Interaction between tissue transglutaminase and phospholipid vesicles

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A specific interaction between purified liver transglutaminase and small unilamellar phospholipid vesicles at the lipid phase transition have been revealed. The enzyme-induced perturbation of the bilayer is sufficient for phase transition release of encapsulated carboxyfluorescein from the vesicles. The size of the enzyme–phospholipid recombinants depends upon the protein–phospholipid ratio as shown on Sepharose 4B elution profile. The activity of transglutaminase inserted into the bilayer is greatly reduced. The interaction does not occur when the phospholipid vesicle are in the solid or liquid phase and it requires the structural integrity of the enzyme.

Transglutaminase *Small unilamellar phospholipid vesicle* *Phase transition*
Enzyme–phospholipid recombinant *Inhibition of activity*

1. INTRODUCTION

Transglutaminases catalyze an acyl-transfer reaction, with the concomitant release of ammonia, between peptide-bound glutamine and a series of primary amines including the ϵ-amino group of lysine in appropriate proteins [1]. In the latter case an ϵ-(γ-glutaminyl)lysine bond is formed, which is a unique example of enzyme-catalyzed intermolecular cross-link formation. The intracellular/tissue form of the enzyme has been localized in the nucleus [2], cytosol and membrane fraction [3] of mammalian cells and its role implicated in such important cellular phenomena like receptor-mediated endocytosis [4], ageing [5], initiation of cell proliferation [6] and surface-related immunological reactions [7]. However, little is known about the intracellular regulation of transglutaminase. It is a Ca^{2+}-dependent enzyme which needs at least 0.1–1.0 mM Ca^{2+} for showing catalytic activity [8]. On the other hand, we could not demonstrate an activation mechanism with calmodulin in purified system [9]. Some recent results [16,10] suggest the regulation of intracellular transglutaminase activity mainly through de novo synthesis–degradation mechanisms, though its translocation within the cell accompanied by abrupt changes of activity has been also demonstrated [2,11]. Here we report that an interaction takes place between transglutaminase and phospholipid vesicles, when the latter is in the crystalline–liquid transition state and the interaction results in a modulation of transglutaminase activity.

2. MATERIALS AND METHODS

2.1. *Phospholipids, proteins and other chemicals*

Dipalmitoylphosphatidylcholine (DPPC), dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), distearylphosphatidylcholine (DSPC), dipalmitoylphosphatidylethanolamine (DPPE), dipalmitoylphosphatidylserine (DPPS), liver phosphatidylcholine (PC), lysophosphatidylcholine (LyPC), phosphatidylethanolamine (PE), lysophosphatidylethanolamine (LyPE) and brain phosphatidylinositol (PI) were obtained from Sigma. Samples of 100 μg gave single spots on thin-layer chromatograms. Agarose was purchased from Bio-Rad; z-L-glutaminyl-gly-

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cine from Vega Chemicals; carboxyfluorescein from Kodak; HSA, glutamate dehydrogenase and metilamine from Sigma. Actin was purified from bovine platelets [12]. All other used reagents were of reagent grade.

2.2. *Preparation of phospholipid vesicles*

Phospholipids were dissolved in chloroform–methanol (2:1), thin-filmed under nitrogen, hydrated (0.1 M NaCl, 0.025 M Tris–HCl, pH 7.5) by vortex mixing, then sonicated (Branson type B30 Sonicator power level 4) under nitrogen for 1 h at 1°C above the transition state temperature (Tc) of the particular phospholipid. The vesicles were centrifuged at $100 \times g$ for 5 min and unilamellar vesicles were obtained either by fractionation in a 1.6×25 cm Sepharose 4B column [13] or by differential high-speed ultracentrifugation [14]. The small unilamellar vesicles fractions did not contain degradation products checked by thin-layer chromatography or autooxidation products detectable by absorbance measurement at 233 nm [15]. The phospholipid content was determined using the method in [16].

2.3. *Phase transition release (PTR)*

DPPC small unilamellar vesicles containing carboxyfluorescein (CF) were prepared as in [17] for the measurements of phase transition release. Changes in fluorescence intensity following the release of CF from the vesicles were measured using a Hitachi Perkin Elmer MPF_4 fluorometer at 515 nm using exciting wavelength at 470 nm as described in [18].

