Limited Proteolysis of Rat Phosphatidylinositol Transfer Protein by Trypsin Cleaves the C Terminus, Enhances Binding to Lipid Vesicles, and Reduces Phospholipid Transfer Activity*

(Received for publication, April 11, 1996, and in revised form, May 29, 1996)

Jacqueline M. Tremblay, George M. Helmkamp, Jr., and Lynwood R. Yarbrough‡

From the Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66160-7421.

Rat phosphatidylinositol transfer protein (PITP) is a 32-kDa protein of 271 amino acids that transfers phosphatidylinositol and phosphatidylcholine between membranes. The α isoform of rat PITP was expressed in Escherichia coli and purified in high yields. The purified protein contained 1 mol of phosphatidylylycerol and had a transfer activity for phosphatidylinositol and phosphatidylcholine equal to or greater than that of PITP purified from mammalian brain. Limited protease digestion was used to further define structure, activity, and function relationships in PITP. PITP alone is relatively resistant to digestion by chymotrypsin, trypsin, and Staphylococcus V8 protease but is readily cleaved by subtilisin. Phospholipid vesicles containing phosphatidic acid enhance susceptibility to digestion by all four proteases. In the presence of vesicles, PITP, which migrates as a 36-kDa protein in SDS-polyacrylamide gel electrophoresis, is cleaved rapidly by trypsin to a form that appears to be 2-3 kDa smaller than the native form. The tryptic fragment retains partial phospholipid transfer activity and shows an enhanced affinity for phospholipid vesicles containing phosphatidic acid. Analysis of the tryptic digestion products by immunoblotting, N-terminal sequencing, and electrospray mass spectrometry showed that trypsin cleaves the C terminus of PITP at Arg253 and Arg259. Thus, removal of the C terminus enhances the affinity of PITP for vesicles and results in a diminution of transfer activity. Overall, the data show that PITP undergoes conformation changes and that the C terminus becomes more accessible to trypsin when bound to vesicles. Hence, the C terminus is not an essential component of the membrane binding site and may be located distal to it.

All eukaryotic cells contain proteins that are capable of binding phospholipids. Phosphatidylinositol transfer protein (PITP) binds phosphatidylinositol and phosphatidylcholine (PtdCho) with relatively high specificity and catalyzes their intermembrane transfer. PITP was first purified over 20 years ago from bovine brain, a tissue in which it is found in relatively high amounts (1). It contains a single binding site for phospholipid, and in SDS-PAGE it exhibits an apparent size of 36 kDa (2, 3).

cDNAs encoding PITP have been cloned and sequenced for rat, human, mouse, and yeast (4–8). The predicted PITPs of rat, mouse, and human consist of 271–272 amino acids, have very highly conserved sequences, and have molecular masses of approximately 32 kDa. More recently, a cDNA encoding another PITP form was isolated from rat brain by complementation in yeast (9). Although the predicted protein is similar in size to the predicted PITPs of rat, human, and mouse, it has only 70% amino acid sequence similarity with those previously reported. Thus, there appear to be two genes encoding PITP in rat and apparently also in bovines (10). PITPs having strong sequence similarity to that sequenced initially by Dicke...

2 The abbreviations used are: PITP, phosphatidylinositol transfer protein; 98/2 PC/PA vesicles, vesicles containing 98/2 mol% phosphatidylcholine/phosphatidic acid; 80/20 PC/PA vesicles, vesicles containing 80/20 mol% phosphatidylcholine/phosphatidic acid; DTT, dithiothreitol; PtdCho, phosphatidycholine; PtdGro, phosphatidylglycerol; rPITP, the α isoform of rat PITP produced in E. coli; HPLC, high pressure liquid chromatography.
have used this approach to characterize recombinant rat PITP. In the absence of lipid vesicles, PITP is highly resistant to digestion by trypsin. In the presence of vesicles, digestion by trypsin is greatly enhanced, and the C terminus is rapidly cleaved at Arg253 and Arg259 to generate a mixture of at least two truncated species. The truncated protein(s) have greatly reduced transfer activity, and the affinity for vesicles is significantly enhanced. The results show that structural changes in the C terminus significantly alter the affinity of PITP for lipid vesicles.

