Mayo, S. L., Olafson, B. D., & Goddard, W. A., III (1989) *Biograf Manual*, BioDesign, Inc.
Mayo, S. L., Olafson, B. D., & Goddard, W. A., III (1991) *J. Phys. Chem.* (in press).
Mosesson, M. W., DiOrio, J. P., Müller, M. F. Shainoff, J. R., Siebenlist, K. R., Amrani, D. L., Homandberg, G. A., Soria, J., Soria, C., & Samama, M. (1987) *Blood 69*, 1073-1081.
Olexa, S. A., & Budzynski, A. Z. (1979) *J. Biol. Chem. 254*, 4925-4932.
Olexa, S. A., & Budzynski, A. Z. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1374-1378.
Olexa, S. A., Budzynski, A. Z., Doolittle, R. F., Cottrell, B. A., & Greene, T. C. (1981) *Biochemistry 21*, 6139-6145.
Pandya, B. V., & Budzynski, A. Z. (1984) *Biochemistry 23*, 460-470.
Pandya, B. V., Rubin, R. N., Olexa, S. A., & Budzynski, A. Z. (1983) *Toxicon 21*, 515-526.
Pandya, B. V., Cierniewski, C. S., & Budzynski, A. Z. (1985) *J. Biol. Chem. 260*, 2994-3000.
Shainoff, J. R., & Dardik, B. N. (1979) *Science 204*, 200-202.
Shainoff, J. R., & Dardik, B. N. (1983) *Ann. N.Y. Acad. Sci. 408*, 254-267.
Shimizu, A., Saito, Y., Matsushima, A., & Inada, Y. (1983) *J. Biol. Chem. 258*, 7915-7917.
Shimizu, A., Saito, Y., & Inada, Y. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 591-593.
Skogen, W. F., & Wilner, G. D. (1986) *Thromb. Res. 41*, 161-166.
Southan, C., Thompson, E., Panico, M., Etienne, T., Morris, H. R., & Lane, D. A. (1985) *J. Biol. Chem. 260*, 13095-13101.
Varadi, A., & Scheraga, H. A. (1986) *Biochemistry 25*, 519-528.
Weisel, J. W. (1986) *Biophys. J. 50*, 1079-1093.

Characterization of cDNA for Human Tripeptidyl Peptidase II: The N-Terminal Part of the Enzyme Is Similar to Subtilisin[†,‡]

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ABSTRACT: Tripeptidyl peptidase II is a high molecular weight serine exopeptidase, which has been purified from rat liver and human erythrocytes. Four clones, representing 4453 bp, or 90% of the mRNA of the human enzyme, have been isolated from two different cDNA libraries. One clone, designated A2, was obtained after screening a human B-lymphocyte cDNA library with a degenerated oligonucleotide mixture. The B-lymphocyte cDNA library and a cDNA library, obtained from human fibroblasts, were rescreened with a 147 bp fragment from the 5′ part of the A2 clone, whereby three different overlapping cDNA clones could be isolated. The deduced amino acid sequence, 1196 amino acid residues, corresponding to the longest open reading frame of the assembled nucleotide sequence, was compared to sequences of current databases. This revealed a 56% similarity between the bacterial enzyme subtilisin and the N-terminal part of tripeptidyl peptidase II. The enzyme was found to be represented by two different mRNAs of 4.2 and 5.0 kilobases, respectively, which probably result from the utilization of two different polyadenylation sites. Furthermore, cDNA corresponding to both the N-terminal and C-terminal part of tripeptidyl peptidase II hybridized with genomic DNA from mouse, horse, calf, and hen, even under fairly high stringency conditions, indicating that tripeptidyl peptidase II is highly conserved.

In 1983, the discovery of a mammalian peptidase, which, at neutral pH, removes tripeptides from the N-terminus of longer peptides, was reported (Bålöw et al., 1983). This peptidase, currently named tripeptidyl peptidase II (TPP II),[1] has since been characterized (Bålöw et al., 1986; Bålöw & Eriksson, 1987; Macpherson et al., 1987; Tomkinson et al., 1987; Tomkinson & Zetterqvist, 1990) and shown to possess a number of unusual properties. TPP II is a high molecular weight serine exopeptidase, consisting of subunits with M_r 135000. The amino acid sequence surrounding the active-site serine residue is similar to the peptidases of the subtilisin class (Tomkinson et al., 1987), while the other mammalian serine peptidases studied so far are of the trypsin class (Neurath, 1984). Furthermore, it has been noted that there is an immunological cross-reactivity between TPP II and the cell binding domain of the extracellular matrix protein fibronectin (Tomkinson & Zetterqvist, 1990).

