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Membrane-promoted unfolding of acetylcholinesterase: A possible mechanism for insertion into the lipid bilayer

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ABSTRACT **Acetylcholinesterase from *Torpedo californica* partially unfolds to a state with the physicochemical characteristics of a "molten globule" upon mild thermal denaturation or upon chemical modification of a single nonconserved buried cysteine residue, Cys^{231}. The protein in this state binds tightly to liposomes. It is here shown that the rate of unfolding is greatly enhanced in the presence of unilamellar vesicles of dimyristoylphosphatidylcholine, with concomitant incorporation of the protein into the lipid bilayer. Arrhenius plots reveal that in the presence of the liposomes the energy barrier for transition from the native to the molten globule state is lowered from 145 to 47 kcal/mol. Chemical modification of Cys^{231} by mercuric chloride produces initially a quasi-native state of *Torpedo* acetylcholinesterase which, at room temperature, undergoes spontaneous transition to a molten globule state with a half-life of 1–2 hr. This permitted temporal resolution of interaction of the quasi-native state with the membrane from the transition of the membrane-bound protein to the molten globule state. The data presented here suggest that either the native enzyme, or a quasi-native state with which it is in equilibrium, interacts with the liposome, which then promotes a fast transition to the membrane-bound molten globule state by lowering the energy barrier for the transition. These findings raise the possibility that the membrane itself, by lowering the energy barrier for transition to a partially unfolded state, may play an active posttranslational role in insertion and translocation of proteins *in situ*.**

The conformation(s) in which proteins insert into and move across biological membranes is a topic of active investigation due to its importance in considering mechanisms of protein traffic (for reviews see refs. 1 and 2). Whether movement occurs cotranslationally or posttranslationally, it is commonly accepted that proteins do not undergo such translocation in their native conformation (3–5). Various mechanisms evoke a signal peptide or prosequence in the translocation process (6), and complexation of the protein with an appropriate chaperone may be involved either in presentation of a partly unfolded conformation of the protein to the membrane or directly in the translocation process (7, 8). A direct involvement of the membrane lipids has also been proposed. Thus, Endo *et al.* (9) have demonstrated that binding of a tightly folded precursor protein to the mitochondrial outer membrane involves a lipid-mediated conformational change, and it has been shown that at acid pH, a globular fragment of the bacterial toxin, colicin, undergoes a liposome-induced conformational transition occurring concomitantly with insertion into the membrane (10, 11). Several studies have also reported occurrence of partially unfolded states of proteins at the interface of anionic phospholipid vesicles (12, 13).

We showed recently that a dimeric form of *T. californica* acetylcholinesterase (AcChoEase), in a partially unfolded state displaying the physicochemical characteristics of a "molten globule" (MG) (14, 15), interacted with dimyristoylphosphatidylcholine (DMPC) liposomes (16). It is demonstrated below that under conditions in which native AcChoEase alone is stable and catalytically active for many hours, DMPC liposomes promote a rapid transition to the partially unfolded MG state, in which the protein remains bound to the membrane.

MATERIALS AND METHODS

Materials. AcChoEase was the G_2 dimer, purified from electric organ tissue of *T. californica* by affinity chromatography subsequent to solubilization with phosphatidylinositol-specific phospholipase C (17).

Mercuric chloride ($HgCl_2$) was purchased from BDH. Tris, reduced glutathione (GSH), and 5,5′-dithiobis(2-nitrobenzoic acid) were from Sigma. Synthetic DMPC was from Avanti Polar Lipids and bovine spinal cord phosphatidylserine was from Lipid Products (South Nutfield, U.K.). Guanidine hydrochloride (Gdn·HCl) (ultra pure) was from Schwartz/Mann. All other reagents were of analytical grade or higher.

Assay Methods. AcChoEase concentrations were determined as described (18) or by measuring intrinsic fluorescence, exciting at 280 nm and measuring emission at 340 nm. AcChoEase activity was monitored as before (18).

Buffers. Unless otherwise stated, the buffer employed was 0.1 M NaCl/10 mM Tris, pH 8.0 (buffer 1).

Liposomes. Small unilamellar phospholipid vesicles were prepared as described (16).