**EXPERIMENTAL PROCEDURES**

Materials—PtdCho was purified from crude egg PtdCho (Sigma) by column chromatography on silica gel. Bovine liver phosphatidyl- serine (Staphylococcus aureus V8 protease) and lysed in an SLMA minicolumn (7-8642) with phenylmethylsulfonyl fluoride (P-7626). Soybean trypsin inhibitor with papain (from Cooper Biomed).

Construction of a Recombinant Clone Expressing rPITP—A cDNA encoding rat PITP α-isofrom was ligated into the pET-11C vector of Studier and co-workers (28, 29), which confines resistance to ampicillin, so that when the lac promoter regulating PITP gene expression was induced by isopropyl β-D-thiogalactoside an unfused protein of 271 amino acids (31.9 kDa) would be produced. For protein expression, the resulting plasmid (pET-PITP) was transformed into Escherichia coli BL21-D3E, which contained an additional plasmid encoding the repressor/operator.

**Purification and Characterization of rPITP from E. coli—**Cells were grown in the presence of both ampicillin and chloramphenicol to an optical density of one at 595 nm and induced with 200 μM isopropyl β-D-thiogalactoside for 18 h at 20°C. Following induction, cells were harvested by centrifugation at 4°C, and the resulting cell pellets were resuspended in buffer A (0.05 mM Tris-HCL, pH 7.5, 0.1 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF), and lyzed in an SLM Amino French pressure cell press (SLM Instruments, Inc.). The resulting lysate was centrifuged at 30,000 rpm for 30 min at 4°C in a type 50.2 Ti rotor (Beckman). The lysate supernatant from the centrifugation step (sample volume, ≤5% of bed volume) was loaded on a Sephadex G-100 column (52 mm × 90 cm) that was equilibrated with buffer B (0.05 mM sodium phosphate, pH 7.4, 0.1 mM EDTA, 0.05 mM phenylmethylsulfonyl fluoride, 0.1 mM DTT). The column was eluted with buffer B, and column fractions were analyzed by SDS-PAGE.

**Purification of Trypsin-Digested rPITP**—The α isofrom of rat PITP was expressed in E. coli using the procedures outlined under “Experimental Procedures.” Crude cell extracts were fractionated by gel chromatography and assayed by SDS-PAGE, and peak fractions were pooled. This material was then further purified by chromatography on Q Sepharose Fast Flow and hydroxyapatite as described above. Analysis of bound phospholipid following exchange showed 0.90 ± 0.02 mol of PtdCho/mol of PITP. Phospholipid transfer activities were measured between two populations of small unilamellar vesicles, as described by Kasper and Helmkkamp (32). Vesicles used in assays were prepared by injection of chloroform solutions into buffer; vesicles used for binding studies were prepared by extensive sonication. Radiolabeled phospholipid substrates for assays were phosphatidyl[3H]inositol and [1-3H]oleoyl-PtdCho. Control incubations were carried out in the absence of transfer protein. Activity is calculated as the percentage of donor phospholipid (40 nmol) transferred in a 30-min assay. Recovery of acceptor vesicles ranged between 94 and 100% activity in the absence of transfer protein rarely exceeded 6%.

**Transfer Activity of Trypsin-Digested rPITP—**PITP (final concentration, 0.25 mg/ml) was digested with a 1:150 (w/w) ratio of trypsin/PITP for 5 min at 37°C in the presence or the absence of 1 mg/ml PC/PA vesicles. Digestion was performed in 10 mM HEPES, pH 7.4, 1 mM EDTA, 50 mM NaCl at 37°C using the following ratios of protease to PITP: trypsin and chymotrypsin (1:25 (w/w) ratio); subtilisin and S. aureus V8 protease (1:100 (w/w) ratio). Aliquots were removed at specific times, and the reaction was stopped by addition of 20% SDS-PAGE sample buffer and boiling at 100°C for 10 min. Samples were fractionated by SDS-PAGE and stained with Coomassie Blue.