In order to investigate the nature, as well as the extent, of the similarity of TPP II to subtilisin and fibronectin, respectively, and to reveal possible similarities to other proteins, it was considered necessary to determine the amino acid sequence of the peptidase. The knowledge of part of the amino acid

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[1] Abbreviations: EBV, Epstein-Barr virus; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl and 15 mM sodium citrate; TPP II, tripeptidyl peptidase II.

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sequence of a 20-kDa fragment of the human enzyme (Tomkinson & Zetterqvist, 1990), allowed, in the present work, the synthesis of an oligonucleotide mixture of low heterogeneity which was used as a probe for screening a cDNA library from human B-lymphocytes.

Materials and Methods

Screening of cDNA Libraries. A cDNA library, containing 5×10^5 independent clones, was constructed in the λgt 10 vector, with mRNA from human Epstein–Barr virus (EBV) transformed B-lymphocytes, as previously described (Jonsson et al., 1989). A degenerated synthetic oligonucleotide with the antisense sequence 5′-TG$^{T}_{C}$TT$^{A}_{G}$TCCAT$^{A}_{G}$TC$^{A}_{G}$TT$^{T}_{C}$TT-3′, based on residues 12–18 of the N-terminal amino acid sequence of the 20-kDa TPP II fragment (Tomkinson & Zetterqvist, 1990), was labeled at its 5′ end with [γ-^{32}P]ATP and T4 polynucleotide kinase and used for screening (Maniatis et al., 1982). The recombinant clones were screened at a density of 100000 on 24 × 24 cm screening plates. Hybridization with the labeled oligonucleotide was performed for 4 h at 43 °C in 6 × SSC (1 × SSC = 0.15 M NaCl in 0.015 M sodium citrate buffer, pH 7.0), 10 × Denhardt's solution [1 × Denhardt's solution = 0.02% bovine serum albumin, 0.02% Ficoll, and 0.02% poly(vinylpyrrolidone)], and 50 μg/mL single-stranded salmon sperm DNA, as described (Mason & Williams, 1985). The library was also screened by using a 147 bp *Eco*RI–*Bgl*II fragment from the 5′ end of the A2 clone (see Figure 2A), labeled to high specific activity with [α-^{32}P]dCTP by random priming (Feinberg & Vogelstein, 1984). Hybridization with this probe and washing of the nitrocellulose filters were carried out as previously described (Jonsson et al., 1989). The *Eco*RI–*Bgl*II fragment was also used to screen a λgt11 library, made from human fibroblasts.

λ phages were prepared by the liquid culture method, and the DNA was extracted by standard methods (Maniatis et al., 1982). The fragments obtained after digestion of positive clones with *Eco*RI, *Stu*I (A2), or *Bam*HI + *Hin*dIII (F5) were subcloned into the plasmid vector pUC 19 (Vieira & Messing, 1982).

Nucleotide Sequence Determination. The nucleotide sequence was determined on both strands in pUC 19, by using the dideoxy chain termination reaction with [α-^{35}S]dATP and the modified T7 DNA polymerase ("Sequenase", United States Biochemical Corp., Cleveland, OH) (Tabor & Richardson, 1987). Specific or universal oligonucleotide primers (binding on either side of the pUC 19 polylinker), were synthesized on an applied Biosystems 380A oligonucleotide synthesizer (Foster City, CA) and used as indicated in Figure 2.

Northern Blot Analysis. Polyadenylated mRNA (10 μg) was separated in a 1.2% agarose gel containing 2.2 M formaldehyde (Maniatis et al., 1982) and transferred onto nitrocellulose membranes. Hybridization was carried out for 16–40 h at 42 °C in 50% formamide, 10 mM Hepes, pH 7.4, 1 × Denhardt's solution, 3 × SSC, 0.1% SDS, 1 mg/mL yeast RNA, 0.1 mg/mL single-stranded salmon sperm DNA, and 2 mM EDTA. Probes, i.e., fragments of the A2 clone, as indicated in the legend of Figure 5, were labeled by random priming (Feinberg & Vogelstein, 1984).