Modification of AcChoEase by $HgCl_2$. Modification of AcChoEase by $HgCl_2$ in the absence and presence of liposomes, and demodification by GSH of the modified enzyme, were performed as described (18, 19). When the reaction was performed in the presence of liposomes, the reaction mixture was subsequently passed through a Sephacryl S-300 column (27×0.7 cm). The liposome fraction, together with adhering AcChoEase, came through in the excluded volume, whereas unbound protein was retarded.

Separation of Bound AcChoEase from Liposomes. Liposomes containing bound AcChoEase were solubilized in 0.4% sodium cholate in buffer 1 and washed four times by concentration in a Centricon 30 microconcentrator followed, each time, by redilution in buffer 1 containing 0.4% sodium cholate, to remove all traces of lipid. The procedure was repeated four times, using buffer 1 alone, to remove all traces of cholate.

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Abbreviations: AcChoEase, acetylcholinesterase; MG, molten globule; DMPC, dimyristoylphosphatidylcholine; GSH, reduced glutathione; Gdn·HCl, guanidine hydrochloride; CD, circular dichroism.

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Spectroscopic Measurements. Measurements of circular dichroism (CD) and of intrinsic protein fluorescence were performed at 22°C and 24°C, respectively, as described (20).

Monitoring of Transition of AcChoEase from the Native to the MG State. Thermal denaturation of native AcChoEase, producing an MG state, was monitored and analyzed as described (21). Denaturation followed first-order kinetics, both in the absence and presence of DMPC liposomes. Activation energies were calculated from Arrhenius plots.

As previously demonstrated, chemical modification by mercurials generates initially a "quasi-native" state, N*, devoid of catalytic activity but amenable to rapid reactivation by GSH (19). This initial process follows pseudo-first-order kinetics. Subsequently, N* decays spontaneously to an MG state, and this latter transition obeys first-order kinetics. The MG species thus obtained cannot be reactivated by GSH.

If the kinetic constants for these two processes are represented by k and κ, respectively, and the total concentration of AcChoEase is taken as unity, the system can be described by the following equations:

$$d[\mathrm{N}]/dt = -k[\mathrm{N}] \quad \textbf{[1]}$$

$$d[\mathrm{N}^*]/dt = k[\mathrm{N}] - \kappa[\mathrm{N}^*] \quad \textbf{[2]}$$

$$d[\mathrm{MG}]/dt = \kappa[\mathrm{N}^*] \quad \textbf{[3]}$$

$$[\mathrm{N}] + [\mathrm{N}^*] + [\mathrm{MG}] = 1, \quad \textbf{[4]}$$

where [N], [N*], and [MG] are the concentrations of the corresponding species at each time, being taken as equal to 1, 0, and 0, respectively, at the time of the addition of the modifying agent. The above equations, in integrated form, yield the concentrations of N, N*, and MG at time t. In particular, it is possible to derive the following expressions for [N] and for $y(t) = [\mathrm{N}] + [\mathrm{N}^*]$, both of which can be monitored experimentally:

$$[\mathrm{N}] = \exp(-kt) \quad \textbf{[5]}$$

$$y(t) = [k/(k - \kappa)]\exp(-\kappa t) + [\kappa/[\kappa - k)]\exp(-kt). \quad \textbf{[6]}$$

Assuming that the AcChoEase activity measured directly represents [N] (taken as 1 at zero time), and the additional activity obtained upon addition of GSH is equivalent to [N*], one can monitor simultaneously both the rate of chemical modification, assaying catalytic activity in aliquots diluted into Tris (pH 7.0), and $y(t)$, assaying aliquots diluted into Tris (pH 8.0), containing GSH (1 mM). Fitting of Eq. **5** to the experimental points yields k. κ can then be determined by fitting the experimental data to Eq. **6** at a fixed value of k.

RESULTS

Effect of Liposomes on Thermal Inactivation of AcChoEase. Fig. 1 shows the influence of liposomes on the enzymic activity of *Torpedo* AcChoEase. At pH 8.0 and 28°C, a control AcChoEase sample retained full catalytic activity for at least 8 hr. In the presence of unilamellar vesicles of DMPC, activity decreased rapidly, with a half-life of ≈6 min. A control experiment, in which the supernatant was added from a sample of liposomes that had been centrifuged for 5 hr at 45,000 rpm in a Beckman SW50.1 rotor, produced no detectable deactivation. Similar experiments were performed at 28°C–37°C, a temperature range in which DMPC is in the fluid state (22, 23). Arrhenius plots, constructed using the data so obtained, are displayed in Fig. 1 (*Inset*), and were used to calculate that in the presence of the liposomes, the activation energy for thermal denaturation is lowered from 145 kcal/mol, a value similar to that reported previously (21), to 47 kcal/mol.

