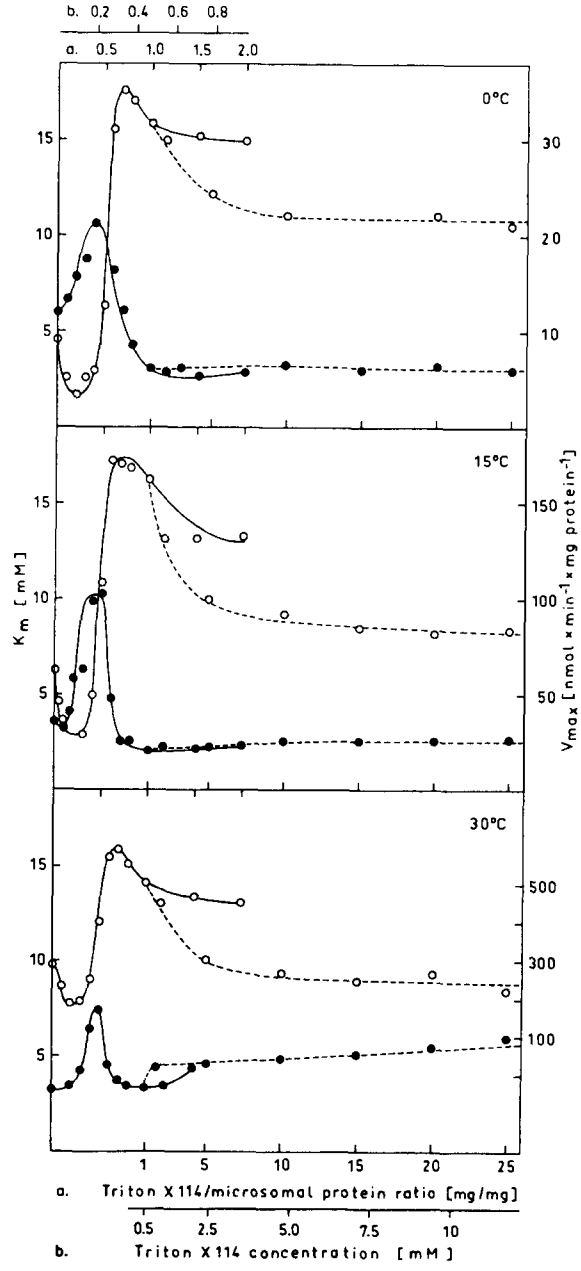

Fig. 7. Kinetic analyses of Triton X-114-treated microsomes. Incubations were carried out at 0°C (60 min), 15°C (15 min), and 30°C (15 min) and started by the addition of 0.1 ml microsomes (0.2 mg of protein) in 0.1 M Tris-HCl (pH 7.4) to the assay medium containing in a final volume of 0.8 ml 175 mM imidazole-HCl (pH 6.5) Triton X-114 between 0 (control) and 12.1 mM as indicated, and 1, 1.5, 2, 3, 5, 10, and 20 mM glucose 6-phosphate, respectively. Upper abscissae for solid line curves and lower abscissae for dashed line curves reflecting the Triton/protein ratios up to 2.0 and from 1 to 25, respectively. Ordinates: K_m (●) and V_{max} (○).

Since the affinity is initially still increased, the inhibition apparently starts before the full latency is released. The inhibition is reversible for some time, at least partially, as demonstrated by albumin supplementation [5] and ultrafiltration experiments (Schulze, unpublished results). (4) Micellar concentrations of detergent (> 1.8 mM) solubilizing completely the membranes (Fig. 4) resulted in formal noncompetitive inhibition although a marked enzyme protection within the mixed tenside-lipid-protein micelles occurs which is also seen in Fig. 5 with short-term preincubation, especially at lower temperatures. This indicates that the unspecific phosphohydrolase as created by interactions of detergent with (97% intact) microsomes is, in principle, independent of a fixed membrane structure itself; mixed micelles as occurring in microsomal solubilizates are obviously capable of serving as matrix for a catalytic active enzyme protein, at least for a limited time. It should be noted too that the K_m in such an unphysiological microenvironment is nearly unchanged compared to the K_m in reconstituted structures obtained after dilution of the preincubated microsomes for the enzyme test (as in Fig. 5; Schulze, unpublished results).

