Is phospholipid a required cofactor for the activity of mammalian signal peptidase?

Masahide Uchida, Yasuhiro Watanabe and Yukio Fujimoto*

Department of Clinical Biochemistry, Hokkaido Institute of Pharmaceutical Sciences, Katsuraoka-cho, Otaru, Hokkaido 047-02, Japan

Received 20 March 1986

To determine whether phospholipid is required for the activity of mammalian signal peptidase, the enzyme was partially purified from porcine pancreas and then extensively freed of phospholipid by SP-Sephadex C-50 chromatography. The delipidated enzyme showed signal peptidase activity, with a low concentration of detergent. Phospholipid was found to release the enzyme from the inhibition due to excess detergent.

Peptidase *Phospholipid* *Delipidation* *Detergent*

1. INTRODUCTION

Signal peptidase is the enzyme responsible for removing the NH_2-terminal hydrophobic signal peptidase portions of newly synthesized secretory proteins during their translocation across the membrane [1]. The enzyme is an integral membrane protein [2–4], which is located in the endoplasmic reticulum [5,6], that can be solubilized with detergents [7,8]. Recently, we reported [8] the enrichment of signal peptidase from microsomal membranes of porcine pancreas through solubilization with Nonidet P-40 (NP-40).

Signal peptidase is considered to depend upon phospholipid for its activity [1–3]. Jackson and White [1] reported that phospholipid is a required cofactor for signal peptidase activity. On the other hand, Lively and Walsh [2] illustrated that an excess of detergent inhibits the enzyme in such a way that it can be partially overcome by the addition of purified phosphatidylcholine, and further concluded that signal peptidase requires phospholipid for its activity [3].

In this study, we investigated the phospholipid requirement for the signal peptidase activity using a delipidated enzyme preparation. Our results suggested that phospholipid is not essential for the activity of signal peptidase, but that it counteracts the inhibition of the enzyme activity due to a detergent.

2. EXPERIMENTAL

SP-Sephadex C-50 was a Pharmacia product. [^{35}S]Methionine was obtained from Amersham and soybean phosphatidylcholine (type III-S) from Sigma.

2.1. *Partial purification of signal peptidase and removal of phospholipid*

Signal peptidase was solubilized as described in [8] from microsomal membranes of porcine pancreas. The solubilized enzyme was applied to a column of DEAE-cellulose and then eluted as in [8]. The pooled active fractions were dialyzed against 10 mM Mes-NaOH (pH 6.0) containing 0.1% NP-40 and 1 mM PMSF (buffer A). The buffer

* To whom correspondence should be addressed

Abbreviations: NP-40, Nonidet P-40; PMSF, phenylmethylsulfonyl fluoride; Mes, 2-(*N*-morpholino)ethanesulfonic acid; pre-hPL, pre-human placental lactogen; SBPC, soybean phosphatidylcholine

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

was changed twice at 3 h intervals and then the dialyzate was left overnight for a total period of 18 h in buffer A. The dialyzed solution was applied to a SP-Sephadex C-50 column equilibrated with buffer A. Protein was estimated according to Lowry et al. [9] with crystalline serum albumin as a standard, in the presence of 2% SDS. Phospholipid phosphorus was determined by the ashing procedure of Bartlett [10]: 23.5 μg phospholipid was assumed to contain 1 μg phosphorus [11]. The amount of NP-40 was determined by the method of Horigome and Sugano [12].

2.2. *Signal peptidase assay*

The procedure was essentially as described in [8]. Protein (pre-hPL) was synthesized in a wheat germ cell-free system programmed by human placental poly(A^+) RNA. For the estimation of signal peptidase activity, 100 μl of assay mixture contained 5 μl translation mixture, 10 μl enzyme preparation, and varying amounts of detergent and phospholipid, in 50 mM Tris-HCl buffer (pH 8.0). The conversion of pre-hPL to hPL was analyzed by fluorography of the SDS-polyacrylamide gel (13%) plate after electrophoresis, and was quantified with a densitometer and expressed as the ratio of the precursor to the mature form [8].

3. RESULTS AND DISCUSSION

Although the signal peptidase reaction appears to be tightly coupled to the transport of nascent proteins across the membrane, little is known about the enzyme itself. The signal peptidase seemed to be an entirely new type of enzyme [7,8]. In an attempt to determine the role of phospholipid in the enzyme's activity, we tried to delipidate the signal peptidase and to reconstitute the enzyme.