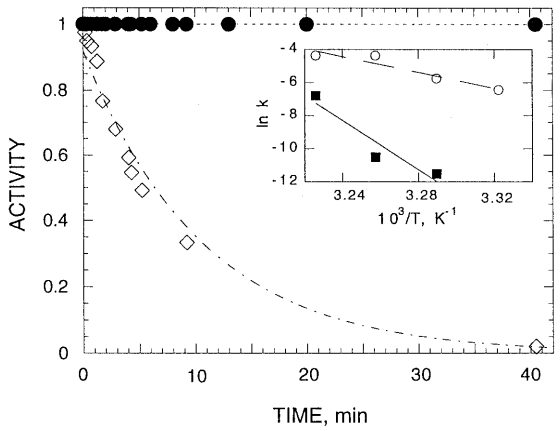

FIG. 1. Effect of liposomes on thermal inactivation of *T. californica* AcChoEase. Samples of AcChoEase (4×10^{-7} M) in buffer 1 were incubated at 28°C. Aliquots were withdrawn at appropriate times for assay of enzymic activity. (●), Control; (◇), in the presence of DMPC liposomes (5 mg/ml). (*Inset*) Arrhenius plots of the temperature dependence of the rate of thermal inactivation of native AcChoEase in the presence (○) and absence (■) of DMPC liposomes.

Binding of Thermally Inactivated AcChoEase to Liposomes. Gel filtration revealed that the accelerated deactivation obtained in the presence of DMPC liposomes was accompanied by binding of the inactive enzyme to the liposomes (Fig. 2). Experiments performed at high ionic strength, namely in the presence of 1 M NaCl, yielded similar results, and the deactivated AcChoEase remained fully bound to the liposomes (not shown). Thus, binding seems to involve hydrophobic rather than electrostatic forces. If DMPC was replaced by DMPC/phosphatidylserine (1:1), producing negatively charged liposomes, the first-order rate constant for deactivation of AcChoEase at 34°C was 0.80 ± 0.12 min^{-1}, compared with 0.76 ± 0.12 min^{-1} for the pure DMPC liposomes and 0.020 ± 0.005 min^{-1} for spontaneous thermal deactivation in the absence of liposomes.

Spectroscopic Characteristics of Partially Unfolded AcChoEase. Both mild thermal denaturation and chemical modification convert native *Torpedo* AcChoEase to a partially unfolded state with the physicochemical characteristics of an MG (18, 19, 21). Various physicochemical techniques show that the

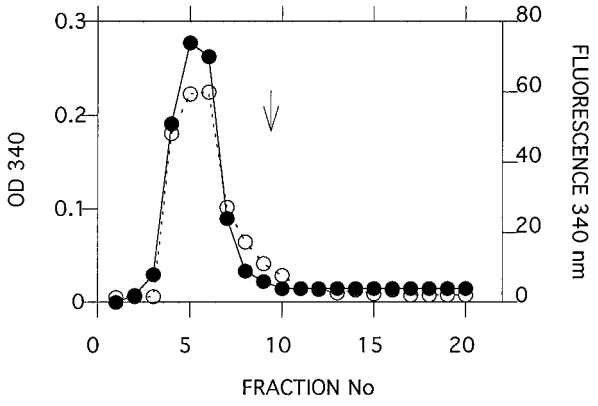

FIG. 2. Interaction of thermally inactivated AcChoEase with DMPC liposomes as revealed by gel filtration. Samples of *Torpedo* AcChoEase (4×10^{-7} M) in buffer 1 were incubated at 34°C in the presence of DMPC liposomes (5 mg/ml). After 10 min, when AcChoEase activity was <0.5%, the reaction mixture was applied to a Sephacryl S-300 column; protein and lipid in the effluent were monitored by measuring, respectively, fluorescence and absorbance at 340 nm. (●), Fluorescence at 340 nm; (○), OD at 340 nm. Arrow marks the position of thermally inactivated AcChoEase in the absence of liposomes.