Transfer Activity of Trypsin-digested rPITP—(Final concentration in reaction, 0.25 mg/ml) was digested with a 1:150 (w/w) ratio of trypsin/PITP at 37°C in the presence or the absence of 3 mg/ml 90/20 PCE/PA vesicles. Digestions were performed in 10 mM HEPES, pH 7.4, 1 mM EDTA, 50 mM NaCl at 37°C using the following ratios of protease to PITP: trypsin and chymotrypsin (1:25 (w/w) ratio); subtilisin and S. aureus V8 protease (1:100 (w/w) ratio). Aliquots were removed at specific times, and the reaction was stopped by addition of 20% SDS-PAGE sample buffer and boiling at 100°C for 10 min. Samples were fractionated by SDS-PAGE and stained with Coomassie Blue.

**Results**

Purification and Characterization of rPITP Purified from E. coli—The α isofrom of rat PITP was expressed in E. coli using the procedures outlined under “Experimental Procedures.” Crude cell extracts were fractionated by gel chromatography and assayed by SDS-PAGE, and peak fractions were pooled. This material was then further purified by chromatography on Q Sepharose Fast Flow anion exchanger followed by chromatography on hydroxylapatite. After purification, PITP appeared homogeneous in SDS-PAGE. Yields normally ranged from 25–30 mg of purified PITP/liter of cell culture, if groES was overexpressed and cells were grown at 20°C. PITP was analyzed for bound lipid and found to contain 0.92 ± 0.08 mol of PtdGro/mol of protein (n = 4). PITP purified from mammalian brain exists as isoforms containing PtdCho and phosphatidylinositol. Prior to determination of transfer activity, PITP was incubated with...
vesicles containing 100 mol% PtdCho to exchange PtdGro. Vesicles were removed, and the protein was shown to contain 0.90 ± 0.02 mol of PtdCho/mol of protein. Transfer activities were then measured before and after lipid exchange. Both the PtdCho and PtdGro forms have approximately equal transfer activity in the standard assay, and the activity is equal to or greater than the activity of PITP purified from mammalian brain (data not shown).

Vesicles Enhance Digestion of rPITP by Proteases—Often the susceptibility to protease digestion is altered by interaction with ligands. We have therefore examined the digestion of PITP by chymotrypsin, trypsin, subtilisin, and S. aureus V8 protease in the presence or the absence of phospholipid vesicles. The products were analyzed by SDS-PAGE, and the results are shown in Fig. 1. In the absence of vesicles, PITP is resistant to digestion by chymotrypsin, trypsin, and V8 protease. Even after 1 h there is little digestion. In contrast, subtilisin produces two major fragments of approximately 25-30 kDa in size, and the digestion appears to be complete by 30 min. Thus, PITP is relatively resistant to protease digestion in the absence of vesicles, with the exception of subtilisin. Vesicles containing the anionic lipid, phosphatidic acid, enhance digestion by all proteases (Fig. 1B). Some new fragments are observed in the chymotrypsin-digested PITP and less of the undigested material remains. In the case of trypsin (lanes 4 and 5), conversion to a form that has an apparent size 2–3 kDa smaller than PITP is complete within 5 min. This product(s) then appears to be digested more slowly to smaller fragments. Subtilisin digestion is also enhanced; after 30 min little undigested material remains. V8 protease also digests PITP more readily in the presence of vesicles. Thus, PITP is more susceptible to digestion by a number of proteases if vesicles are present during the digestion. Moreover, both the PtdCho and PtdGro isoforms of PITP show the same patterns of protease sensitivity.