Southern Blot Analysis. DNA (10 μg), prepared as described (Andersson et al., 1987), was digested overnight with 30 U of restriction endonuclease, as indicated. The DNA-fragments were separated in an 0.7% agarose gel and transferred onto Biodyne hybridization membranes (Pall, New York). Hybridization, using probes labeled by random priming, was carried out overnight at 42 °C in 40% formamide, 10% dextran sulfate, 1% SDS, 0.2 mg/mL single-stranded salmon sperm DNA, 50 mM sodium phosphate buffer, pH 6.4, 5 × Denhardt's solution, and either 0.5 M NaCl ("high stringency"-Figure 6) or 1 M NaCl ("low stringency"-Figure 7). The filters were washed twice for 5 min with 2 × SSC, 0.2% SDS at room temperature, and twice for 25 min at 60 °C with 0.7 × SSC, 0.5% SDS.

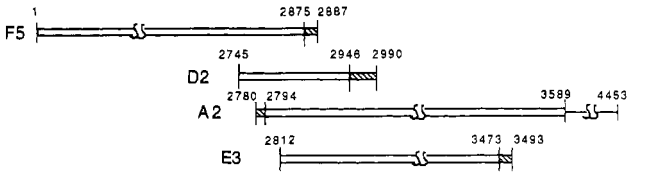

FIGURE 1: Diagram showing the position of the different clones in relation to each other. (—) indicates a noncoding region; open bars indicate a coding region; hatched bars indicate differing nucleotide sequence; SS indicates discontinuity in scale.

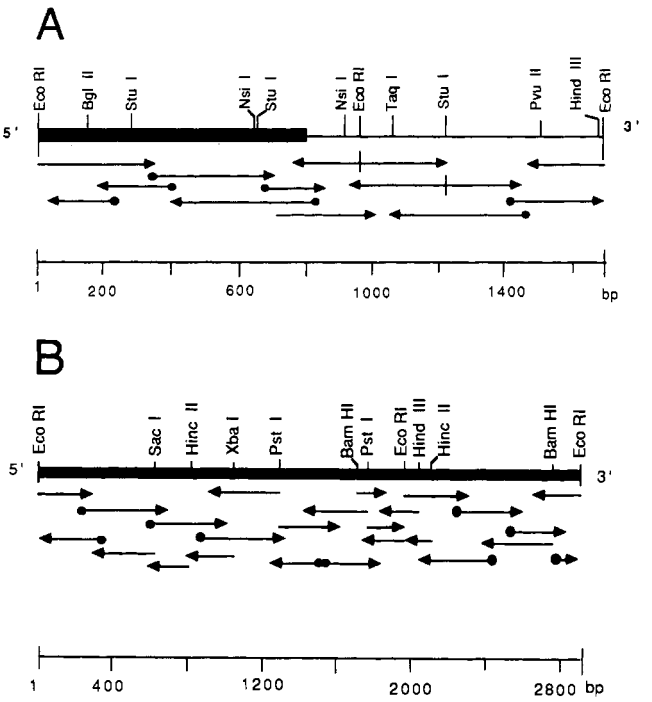

FIGURE 2: Restriction sites relevant to the determination of the nucleotide sequence of the insert of clone A2 (A) and clone F5 (B) and sequencing strategy. Bold and fine lines represent coding and noncoding regions, respectively. Arrows indicate the extent and direction of each sequencing reaction. (●) indicates use of a specific sequencing primer.

Results and Discussion

Isolation and Characterization of cDNA Clones Coding for Human Tripeptidyl Peptidase II. When the mixed oligonucleotide, prepared as described under Materials and Methods, was used for screening the human lymphocyte cDNA library, one positive clone (A2) was identified out of 1×10^6 clones screened.