Effect of organic solvents on the glucose-6-phosphatase activity

Owing to their ability to separate membrane lipids from protein [30], organic solvents have been utilized for the modification of microsomal membranes. Fig. 8 shows the effects of several alcohols on the activity of glucose-6-phosphatase including the pattern of behavior at different temperatures as a function of concentration at fixed protein content. In comparison with Triton X-114, two conspicuous differences should be emphasized. First of all, 2-propanol and ethanol at 0°C are more potent tools for releasing latency than Triton X-114. This provides further evidence for the instability of glucose-6-phosphatase in the presence of detergents even at lower temperatures, unless one wishes to take into account some changes in the active conformational status of the enzyme protein, e.g. by weakening of intramolecular hydrophobic bonds. Secondly, as the profiles in Fig. 8 also show, low concentrations of the alcohols used give a more differentiated response;

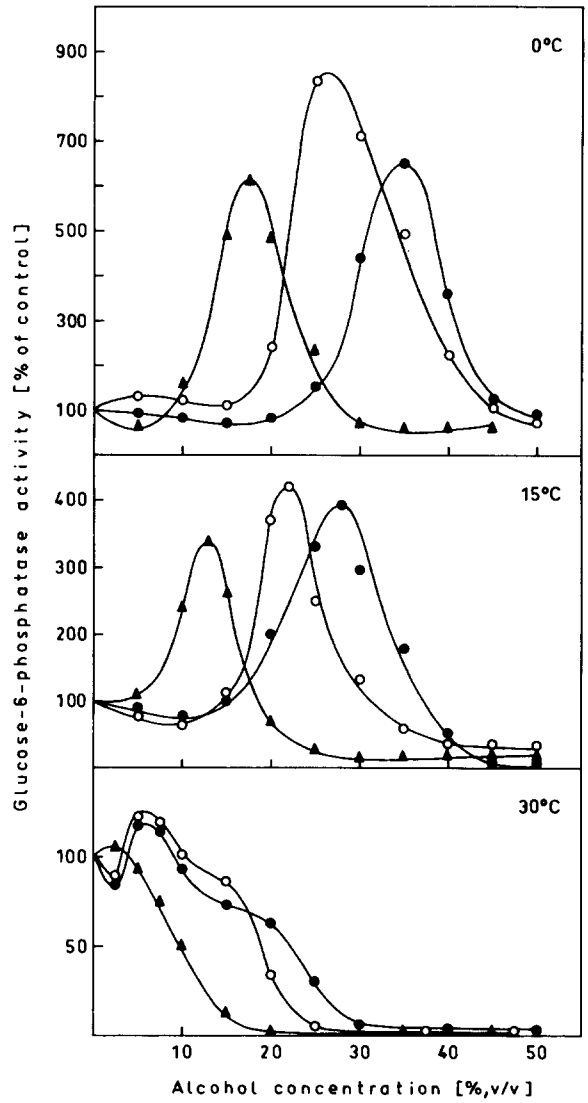

Fig. 8. Effects of organic solvents on the glucose-6-phosphatase activity. Incubations were carried out at 0°C (60 min), 15°C (15 min), and 30°C (15 min) and started by the addition of 10 µl microsomes (0.2 mg of protein) in 0.1 M Tris-HCl (pH 7.4) to the assay medium containing in a final volume of 0.8 ml 20 mM glucose 6-phosphate and with increasing concentrations of organic solvents as indicated from 0.2 M to 0.1 M decreasing concentrations of imidazole-HCl (pH 6.5). Ethanol (●), 2-propanol (○), *n*-propanol (▲). Acetone provided curves nearly identical to ethanol and is therefore not shown.

some of them elicit a small but reproducible enhancement of the glucose-6-phosphatase activity, others have a depressing effect like that evoked by very low concentrations of detergent. Incubation

of solvent-supplemented microsomes at 30°C rapidly destroys the enzymatic activity of glucose-6-phosphatase. However, the weak initial activation mentioned is retained. These characteristic features could reflect, as already suggested above for tensides, interactions of the solvents with the permeability barrier (carrier moiety) of microsomal glucose-6-phosphatase.

Conclusion

The application of detergents to disrupt the microsomal membrane in order to characterize glucose-6-phosphatase kinetically and to analyse its molecular organization has become a commonly used technique. The interactions of detergents with the components studied, however, are frequently negated or at least neglected. The results obtained are interpreted as the properties of the natural protein itself. Our studies reveal the high sensitivity of glucose-6-phosphatase to the nonionic surfactant Triton X-114. This is probably a consequence both of the high affinity of the glucose-6-phosphatase system to detergents with direct actions of the surfactants on the components and the drastic changes within the microenvironment around the enzyme protein caused by nearly complete solubilization under preincubation and subsequent reconstitution in the test medium including possible changes in the natural orientation of the molecules. The very high affinity site of the glucose-6-phosphatase system to Triton X-114 (10^{-4} M to give a Triton/protein ratio of 0.25) as revealed by kinetic analyses might suggest the interaction of detergent with phospholipids which as a permeability barrier limit the substrate flux to the integral hydrolase. The results obtained with organic solvents (Fig. 8) and earlier observations [9], especially the effects of controlled proteolysis, phospholipase C action, and the use of antibodies to microsomes indicating that phospholipids may be involved in glucose-6-phosphate hydrolysis as well as transport are easily reconciled with this interpretation.