MG states generated by the two procedures are indistinguishable (21), and we earlier demonstrated interaction of the MG preparation obtained by chemical modification with liposomes (16). It thus seemed reasonable to assume that liposome-induced inactivation involved binding of the enzyme to the liposomes, followed by transition to an MG state that would remain bound to the bilayer. CD measurements, in the far UV (Fig. 3), showed a reduced ellipticity of the bound protein, as compared with native AcChoEase, but greater ellipticity than for AcChoEase in a fully unfolded (U) state in 5 M Gdn·HCl, as would be expected for AcChoEase in an MG state (24). It is well known that CD spectroscopy of membrane preparations must take into account possible scattering artifacts (25, 26). This precluded accurate CD measurements in the near UV, where the contribution of the aromatic bands produces relatively low ellipticity. To overcome this problem, the liposomes were solubilized by use of 0.4% sodium cholate, which was subsequently removed. Control experiments showed that 0.4% cholate had no effect on either native AChE or on the MG (not shown). The CD spectrum of the solubilized protein in the far UV was very similar to its CD spectrum when bound to the liposomes, and identical to that of MG preparations of AcChoEase obtained directly by thermal denaturation (ref. 21; not shown). The CD spectrum of the solubilized protein in the near UV was also similar to that of MG preparations obtained by thermal denaturation, permitting us to infer that the CD spectrum of the membrane-bound AcChoEase in the near UV was also that to be expected of an MG state. Independent evidence that the liposome-bound AcChoEase was in an MG state came from intrinsic fluorescence measurements. These revealed a red shift of the emission maximum from 333 to 340 nm, in agreement with our previous observations for MG states of AcChoEase in solution, produced both by thermal denaturation and chemical modification, as compared with an emission maximum of 353 nm for the protein unfolded in 5 M Gdn·HCl (19, 24).

Temporal Resolution of Binding to Liposomes and Unfolding of Chemically Modified AcChoEase. It was earlier shown that chemical modification of *Torpedo* AcChoEase by mercurials generates a quasi-native state, N* (19). The spectroscopic characteristics of N* resemble those of native, N, AcChoEase, and reversal of chemical modification by GSH regenerates catalytic activity. Yet the mercurial-modified enzyme is metastable and, with time, N* decays to an MG state from which no catalytic activity can be regenerated upon demodification (19). The spectroscopic characteristics of this latter state are identical to those of the partially unfolded species obtained by thermal inactivation (21). Despite its spectroscopic resemblance to N, N* binds the amphiphilic probe, 1-anilino-8-naphthalenesulfonic acid, almost as well as does the MG state (19). This implies that it displays exposed hydrophobic surfaces that might enhance its interaction with liposomes. Fig. 4 shows the rates of reversible and irreversible inactivation of AcChoEase by $HgCl_2$, in the absence and presence of DMPC liposomes. $HgCl_2$ rapidly inactivates both the control and liposome-containing samples at equal rates. As expected, the enzymic activity of the control sample could be >90% recovered upon immediate treatment with reduced GSH, and the extent of recovery obtained decreased gradually, with $\tau_{1/2} \sim 10$ min, in agreement with the data already published (19). In the presence of liposomes, however, irreversible inactivation occurred much more rapidly, implying that the rate of transition to an MG state had been accelerated. In this case, too, temperature-dependence was studied and Arrhenius plots constructed (Fig. 4 *Inset*). In the presence of liposomes, the activation energy of the N* → MG transition decreased from 40 to 16 kcal/mol.

Fig. 4 shows that irreversible inactivation, reflecting the N* → MG transition, occurred much more slowly than the reversible step, even in the presence of liposomes. This permitted temporal resolution of membrane insertion and of the N* → MG transition. Gel filtration showed that, as demonstrated above for heat-denatured AcChoEase, the $HgCl_2$-modified enzyme was fully bound to the liposomes (Fig. 5*A*). If, however, reactivation by GSH was performed shortly after inactivation, not only was a large part of the enzymic activity recovered, but gel filtration clearly showed that the reactivated enzyme had been released from the liposomes (Fig. 5*B*).