2.4. *Transglutaminase*

Purified guineapig liver transglutaminase was prepared as in [19]. It was electrophoretically pure and had a spec. act. of 12.5 units/mg as determined by an assay in which the ammonia released during the transfer reaction between z-L-glutaminylglycine and methylamine was measured as described in [20]. One unit of the enzyme was defined as the amount catalyzing the release of 1 μmol NH_3/min at 37°C.

3. RESULTS AND DISCUSSION

Since the introduction of the phase transition release (PTR) technique [17] it has been successfully used to demonstrate specific interactions at the lipid phase transition temperature (Tc) between lipid bilayers and proteins like tubulin [17], actin [21] and lipoproteins [18]. Tissue transglutaminase can also induce a smooth, rapid release of water-soluble carboxyfluorescein (the encapsulated dye is quenched inside the vesicles but gives fluorescence when released and diluted into the external medium) from small unilamellar DPPC vesicles during scans through Tc (fig.1). The PTR is enzyme concentration-dependent (fig.2). The lipid/protein molar ratio sufficient for 50% protein-induced release is about 250:1 using M_r's of 80 000 for TGase [1] and 733 for DPPC in the calculation. At this ratio, recycling of the enzyme–lipid complex through Tc does not induce further release of carboxyfluoresceine (not shown); i.e., the interaction is all or none and irreversible [18]. The vesicles show the same response to actin as in [21] and do not respond to HSA. Heat-inactivated TGase can not induce PTR; i.e., a structural integrity possessed by the intact enzyme molecule is essential for the interaction. Ca^{2+}-induced conformational change of TGase (which is needed for the catalytic activity) is

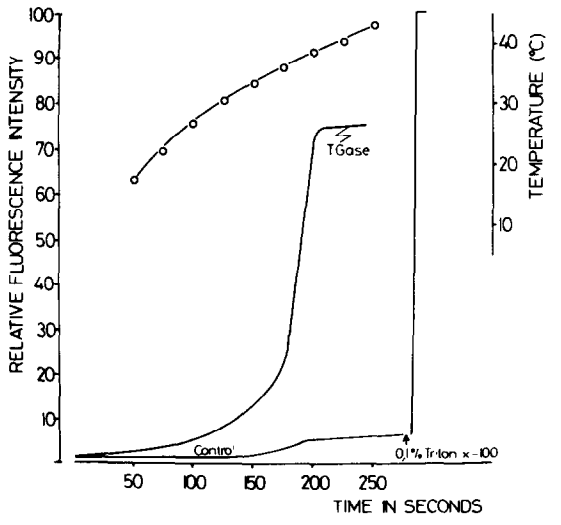

Fig.1. Phase transition release (PTR) profile of small unilamellar DPPC vesicles (50 μM) obtained in the presence or absence (control) of TGase (50 μg/ml). Vesicles and the enzyme were mixed at room temperature and the PTR curve was obtained as in [17]; (○) temperature in the cuvette.

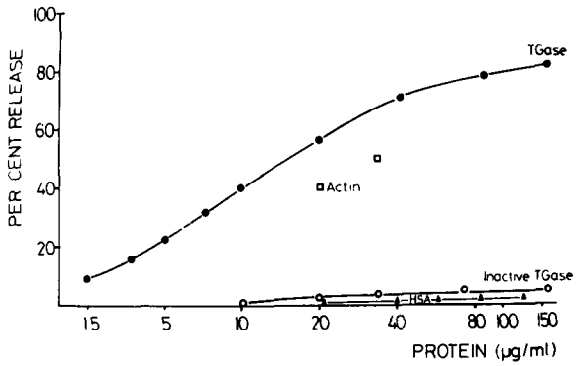

Fig.2. Dependence of phase transition release (PTR) from small unilamellar DPPC vesicles on TGase concentration. Various concentrations of intact or inactive (heat denatured for 30 min at 60°C) TGase, platelet actin and HSA were added to the vesicles; then PTR curves were obtained. The vesicle/lipid concentration was 50 μM. Per cent release of carboxyfluorescein was calculated after complete release by Triton X-100.

not a prerequisite. Besides, neither does it reduce the PTR-inducing capacity (not shown).