When the dialyzed solution (1 ml of the solution contained 194 μg protein and 656 μg phospholipid), obtained on DEAE-cellulose chromatography, was subjected to SP-Sephadex C-50 column chromatography, the elution profile shown in fig.1 was seen. As can be seen, the phospholipid was clearly separated from most of the protein, under the conditions used. Pool II (total protein: 76 μg) was freed from endogenous

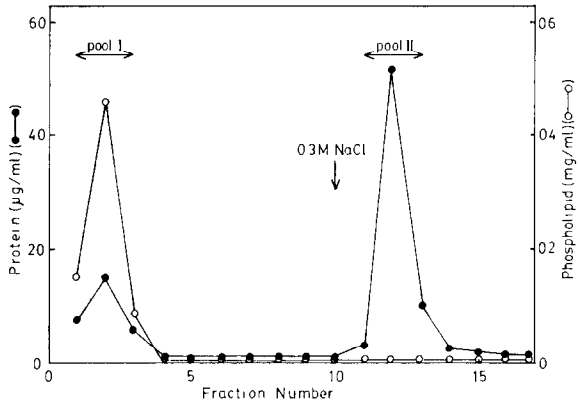

Fig.1. Removal of phospholipid by chromatography on SP-Sephadex C-50. 1 ml of the dialyzed solution of the partially purified signal peptidase was applied to a column of SP-Sephadex C-50 (1 × 3 cm) equilibrated with buffer A. The column was washed with the above buffer and then eluted with a stepwise salt gradient (300 mM NaCl) in the same buffer. Fractions of 1 ml were collected and aliquots of each fraction were assayed for protein and phospholipid.

phospholipid. When this preparation was assayed for enzyme activity with a final detergent concentration of 0.01% NP-40, it showed signal peptidase activity (fig.2A, lane 3). On the other hand, pool I (the total protein and phospholipid were 31 and 640 μg, respectively) did not show any signal peptidase activity (not shown). As can be seen in fig.2B, when the detergent concentration was increased to 1% in the assay system, pool II did not exhibit the signal peptidase activity (lane 2). However, interestingly, on combination pools I and II showed the activity (fig.2B, lane 3 and 2C, lane 1). The signal peptidase was found to be labile on heating: treatment at 60°C for 1 min caused a 90% loss of activity, and a 50% loss was seen at 40°C [8]. As can be seen in fig.2C, pool II after heating at 60°C for 1 min was inactive, even in the presence of pool I (lane 3). On the other hand, the signal peptidase activity could be restored in pool II by the addition of heated pool I (lane 4). Phosphatidylcholine was assumed to be the most probable endogenous phospholipid in pool I, although it seemed to be worthy of further study. The addition of exogenous phospholipid (SBPC) to pool II resulted in the restoration of the signal peptidase activity (fig.2D, lane 2), with 1% NP-40.

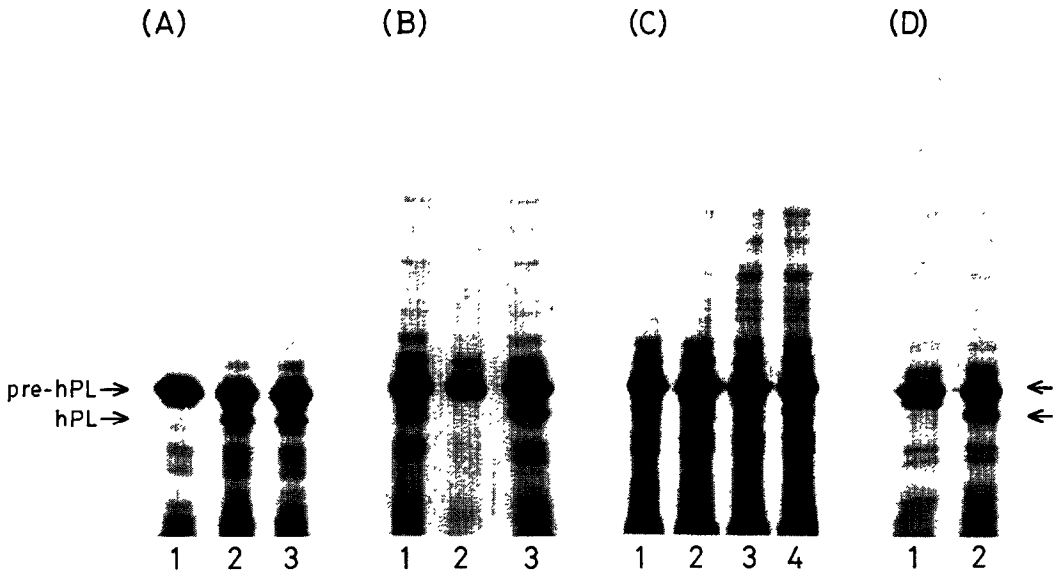

Fig.2. Post-translational cleavage of pre-hPL. (A) Assay performed in the presence of 0.01% NP-40. Lanes: 1, without enzyme (control); 2, with enzyme preparation before the delipidation; 3, with the delipidated signal peptidase, i.e., pool II in fig.1. (B–D) Assays performed in the presence of 1% NP-40. (B) Lanes: 1, pool I; 2, pool II; 3, pool I + pool II. (C) Lanes: 1, pool I + pool II; 2, heated pool I + heated pool II; 3, pool I + heated pool II; 4, heated pool I + pool II. (D) Lanes: 1, pool II; 2, pool II + SBPC (200 μg).