Protease Digestion of C Termminus of PITP

Trypsin Digestion of rPITP Is Enhanced by Vesicles, Is Dependent on Vesicle Composition, and Cleaves the C Terminus—To further characterize the digestion of PITP by trypsin, samples were digested in the absence of vesicles, with 98/2 PC/PA vesicles, or with 80/20 PC/PA vesicles, as described in Fig. 1. Samples were then fractionated by SDS-PAGE and stained with Coomassie Blue (Fig. 2A), immunoblotted with antibodies against the N terminus of PITP (Fig. 2B), or immuno- blotted with antibodies to the C-terminal 12 amino acids of PITP (Fig. 2C). In the absence of vesicles (lanes 1–3), PITP is relatively resistant to digestion by trypsin, as described above. The immunoblots (Fig. 2, B and C) confirm that there is little if any digestion in the absence of vesicles. If, however, digestion is performed in the presence of vesicles containing 98/2 PC/PA, a band appears after 5 min of digestion that runs slightly below the main band in Coomassie-stained gels (lanes 4–6). This band reacts with antibody to the N terminus (Fig. 1B) but does not react with the antibody to the C terminus (Fig. 2C). When digestion is performed in the presence of vesicles containing 80/20 PC/PA the conversion to the slower migrating band is complete within 5 min (lanes 8 and 9). This slower migrating band, which appears to be smaller than PITP by about 2–3 kDa, reacts with antibody to the N terminus of PITP but shows no reaction with the antibody to the C terminus (lanes 8 and 9, Fig. 2C). Thus, the data suggest that trypsin cleaves rapidly the C terminus of PITP in the presence of 80/20 vesicles, thereby eliminating the antigenic determinant(s) associated with the C-terminal 12 amino acids of PITP. Conceivably, the C-terminal peptide produced by trypsin might remain associated noncovalently with the larger N-terminal fragment. Attempts to detect a small peptide of 13–18 amino acids by SDS-PAGE were not successful. Thus, we presume that the C-terminal peptide is released and degraded or that cleavage occurs from the C terminus in a sequential manner. Control experiments were performed to determine whether vesicles affected the action of trypsin on another protein, beef brain tubulin; no significant effects were observed upon the rate of digestion or the products produced. Thus, the enhanced digestion of PITP in the presence of vesicles is apparently a specific effect and not a general effect of vesicles on trypsin digestion of
proteins.

Fig. 2 shows that digestion of PITP by trypsin (1:15 (w/w) ratio) is complete by 5 min. To further characterize the enhanced sensitivity to trypsin in the presence of vesicles, digestion was performed with 80/20 PC/PA vesicles using smaller ratios of trypsin to PITP. Digestion with a ratio of trypsin to PITP of 1:200 was complete by 5 min, as measured by SDS-PAGE, (data not shown). Thus, vesicles with a high content of phosphatidic acid enhance susceptibility to trypsin by orders of magnitude.

N-terminal Analysis and Mass Spectrometry of Proteolytic Products—The immunoblotting studies suggested that digestion by trypsin occurred at the C terminus of the molecule. To further characterize the digestion products, PITP was digested briefly with trypsin in the presence of 80/20 PC/PA vesicles, which completely converted it into the smaller form, as measured by SDS-PAGE. The protein was then isolated, and N-terminal sequence analyses were performed. These studies showed that the majority of the digested protein retained the initiating Met residue, as was found in undigested controls (data not shown). Further characterization of the tryptic digestion products was performed using electrospray mass spectrometry. The molecular mass of PITP, which had not been digested with trypsin, was determined to be 31,914 Da, in good agreement with a mass calculated for the predicted protein of 31,909, based on amino acid sequence. PITP, which had been subjected to trypsin digestion displayed two components of lesser mass. One component had a mass of 30,607 ± 3; a second had a mass of 29,846 ± 4. These data were consistent with two components resulting from cleavage at Arg253 and Arg259. The masses for these fragments were calculated to be 30,622 Da and 29,850 Da, respectively.

Table I

| Trypsin | Vesicles | Transfer activity % transfer/μg 30 min |
|---------|----------|---------------------------------------|
|         |          |                                       |
| ------  | -------- |                                       |
| -      | -        | 8.5 ± 3.3                             |
| +      | -        | 8.5 ± 0.4                             |
| +      | +        | 2.1 ± 0.5                             |
| -      | +        | 6.8 ± 0.1                             |

Transfer Activity of Trypsin Digestion Products—The in vitro transfer activity of control and trypsin digested PITP was examined using standard assay conditions and 98/2 PC/PA donor and acceptor vesicles (Table I). PITP showed about a 75% reduction in transfer activity following digestion with trypsin. Control experiments showed that this reduction in activity was dependent on digestion with trypsin. The addition of 80/20 PC/PA vesicles to undigested PITP had no inhibitory effect in the assay. Thus, removal of the C-terminal 12–18 amino acids of PITP greatly reduced but did not eliminate transfer activity as measured in the standard assay.