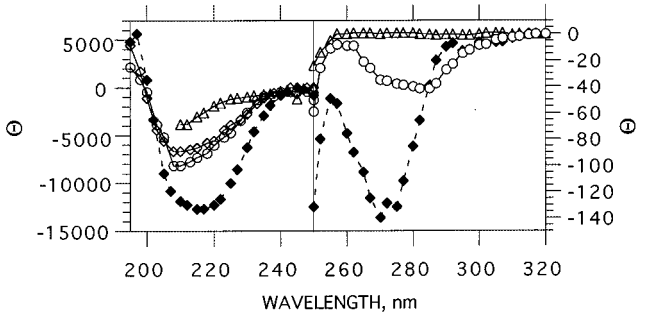

FIG. 3. CD spectra in the far and near UV of native and unfolded preparations of *Torpedo* AcChoEase. (♦), Native AcChoEase; (Δ), AcChoEase in 5 M Gdn·HCl; (◇), AcChoEase after thermal inactivation in the presence of DMPC liposomes; (○), AcChoEase after thermal inactivation in the presence of DMPC liposomes followed by removal of the liposomes (see *Materials and Methods*). Thermal inactivation was for 30 min at 37°C. Protein concentrations were 3–4.5 μM in buffer 1.

DISCUSSION

The data presented here clearly show that a lipid bilayer can lower the activation energy for unfolding of a native protein to an MG species, which will then insert into the bilayer. This finding raises the possibility that the plasma membrane could play a direct role in insertion and translocation of native

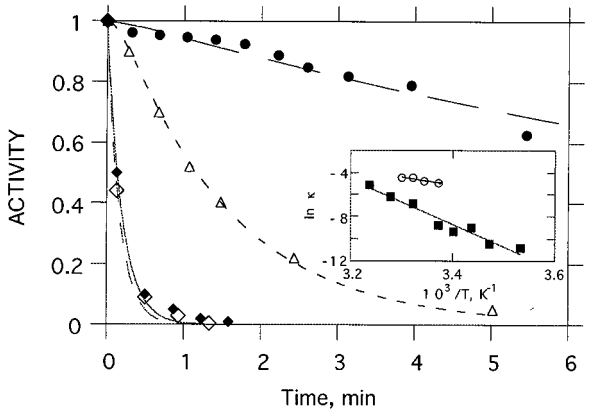

FIG. 4. Kinetics of chemical modification and irreversible inactivation of *T. californica* AcChoEase by $HgCl_2$ in the presence and absence of liposomes. Samples of *Torpedo* AcChoEase (4×10^{-7} M) in buffer 1 were incubated at 28°C with 0.1 mM $HgCl_2$ in the presence and absence of DMPC liposomes (5 mg/ml). Aliquots were withdrawn at appropriate times and either assayed directly for enzymic activity or diluted into 1 mM GSH in 0.01 M Tris buffer (pH 8.0) prior to the assay, so as to monitor the decrease in recoverable enzymic activity (see *Materials and Methods*). (♦), Decrease in AcChoEase activity in the absence of liposomes; (◇), decrease in AcChoEase activity in the presence of liposomes; (●), decrease in recoverable AcChoEase activity in control aliquots exposed to reduced GSH prior to assay; (Δ), decrease in recoverable AcChoEase activity in liposome-containing aliquots exposed to reduced GSH prior to assay. (*Inset*) Arrhenius plots of the temperature-dependence of the rate of the N* → MG transition in the presence (○) and absence (■) of DMPC liposomes.