These results suggested that phospholipid was not essential for the signal peptidase activity, but it might cause the release of the enzyme from the detergent inhibition. An attempt was therefore made to release the delipidated signal peptidase from the enzyme inhibition through the addition of SBPC.

Prior to this study, the relationships between the amounts of enzyme protein, detergent and phospholipid, and the enzyme activity were examined. Signal peptidase is not able to be purified or its properties determined, unless it is solubilized and assayed in its soluble form [4,13]. Detergents have been extensively used for this purpose. However, a delicate balance may exist between solubilization and inactivation, with respect to the activity of signal peptidase. The enzyme activity increased with increasing protein amount and reached a plateau (not shown). A maximum of approx. 30% of the pre-hPL was converted to hPL. The minimum enzyme protein required for the maximum signal peptidase activity was found to be about 70 ng, in 100 μl of test mixture, in the presence of 1% NP-40 and 200 μg SBPC.

Fig.3 shows that, as exogenous phospholipid (SBPC) was added in increasing amounts, there was a concomitant increase in the signal peptidase activity, under the conditions of 70 ng protein in the presence of 1% NP-40. On the other hand, when the assay was performed with a final concentration of 0.01% detergent, the signal peptidase activity was independent of phospholipid (SBPC).

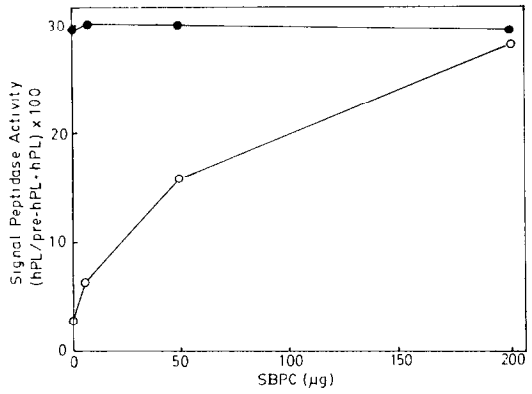

Fig.3. Apparent phospholipid requirement of the delipidated signal peptidase. The signal peptidase assays were performed with 70 ng of delipidated enzyme in the presence of 0.01% NP-40 (●) or 1% NP-40 (○).

We concluded that the apparent loss of the signal peptidase activity is not a direct result of the phospholipid depletion, but is due to the detergent inhibition at high concentration.

Jackson and Blobel [13] reported that the DOC-solubilized signal peptidase of dog pancreatic microsomes is inhibited by increasing concentrations of DOC. Recently, Hikawa et al. [14] reported the DOC-solubilization of the signal peptidase of rat liver microsomes, and also suggested that the enzyme was labile to show a detergent-dependent conformational change at increased temperature [13,14]. As to our observation of the phospholipid release of the detergent inhibition of signal peptidase activity, it is not yet clear whether restoration of the conformation occurred or not. However, phospholipid probably does not play an essential role itself in the activity of the signal peptidase, although it appears to be very important for stabilization of the active enzyme.

This study takes us one step closer to a full understanding of the signal peptidase and of the transfer of a nascent protein across the membrane.

REFERENCES

[1] Jackson, R.C. and White, W.R. (1981) J. Biol. Chem. 256, 2545–2550.
[2] Lively, M.O. and Walsh, K.A. (1983) J. Biol. Chem. 258, 9488–9495.
[3] Lively, M.O. and Walsh, K.A. (1983) J. Cell. Biochem. 7B, 331.
[4] Jackson, R.C. (1983) Methods Enzymol. 96, 784–794.
[5] Jackson, R.C. and Blobel, G. (1977) Proc. Natl. Acad. Sci. USA 74, 5598–5602.
[6] Bielinska, M., Rogers, G., Rucinski, T. and Boime, I. (1979) Proc. Natl. Acad. Sci. USA 76, 6125–6156.
[7] Kreil, G. (1981) Annu. Rev. Biochem. 50, 317–348.
[8] Fujimoto, Y., Watanabe, Y., Uchida, M. and Ozaki, M. (1984) J. Biochem. 96, 1125–1131.
[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[10] Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
[11] Bachmann, E., Allmann, D.W. and Green, D.E. (1966) Arch. Biochem. Biophys. 115, 153–164.
[12] Horigome, T. and Sugano, H. (1983) Anal. Biochem. 130, 393–396.
[13] Jackson, R.C. and Blobel, G. (1980) Ann. NY Acad. Sci. 343, 391–404.
[14] Hikawa, A., Hashimoto, M., Horigome, T., Omata, S. and Sugano, H. (1985) J. Biol. Chem. 97, 105–112.