Analysis of Binding of rPITP and Its Trypsin Digestion Product to Lipid Vesicles by HPLC Gel Filtration—Digestion of PITP by trypsin occurred much more rapidly in the presence of 80/20 PC/PA than 98/2 PC/PA vesicles. This suggested differences in interaction of PITP with vesicles of differing lipid composition. To explore the binding of PITP to vesicles, PITP was added to either 80/20 PC/PA or 98/2 PC/PA vesicles to give a final vesicle concentration of 1 mg and incubated for 5 min at 37 °C. Following incubation, the solutions were analyzed by HPLC gel chromatography on a Tosohaas TSK-GEL G2000SW XL column. Elution was monitored by intrinsic fluorescence with excitation at 290 nm and emission at 340 nm. A control sample without vesicles was also chromatographed. The column void volume was eluted at approximately 5.5 min.

**DISCUSSION**

Using the pET vector system, we have developed procedures for expression in E. coli and purification in large amounts of the α isof orm of rat PITP. We have employed a combination of low cell growth temperature and overexpression of groELs to significantly enhance solubility of PITP produced in E. coli. Previous studies by a number of investigators have shown that low cell growth temperature (33–35) and overexpression of molecular chaperones (17, 36, 37) can significantly enhance yields and/or solubility of proteins expressed in E. coli. As reported by Geijtenbeek et al. (6) for mouse PITP, we find that if expressed at 37 °C, PITP is mostly insoluble. In contrast, if expressed at 20 °C with elevated levels of groELs, approximately half the PITP produced is in soluble form. Our yields (25–30 mg of PITP/1 liter of cell culture) are about an order of magnitude greater than the 3 mg of PITP/liter of cell culture reported for mouse.
mouse PITP (6). rPITP is active and contains 1 mol of PtdGro as was previously reported for mouse PITP (6). N-terminal sequencing and mass spectrometry analysis are consistent with the majority of the protein retaining its initiating Met residue.

The data we have obtained demonstrate that PITP is highly resistant to digestion by trypsin, chymotrypsin, and V8 protease in the absence of phospholipid bilayer. However, in the presence of phospholipid vesicles, digestion by trypsin is greatly enhanced, and the conversion to a C-terminally truncated product is complete within 5 min, even at relatively low ratios of enzyme to PITP. Under the conditions used for digestion, a significant fraction of PITP is bound to vesicles. Moreover, increased binding to vesicles, as measured by gel filtration, is associated with a more rapid rate of digestion by trypsin. Thus, the C terminus of PITP becomes highly susceptible to digestion by trypsin when bound to vesicles. This would appear to be due to changes in conformation of the C terminus. There may also be conformational changes in regions other than the C terminus of PITP because the susceptibility to other proteases is also enhanced when it is bound to vesicles. Studies of sulfhydryl reactivity and tyrosine second derivative spectra show that binding of PITP to vesicles is associated with increased exposure or reactivity of these residues. Overall, the data suggest that vesicle binding leads to a somewhat looser and more flexible conformation. Similarly, a more relaxed conformation has been described for two C-terminal truncated species (residues 1–253 and residues 1–259).3 However, the changes in conformation of PITP on binding to vesicles apparently do not involve significant changes in secondary structure because the far UV CD spectrum is not significantly altered by binding to vesicles.4

Why some proteins are subject to limited digestion by specific proteases is not clear. Conformational or segmental mobility and exposure or accessibility have been suggested previously as important determinants of proteolytic susceptibility (27). The interaction of trypsin with substrates and protein inhibitors has been studied extensively. X-ray crystal structures for several trypsin-protein inhibitor complexes have been determined. It has been suggested that the susceptible region of a protein that is cleaved by trypsin must assume a conformation similar to the conformation of that portion of the trypsin inhibitor (loop) that binds to the active site of the enzyme (26). Recent studies of tryptic cleavage sites of several native proteins using molecular modeling supports the conclusion that these “nick” sites are able to assume a loop-like conformation similar to that of the bound trypsin inhibitors (26).