The PTR experiments suggest that TGase can be integrated into the lipid membrane at phase transition of the latter to form stable enzyme-vesicle recombinants. The Sepharose 4B elution profiles shown in fig.3 reveal some of the molecular details of the TGase-induced bilayer perturbation . There is no interaction (Panel A) when the enzyme is mixed with solid phase vesicles (T_c of DPPC is 38°C [18]) and the enzyme activity is unchanged. Fluid phase vesicles, in our case DLPC which have a T_c at -1.8°C [21], do not bind significant amounts of TGase either (Panel B), and TGase activity is not influenced. When the enzyme is present during phase transition, the consequence of the perturbation depends upon the lipid/protein ratio. When it is about 100:1 (Panel C) the result is the formation of vesicle-protein recombinants eluting in the void volume and significantly reduced TGase activity. In addition, there is a phospholipid peak representing intact vesicles and a protein one without catalytic activity (most probably the portion of denatured TGase inert in our preparation). When the lipid/protein ratio is about 25:1 the elution profile (Panel D) shows that the small vesicles disappeared, most of the phospholipid material is associated with the enzyme fraction in which the catalytic activity is reduced. Large vesicle-enzyme

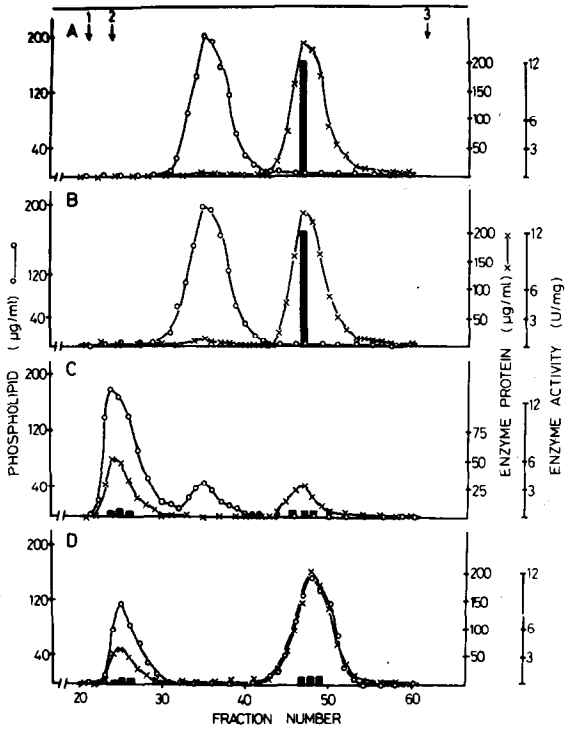

Fig.3. Sepharose 4B profile of the mixture made from small unilamellar phosphatidylcholine vesicles and TGase. Panel A, DPPC (1.2 mg/ml) vesicles and TGase (1.2 mg/ml) mixed at room temperature; Panel B, DLPC (1.2 mg/ml) vesicles and TGase (1.2 μg/ml) mixed at room temperature; Panel C, DPPC (1.2 mg/ml) vesicles and TGase (300 μg/ml) mixed at room temperature then warmed up to 43°C before chromatography; Panel D, DPPC (1.2 mg/ml) vesicles and TGase (1.2 mg/ml) mixed at room temperature then warmed up to 43°C before chromatography. The vesicles were prepared as in [13]. Chromatography was performed at room temperature collecting 1.0-ml fractions from which phospholipid (○) and protein (x-determined by Bio-Rad Protein Assay) concentrations as well as TGase activity (closed bars) was measured. Column size: 1.6 × 30 cm; references: (1) blue dextran; (2) large unilamellar vesicles prepared as in [13]; (3) HSA.

recombinants could be also seen. Therefore, lower lipid/protein ratios (at least lower then 25:1) favour disruption of vesicles at phase transition. High ratios (at least higher than 100:1) favour the formation of large recombinants (fusion?; vesicle aggregation?; increased size with the protein?) accompanied by the association of TGase with them.

It is known, that the perturbation/disruption of

the lipid bilayer can be induced by phospholipases. Since we could not find phospholipid breakdown products by thin-layer chromatography or detect phospholipase activity in any of the fractions or TGase preparations, this possibility was ruled out.