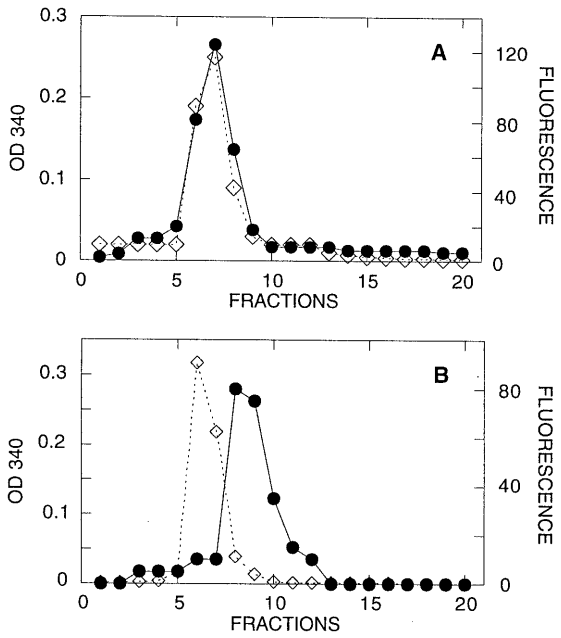

FIG. 5. Interaction of $HgCl_2$-modified *Torpedo* AcChoEase with DMPC liposomes and release by GSH. Samples of AcChoEase (3×10^{-6} M) in buffer 1 were incubated with 0.3 mM $HgCl_2$ at 10°C. After 40 min, when AcChoEase activity was <0.5%, exposure to GSH resulted in ≈90% reactivation. (*A*) A sample of unreactivated enzyme (30 μl) was added to 270 μl of DMPC liposomes (35 mg/ml) in buffer 1. Within 1 min, the reaction mixture was applied to a Sephacryl S-300 column. Protein and lipid in the effluent were monitored as in the legend to Fig. 2. (●), Fluorescence at 340 nm; (◇), absorbance at 340 nm. (*B*) Experimental conditions and symbols as in *A*, except that within 1 min of mixing modified AcChoEase and liposomes GSH was added to a final concentration of 5 mM prior to gel filtration.

proteins, under physiological conditions, in the absence of a signal sequence, pro-region, or other docking device.

Although expression of *Torpedo* AcChoEase in COS cells at 37°C resulted in production of immunoreactive AcChoEase protein, little or no catalytic activity was obtained unless the incubation temperature was lowered to 27°C–28°C (27, 28). This is not surprising in retrospect, since it was earlier shown that thermal inactivation of *Torpedo* AcChoEase occurs with a half-life of ≈45 min at 37.5°C (21). However, even at 28°C, the activity obtained was <5% of that expected from the levels of immunoreactive protein (28), although the soluble enzyme is stable for >8 hr. It is plausible that the enzyme is destabilized by the plasma and/or intracellular membranes by the same mechanism as by DMPC liposomes.

The MG is considered to be a transient intermediate in protein folding, both *in vivo* and *in vitro* (15, 29, 30), and it has also been proposed that proteins may be inserted into or translocated across membranes in the MG state (31). Indeed, Christensen and Pain (32) suggested that "adsorption to the membrane requires interaction of the membrane surface with sites on the protein that are more readily accessed from the partially folded than from the native protein." The studies of Endo *et al.* (9) on an artificial mitochondrial precursor protein, and of van der Goot *et al.* (10) and Shin *et al.* (11) on colicin fragments, suggested a more direct role for the membrane itself in insertion and/or translocation. However, the study of Endo *et al.* (9) involved a protein bearing a precursor sequence, and in the studies on the membrane-inserting fragments of the colicins, insertion was preceded by transformation to an MG state at the acid pH values employed. In the experimental system employed in this study, unfolding and insertion were achieved using a fully processed and folded protein that was both catalytically active and devoid of a precursor and/or anchoring sequence.

It is often assumed that the native conformation of a protein is in equilibrium with the MG state, but that the equilibrium, under physiological conditions, favors the native state (33, 34). It was earlier shown that MG states of *Torpedo* AcChoEase, produced by chemical modification or mild denaturation, interact with DMPC liposomes under conditions where native AcChoEase remains unbound (16). It was thus considered possible that the effect observed in this study, namely pronounced acceleration of unfolding of native AcChoEase, was due to binding to the liposomes of a small fraction of AcChoEase in the MG state, coexisting with the native enzyme. But the high-energy barrier (≈140 kcal/mol) separating the N from the MG state makes such a mechanism unlikely. This led us to postulate that the native enzyme itself, or a state in rapid equilibrium with it, is the species that interacts with the liposome. The experimental system developed earlier (19) permitted us to approach this issue experimentally. Thus, *Torpedo* AcChoEase in which a nonconserved cysteine, Cys^{231}, has been modified by a mercurial is in a quasi-native, N*, state. N* lacks catalytic activity, even though the thiol modified has no functional role; but activity is regenerated upon exposure to GSH, and temperature-dependence of reactivation reveals that the quasi-native is separated from the native state by a low energy barrier (6 kcal/mol) (19). N* is quite similar to native AcChoEase, except that 1-anilino-8-naphthalenesulfonic acid binding reveals exposed hydrophobic surfaces, which should increase its tendency to interact with liposomes (19). Finally, this state is metastable, decaying to an MG state spontaneously at room temperature, with $\tau_{1/2}$ = 1–2 hr. The data presented above (Fig. 5) show that N* indeed interacts rapidly and quantitatively with the DMPC liposomes, with subsequent transition to the MG state. But we also showed that exposure to GSH leads to recovery of AcChoEase activity, with concomitant release of the bound enzyme from the liposomes. Furthermore, the released AcChoEase no longer binds 1-anilino-8-naphthalenesulfonic acid (not shown). Even in native AcChoEase, a small amount of a quasi-native state may exist in equilibrium with the native state, and binding of this putative species to the liposome, followed by its transition to an MG in the bound state, would drive the process. The existence of a native-like state that is in equilibrium with the native state has recently been proposed for apomyoglobin (35). Although the model that we have proposed is attractive, one cannot exclude a simpler model in which the process is driven by weak binding of native AcChoEase itself to the liposome. The scheme below summarizes the intraconversions of the various states of AChE discussed above and their interactions with liposomes, where N, N*, MG, and U are as already designated and L stands for liposome:

$$\begin{array}{ccccc} \mathrm{N} & \Leftrightarrow & \mathrm{N^*} & \Rightarrow & \mathrm{MG} \Leftrightarrow \mathrm{U} \\ +\mathrm{L}\uparrow\downarrow & & +\mathrm{L}\downarrow & & \\ \mathrm{N^*{\cdot}L} & & \Rightarrow & & \mathrm{MG{\cdot}L.} \end{array}$$

Various papers in the literature, which have investigated interaction of proteins with the lipid bilayer with concomitant unfolding, have suggested a requirement for acidic phospholipids (9, 10, 36, 37). The data comparing DMPC/phosphatidylserine liposomes to DMPC liposomes, as well as the absence of an effect of ionic strength, do not support a strong electrostatic component in the phenomenon that we are studying. In our earlier study (16), we showed that a preexisting MG of AcChoEase bound well to small unilamellar DMPC vesicles but poorly to large unilamellar vesicles of the same lipid. Thus, the curvature of the lipid surface seems to play an important role in our model system, suggesting hydrophobic interaction of the partially unfolded AcChoEase with the bilayer itself, rather than with the charged surface. This is in keeping with

the view that transient or sustained exposure of the hydrophobic regions of lipid bilayers is a requirement for incorporation of proteins to occur (38). It also is in line with our suggestion that accelerated unfolding of AcChoEase in the presence of DMPC liposomes involves a quasi-native state, displaying exposed hydrophobic surfaces.

Molecular chaperones are involved not only in folding of nascent polypeptide chains but also in translocation of proteins across plasma membranes (2, 39, 40). Such translocation involves a nonnative state of the protein (41). It has been shown by Laminet *et al.* (42) that GroEL, when added to native pre-β-lactamase, recognizes and stabilizes a partially unfolded conformation of the enzyme, and that the process can be reversed by addition of Mg–ATP. It is not clear whether GroEL is passively trapping spontaneously appearing nonnative conformations or is actually serving as an "unfoldase." Current knowledge invokes chaperones both in insertion into the membrane of the protein to be translocated and in its retrieval and refolding subsequent to translocation (2). The data presented above can be plausibly reconciled with this supposition. Thus, a native protein, upon binding to a molecular chaperone, may assume a partially unfolded conformation (42), and the hydrophobic surfaces thus exposed could promote interaction with the bilayer, as occurs in our system in the absence of a chaperone. As already mentioned, Laminet *et al.* (42) also showed that the addition of ATP to such a complex promotes release of fully folded native protein. Thus, subsequent to translocation, a protein could be released from the membrane by a reverse process, namely, interaction with GroEL of the partially unfolded protein, followed by ATP-driven release of the fully folded native protein from its complex with the chaperone.