Based on the results of these studies and the known specificity of trypsin, we have developed a model for the digestion of PITP by trypsin (Fig. 4). In the absence of vesicles, the C terminus of PITP is bound to the remainder of the protein and hence is resistant to digestion. When PITP binds to vesicles the conformation and/or stability of the C terminus is altered resulting in weaker binding to the protein core. Consequently, the more flexible C terminus can then more readily assume a conformation, which is necessary for binding and cleavage by trypsin. Predictive methods based on the amino acid sequence of PITP suggest that the C-terminal 18 amino acids are highly hydrophilic, located on the surface, and flexible. The model has the membrane binding site distal to the C terminus. This is based on the assumption that the C terminus would be unavailable to trypsin because of steric effects if it were a part of the membrane binding site.

3 P. Voziyan, J. M. Tremblay, L. R. Yarbrough, and G. M. Helmkamp, Jr., Biochemistry, in press.
4 J. M. Tremblay and L. R. Yarbrough, unpublished data.
the effect of the C terminus on transfer activity and membrane binding requires the study of homogeneous truncated derivatives.

The increased affinity of trypsin-digested PITP for vesicles is an important contributor to the decrease in transfer activity. Previous studies have shown that PITP binds more tightly to negatively charged phospholipids and that transfer activity is reduced under these conditions (23). We have found that the transfer activities of derivatives of PITP truncated at Arg253 and Arg250 are highly dependent on the lipid composition of the donor and acceptor vesicles and the position of truncation.2 Both of these derivatives bind much more avidly to vesicles than full-length PITP. Although membrane binding is a necessary condition for lipid transfer, if it becomes too tight, transfer activity may be greatly reduced or eliminated.

The in vivo function of PITP is only now becoming clear. In view of the greatly enhanced binding of truncated PITP derivatives to vesicles, it is possible that there might be significant biological effects if these derivatives were introduced into cells. Because of the greatly enhanced binding affinity they could form tight complexes with membranes, which might then affect interactions with the normal protein. Thus, the truncated PITP derivatives may be useful in further elucidating the in vivo function of this protein.

Acknowledgments—We thank Greg Grant (Washington University, Saint Louis, MO) for performing the mass spectral analysis of PITP and its digestion products. We gratefully acknowledge Paul Vázquez's helpful comments during preparation of the manuscript.