Studying the effect of the interaction between TGase and phospholipid vesicles of various kinds on the enzyme activity a pattern consistent with the above findings has been revealed (fig.4 and 5). Those vesicles which have a Tc above 37°C or below 0°C, that is DPPC, DSPC, DPPE, DPPS and DLPC [22], do not influence TGase activity. Vesicles made from PC, LyPC, PE, LyPE of liver origin or DMPC, which have a phase transition [22] in the temperature range of 0–37°C passed by the enzyme during the procedure of activity measurement, inhibit the enzyme in a concentration-dependent manner. Charged or uncharged, natural or synthetic as well as lysophospholipids were equally effective, provided the Tc criterion was met. Consequently, the main requirement for

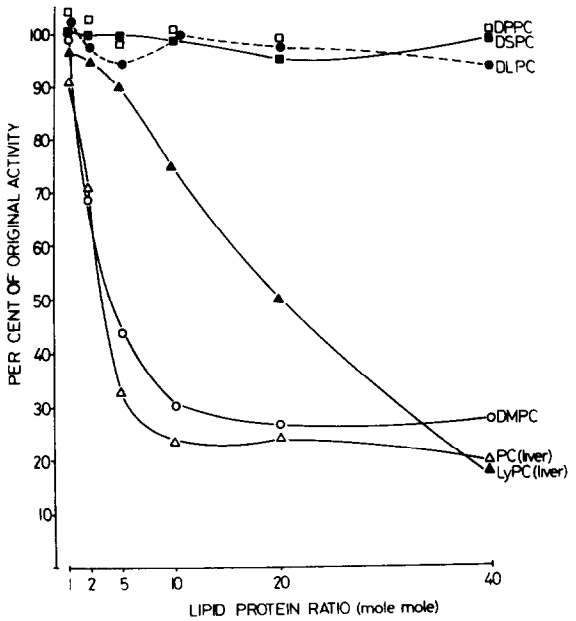

Fig.4. The effect of interaction of TGase with phosphatidylcholine vesicles on its catalytic activity. 20 μg TGase was added at 0°C to small unilamellar vesicles prepared from various phosphatidylcholines as described in [14]. The mixture was incubated at 37°C for 5 min, then added to the assay constituents at 37°C to measure catalytic activity. Original activity means the result was obtained with buffer instead of vesicles.

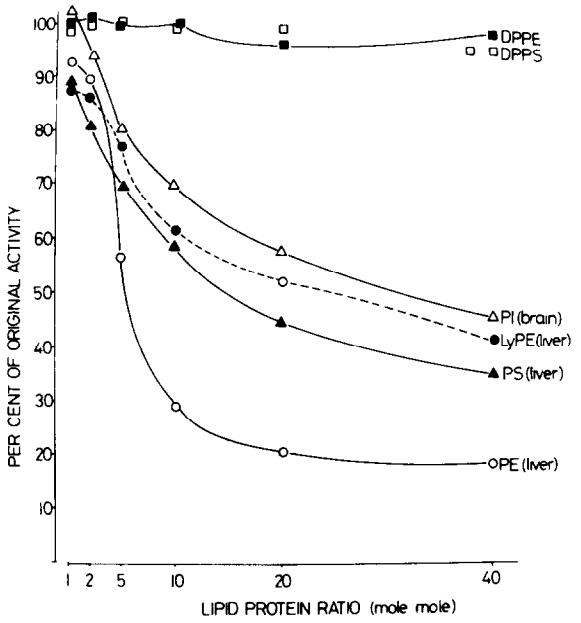

Fig.5. The effect of interaction of TGase with phosphatidylcholine vesicles on its activity. Small unilamellar vesicles prepared as in [14] were mixed to 20 μg TGase at 0°C then warmed up to 37°C for 5 min. TGase activity was measured adding the enzyme-phospholipid mixture to assay constituents.

the inhibition appears to be the hydrophobic environment provided by the phospholipid hydrocarbon chain whenever the enzyme is inserted into the bilayer. The inhibition is never complete and usually reaches its maximum at phospholipid/protein ratios 10–20:1. At this ratio complete PTR and the formation of TGase-phospholipid recombinants can be induced (see above).