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1. Isenman, L., Liebow, C. & Rothman, S. (1995) *Biochim. Biophys. Acta* **241,** 341–370.
2. Schatz, G. & Dobberstein, B. (1996) *Science* **271,** 1519–1526.
3. Neupert, W. & Schatz, G. (1981) *Trends Biochem. Sci.* **6,** 1–4.
4. Pelham, H. R. B. (1986) *Cell* **46,** 959–961.
5. Verner, K. & Schatz, G. (1988) *Science* **241,** 1307–1313.
6. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67,** 835–851.
7. Arkowitz, R. A., Joly, J. C. & Wickner, W. (1993) *EMBO J.* **12,** 243–253.
8. Mayer, A., Neupert, W. & Lill, R. (1995) *Cell* **80,** 127–137.
9. Endo, T., Eilers, M. & Schatz, G. (1989) *J. Biol. Chem.* **264,** 2951–2956.
10. van der Goot, F. G., González-Mañas, J. M., Lakey, J. H. & Pattus, F. (1991) *Nature (London)* **354,** 408–410.
11. Shin, Y.-K., Levinthal, C., Levinthal, F. & Hubbell, W. L. (1993) *Science* **259,** 960–963.
12. Pinheiro, T. J. T. & Watts, A. (1994) *Biochemistry* **33,** 2451–2458.
13. Bañuelos, S. & Muga, A. (1995) *J. Biol. Chem.* **270,** 29910–29915.
14. Ptitsyn, O. B. (1992) in *Protein Folding*, ed. Creighton, T. E. (Freeman, New York), pp. 243–300.
15. Fink, A. L. (1995) *Annu. Rev. Biophys. Biomol. Struct.* **24,** 495–522.
16. Shin, I., Silman, I. & Weiner, L. M. (1996) *Protein Sci.* **5,** 42–51.
17. Sussman, J. L., Harel, M., Frolow, F., Varon, L., Toker, L., Futerman, A. H. & Silman, I. (1988) *J. Mol. Biol.* **203,** 821–823.
18. Dolginova, E., Roth, E., Silman, I. & Weiner, L. M. (1992) *Biochemistry* **31,** 12248–12254.
19. Kreimer, D. I., Dolginova, E. A., Raves, M., Sussman, J. L., Silman, I. & Weiner, L. (1994) *Biochemistry* **33,** 14407–14418.
20. Weiner, L., Kreimer, D., Roth, E. & Silman, I. (1994) *Biochem. Biophys. Res. Commun.* **198,** 915–922.
21. Kreimer, D. I., Shnyrov, V. L., Villar, E., Silman, I. & Weiner, L. (1995) *Protein Sci.* **4,** 2349–2357.
22. Chapman, D. (1975) *Q. Rev. Biophys.* **8,** 185–235.
23. Presti, F. T. (1985) in *Membrane Fluidity in Biology: General Principles*, ed. Aloia, R. C. (Academic, New York), Vol. 4, pp. 97–146.
24. Kreimer, D. I., Szosenfogel, R., Goldfarb, D., Silman, I. & Weiner, L. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12145–12149.
25. Schneider, A., Schneider, M.-J. & Rosenheck, K. (1970) *Proc. Natl. Acad. Sci. USA* **66,** 793–797.
26. Glaser, M. & Singer, S. J. (1971) *Biochemistry* **10,** 1780–1787.
27. Gibney, G. & Taylor, P. (1990) *J. Biol. Chem.* **265,** 12576–12583.
28. Duval, N., Massoulié, J. & Bon, S. (1992) *J. Cell Biol.* **118,** 641–653.
29. Kim, P. S. & Baldwin, R. L. (1990) *Annu. Rev. Biochem.* **59,** 631–660.
30. Bychkova, V. E. & Ptitsyn, O. B. (1993) *Chemtracts Biochem. Mol. Biol.* **4,** 133–163.
31. Bychkova, V. E., Pain, R. H. & Ptitsyn, O. B. (1988) *FEBS Lett.* **238,** 231–234.
32. Christensen, H. & Pain, R. H. (1994) in *Mechanisms of Protein Folding*, ed. Pain, R. H. (Oxford Univ. Press, Oxford), pp. 55–79.
33. Haynie, D. T. & Freire, E. (1993) *Proteins* **16,** 115–140.
34. Bai, Y. & Englander, S. W. (1996) *Proteins* **24,** 145–151.
35. Eliezer, D. & Wright, P. E. (1996) *J. Mol. Biol.* **263**, 531–536.
36. Muga, A., Mantsch, H. H. & Surewicz, W. K. (1991) *Biochemistry* **30,** 7219–7224.
37. Surrey, T. & Jähnig, F. (1995) *J. Biol. Chem.* **270,** 28199–28203.
38. Jain, M. K. & Zakim, D. (1987) *Biochim. Biophys. Acta* **906,** 33–68.
39. Rothman, J. E. (1989) *Cell* **59,** 591–601.
40. Lazdunski, C. J. & Benedetti, H. (1990) *FEBS Lett.* **268,** 408–414.
41. Eilers, M. & Schatz, G. (1986) *Nature (London)* **328,** 800–805.
42. Laminet, A. A., Ziegelhoffer, T., Georgopoulos, C. & Plückthun, A. (1990) *EMBO J.* **9,** 2315–2319.