REFERENCES
1. Helmkamp, G. M., Jr., Harvey, M. S., Wirtz, K. W. A., and van Deenen, L. L. M. (1974) J. Biol. Chem. 249, 6382–6389
2. Helmkamp, G. M., Jr. (1980) Subcell. Biochem. 16, 129–174
3. Wirtz, K. W. A. (1991) Annu. Rev. Biochem. 60, 73–99
4. Dickeson, S. K., Lim, C. N., Schuyler, G. T., Dalton, T. P., Helmkamp, G. M., Jr., and Yarbrough, L. R. (1989) J. Biol. Chem. 264, 16557–16564
5. Dickeson, S. K., Helmkamp, G. M., Jr., and Yarbrough, L. R. (1994) Gene (Amst.) 142, 301–305
6. Geijtenbeek, T. B., de Groot, E., van Baal, J., Brunink, F., Westerman, J., Snoek, G. T., and Wirtz, K. W. A. (1994) Biochim. Biophys. Acta 1213, 309–318
7. Bankaitis, V. A., Malehoun, D. E., Emr, S. D., and Greene, R. (1989) J. Cell Biol. 108, 1271–1281
8. Salama, S. R., Cleves, A. E., Malehoun, D. E., Whitters, E. A., and Bankaitis, V. A. (1990) J. Bacteriol. 172, 4510–4521
9. Tanaka, S., and Hosaka, K. (1994) J. Biochem. (Tokyo) 115, 981–984
10. de Vries, K. J., Heinrichs, A. A., Cunningham, E., Brunink, F., Westerman, J., Somerharju, P. J., Cockcroft, S., Wirtz, K. W., and Snoek, G. T. (1995) Biochim. Biophys. Acta 1210, 643–649
11. Aitken, J. F., van Heusden, G. P. H., Temkin, M., and Dowhan, W. (1990) J. Biol. Chem. 265, 4711–4717
12. Skinner, H. B., Alb, J. G., Jr., Whitters, E. A., Helmkamp, G. M., Jr., and Bankaitis, V. A. (1993) EMBO J. 12, 4775–4784
13. Hay, J. C., and Martin, T. F. Jr. (1993) Nature 366, 572–575
14. Thomas, G. M., Cunningham, E., Fensome, A., Ball, A., Totty, N. F., Truong, O., Hisuan, J. J., and Cockcroft, S. (1993) Cell 74, 919–928
15. Hay, J. C., Fleitte, P. L., j enkins, G. H., Fukami, K., Takenawa, T., Anderson, R. A., and Martin, T. F., Jr. (1995) Nature 374, 173–177
16. Kauffmann-Zeh, A., Thomas, G. M. H., Ball, A., Prosser, S., Cunningham, E., Cockcroft, S., and Hisuan, J. J. (1995) Science 268, 1188–1190
17. Blum, P., Velligan, M., Lin, N., and Martin, A. (1992) Bio/Technology 10, 301–304
18. Ohashi, M., de Vries, K. J., Frank, R., Snoek, G., Bankaitis, V., Wirtz, K., and Huttner, W. B. (1995) Nature 377, 544–547
19. Liscovitch, M., and Cantley, L. C. (1995) Cell 85, 659–662
20. Cunningham, E., Thomas, G. M. H., Ball, A., Hiles, I., and Cockcroft, S. (1995)Curr. Biol. 5, 775–783
21. Kasurinen, J., van Paridon, P. A., Wirtz, K. W. A., and Somerharju, P. (1990) Biochimica 28, 8548–8554
22. van Paridon, P. A., Gadella, T. W. J., Jr., Somerharju, P. J., and Wirtz, K. W. A. (1988) Biochimica 27, 6238–6241
23. Somerharju, P., Van Paridon, P., and Wirtz, K. W. A. (1983) Biochim. Biophys. Acta 731, 186–195
24. DiCorleto, P. P., Warach, J. B., and Zilversmit, D. B. (1979) J. Biol. Chem. 254, 7795–7802
25. Ellison, D., Hinton, J., Hubbard, S. J. Jr., and Beynon, R. J. (1995) Protein Sci. 4, 1337–1345
26. Hubbard, S. J., Eisenmenger, F., and Thornton, J. M. (1994) Protein Sci. 3, 757–768
27. Price, N. C., and Johnson, C. M. (1989) Proteolytic Enzymes: A Practical Approach (Beynon, R. J., and Bond, J. S., eds), pp. 163–180, IRL Press, Oxford, UK
28. Studier, F. W., and Moffatt, B. A. (1986) J. Mol. Biol. 189, 113–130
29. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
30. Goloubinoff, P., Gatenby, A. A., and Lorimer, G. H. (1989) Nature 337, 44–47
31. Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–468
32. Kasper, A. M., and Helmkamp, G. M., Jr. (1981) Biochim. Biophys. Acta 664, 22–32
33. Schein, C. H., and Noteborn, M. M. H. (1988) Bio Technology 6, 291–294
34. Schein, C. H. (1989) Bio Technology 7, 1141–1149
35. Mitra, A., Fane, B., Haase-Pettingell, C., Sturtevant, J., and King, J. (1991) Science 253, 54–58
36. Lee, S. C., and Olins, P. O. (1992) J. Biol. Chem. 267, 2849–2852
37. Wyen, R. M., Davie, J. R., Cox, R. P., and Chuang, D. T. (1992) J. Biol. Chem. 267, 12400–12403
Limited Proteolysis of Rat Phosphatidylinositol Transfer Protein by Trypsin Cleaves the C Terminus, Enhances Binding to Lipid Vesicles, and Reduces Phospholipid Transfer Activity

Jacqueline M. Tremblay, Helmkamp M. George, Jr. and Lynwood R. Yarbrough

*J. Biol. Chem.* 1996, 271:21075-21080.
doi: 10.1074/jbc.271.35.21075

Access the most updated version of this article at [http://www.jbc.org/content/271/35/21075](http://www.jbc.org/content/271/35/21075)

**Alerts:**
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/271/35/21075.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 36 references, 12 of which can be accessed free at [http://www.jbc.org/content/271/35/21075.full.html#ref-list-1](http://www.jbc.org/content/271/35/21075.full.html#ref-list-1)