The substrate transport model of the multifunctional glucose-6-phosphatase developed by Arion and his group [2], which culminates in the presentation of a miniature metabolic system [10], is a fascinating alternative to the conformational concept of Stetten and Burnett [4] as well as Nordlie and his co-workers [1,13] and is likewise consistent with a large body of existing data, especially manifold differences in the kinetic properties of the enzyme of intact and detergent-treated microsomes. However, two of the most important prerequisites of this attractive hypothesis favored at present have still not been proved experimentally. On the contrary, immunological studies have suggested that the phosphohydrolase is not attached to the luminal membrane surface rather than burried within the microsomal membrane [9,12] and from the results of the present investigation it is at least dubious whether detergent induced structural modifications in the membrane do indeed preserve the functional integrity of the enzyme protein. Therefore, the discussion, which of the offered concepts [1,13,2,9] describes the real molecular organisation of the glucose-6-phosphatase system in the microsomal membrane should be left open at this time.

Acknowledgements

We are grateful to Mrs. E. Jentzsch for help in the high performance liquid chromatography measurements and Mrs. I. Schulze for correcting the English of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (Schu 54/1 and 54/7).

References

1 Nordlie, R.C. (1971) in The Enzymes (Boyer, P.D., ed.), 3rd Edn., Vol. 4, pp. 543–610, Academic Press, New York and London
2 Arion, W.J., Wallin, B.K., Lange, A.J. and Ballas, L.M. (1975) Mol. Cell. Biochem. 6, 75–83
3 Nordlie, R.C., Arion, W.J. and Glende, E.A., Jr. (1965) J. Biol. Chem. 240, 3479–3484
4 Stetten, M.R. and Burnett, F.F. (1967) Biochim. Biophys. Acta 132, 138–147
5 Arion, W.J., Carlson, P.W., Wallin, B.K. and Lange, A.J. (1972) J. Biol. Chem. 247, 2551–2557
6 Arion, W.J., Wallin, B.K., Carlson, P.W. and Lange, A.J. (1972) J. Biol. Chem. 247, 2558–2565
7 Goldsmith, P.K. and Stetten, M.R. (1979) Biochim. Biophys. Acta 583, 133–147
8 Nordlie, R.C., Johnson, W.T., Cornatzer, W.E., Jr. and Twedell, G.W. (1979) Biochim. Biophys. Acta 585, 12–23
9 Schulze, H.-U. and Speth, M. (1980) Eur. J. Biochem. 106, 505–514

10 Arion, W.J., Lange, A.J., Walls, H.E. and Ballas, L.M. (1980) J. Biol. Chem. 255, 10396–10406
11 Gunderson, H.M. and Nordlie, R.C. (1975) J. Biol. Chem. 250, 3552–3559
12 Speth, M. and Schulze, H.-U. (1982) FEBS Lett. 144, 140–144
13 Nordlie, R.C. (1982) in Membranes and Transport (Martonosi, A.N., ed.), Vol. 1, pp. 263–268, Plenum Publishing Corporation, New York
14 Schulze, H.-U., Gallenkamp, H. and Staudinger, Hj. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 391–406
15 Pönnighaus, J.M., Schulze, H.-U. and Staudinger, Hj. (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 815–824
16 Strittmatter, P. (1967) Methods Enzymol. 10, 553–556
17 Schulze, H.-U. and Staudinger, Hj. (1971) Hoppe-Seyler's Z. Physiol. Chemie 352, 1659–1674
18 Arion, W.J., Ballas, L.M., Lange, A.J. and Wallin, B.K. (1976) J. Biol. Chem. 251, 4901–4907
19 Schulze, H.-U., Gallenkamp, H. and Staudinger, Hj. (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 809–817
20 Harper, A.E. (1962) in Methoden der Enzymatischen Analyse (Bergmeyer, H.-U., ed.), pp. 788–792, Verlag Chemie, Weinheim/Bergstr.
21 Bode, C., Goebell, H. and Stähler, E. (1968) Z. Klin. Chem. Klin. Biochem. 6, 418–422
22 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
23 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
24 Schulze, H.-U. and Staudinger, Hj. (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 184–193
25 Hutchinson, E. and Shinoda, K. (1967) in Solvent Properties of Surfactant Solutions (Shinoda, K., ed.), pp. 1–26, Marcel Dekker, Inc., New York
26 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
27 Hallam, V. and Wrigglesworth, J.M. (1976) Biochem. J. 156, 159–165
28 Arion, W.J. and Walls, H.E. (1982) J. Biol. Chem. 257, 11217–11220
29 Wallin, B.K. and Arion, W.J. (1972) Biochem. Biophys. Res. Commun. 48, 694–699
30 Razin, S. (1972) Biochim. Biophys. Acta 265, 241–296