There are several data which indicate that the demonstrated interaction of TGase with phospholipid bilayer may have cellular significance; e.g., the translocation of the enzyme [2,11], and the abrupt change of its activity [2,7,10] in various cell conditions, the presence of a significant amount of inhibited enzymes in cells [10]. The in vivo formation of TGase–phospholipid recombinants may be responsible for the demonstrated presence of more than one form of the enzyme in a cell [23,24], especially the ones which differ only slightly in electrophoretic mobility [25]. In addition, one can not exclude the possibility that some of the reported differences of enzyme activity in various cells or in a cell type

under various conditions [2,5–7,11] are merely the consequences of the in vitro homogenizing procedures inducing TGase-lipid interactions to various degrees. Obviously, further studies are needed to clarify the cellular significance of the results reported here, especially in relation to the implication of TGase in the mentioned cellular phenomena [3–7, 26]. Such experiments are in progress in our laboratory.

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REFERENCES

[1] Folk, J.E. and Finlayson, J.S. (1977) Adv. Protein Chem. 31, 1–133.
[2] Haddox, M.K. and Russel, D.H. (1981) Proc. Natl. Acad. Sci. USA 78, 1712–1716.
[3] Folk, J.E. (1980) Annu Rev. Biochem. 49, 517–531.
[4] Davies, P.J.A., Davies, D.R., Levitzki, A., Maxfield, F.R., Milhaud, P., Willingham, M.C. and Pastah, J. (1980) Nature 283, 163–167.
[5] Lorand, L., Hsu, L.K.H., Siefring, G.E. and Rafferty, J. (1981) Proc. Natl. Acad. Sci. USA 78, 1356–1360.
[6] Birckbichler, P.J., Orr, G.R., Patterson, M.K., Conway, E. and Carter, H.A. (1981) Proc. Natl. Acad. Sci. USA 78, 5005–5008.
[7] Fésüs, L. (1982) Surv. Immunol. Res. 1, 297–304.
[8] Fésüs, L. and Laki, K. (1977) Biochemistry 16, 4061–4066.
[9] Fésüs, L., Horváth, A. and Muszbek, L. (1982) Acta Biochem. Biophys. Hung. 17, 107–108.
[10] Birckbichler, P.J., Orr, G.R., Patterson, M.K., Conway, E. and Carter, H.A. (1982) Fed. Proc. 41, 754.
[11] Remington, J.A. and Russel, D.H. (1982) J. Cell. Physiol. 1113, 252–256.
[12] Rosenberg, S., Stracher, A. and Lucas, R.C. (1981) J.Cell. Biol. 91, 201–211.
[13] Huang, C. (1969) Biochemistry 8, 345–352.
[14] Barenholz, Y., Gibbes, D., Litman, B.J., Goll, J., Thompson, T.E. and Carlson, F.D. (1977) Biochemistry 16, 2806–2810.
[15] Klein, R.A. (1970) Biochim. Biophys. Acta 210, 486–489.
[16] Vaskovsky, V.E., Kostetsky, E.Y. and Vasendin, I.M. (1975) J. Chromatography 114, 129–141.
[17] Klausner, R.D., Kumar, N., Weinstein, J.N., Blumenthal, R. and Flavin, M. (1981) J. Biol. Chem. 256, 5879–5885.
[18] Weinstein, J.N., Klausner, R.D., Innerarity, T., Ralston, E. and Blumenthal, R. (1981) Biochim. Biophys. Acta 647, 270–284.
[19] Connellan, J.M., Chung, S.I., Whetzel, N.K., Bradley, L.M. and Folk, J.E. (1970) J. Biol. Chem. 246, 1093–1098.
[20] Fésüs, L. and Muszbek, L. (1983) Anal. Biochem., submitted.
[21] Utsumi, K., Okimasu, E., Morimoto, Y.M., Nishihara, Y. and Mivahara, M. (1982) FEBS Lett. 141, 176–180.
[22] Szoka, F. Jr. and Paphadjopoulos, D. (1980) Ann. Rev. Biophys. Bioeng. 9, 467–508.
[23] Kannagi, R., Teshigawara, K., Noro, N. and Masuda, R. (1982) Biochem. Biophys. Res. Commun. 105, 164–171.
[24] Chang, S.K. and Chung, S.I. (1982) Fed. Proc. 41, 641.
[25] Lorand, L., Siefring, G.E. Jr., Tong, Y.S., Brunner-Lorand, J. and Gray, A. Jr. (1979) Anal. Biochem. 93, 453–458.
[26] Fésüs, L., Falus, A., Erdei, A. and Laki, K. (1981) J. Cell Biol. 89, 706–710.