In a new screening of 1×10^6 clones of the lymphocyte cDNA library, a 147 bp *Eco*RI–*Bgl*II fragment containing the 5′ end of the sense strand of the A2 clone was used as a probe. Only one new positive clone (D2) was obtained. Since no clones of extended cDNA could be detected in this library, another cDNA library, constructed from human fibroblasts, was investigated. Out of 2×10^6 screened clones, 2 positive clones were identified and designated E3 and F5. The length and the position of the different clones in relation to each other are indicated in Figure 1. The nucleotide sequence was determined on both strands, with an overlap covering 32% of

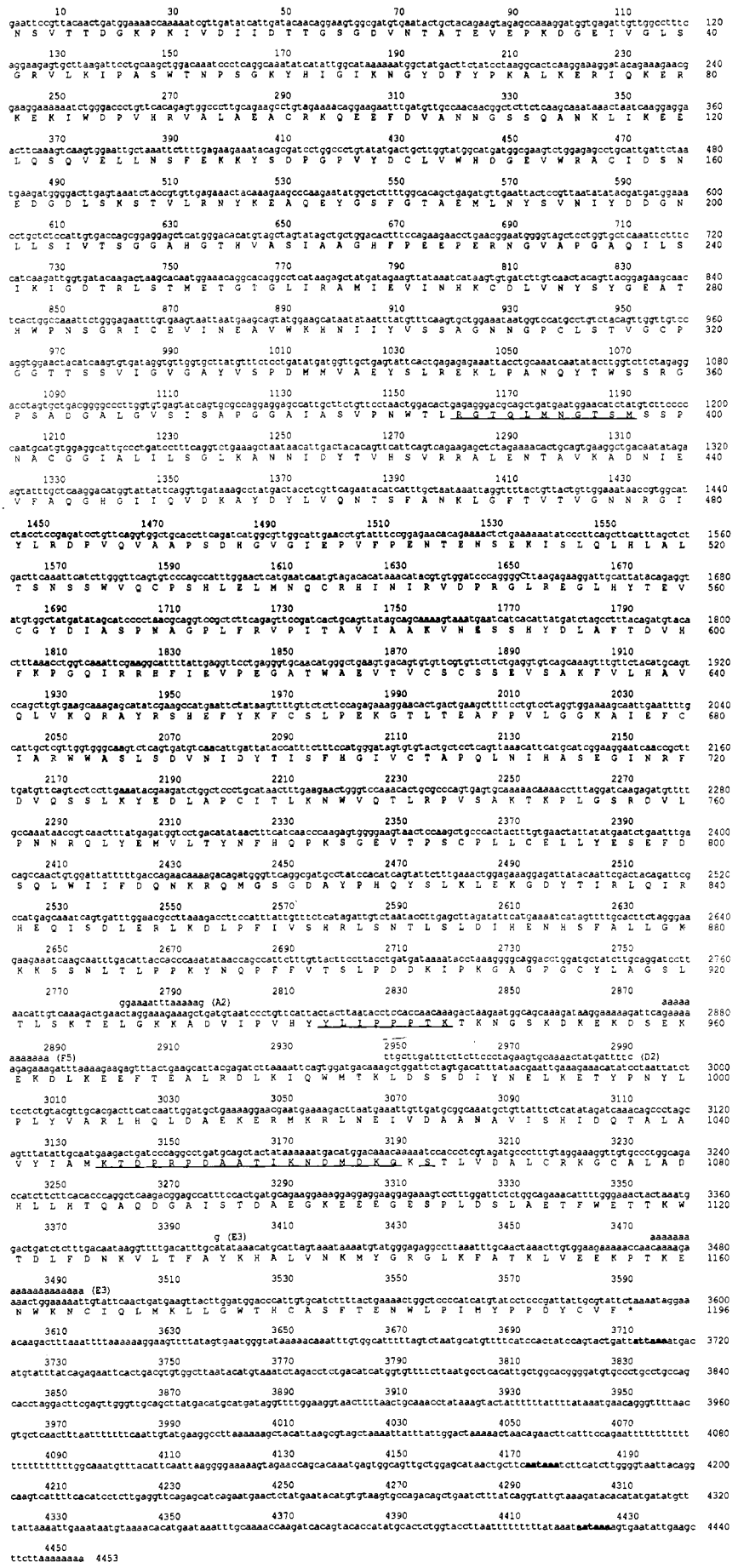

FIGURE 3: Nucleotide sequence and predicted amino acid sequence of human TPP II. Differing nucleotide sequences in the clone (indicated in parentheses) are shown above the nucleotide sequence (cf. Figure 1). Underlined amino acid sequences have been identified at the protein level. Three possible polyadenylation signals are in boldfaces. Asterisk indicates termination codon.

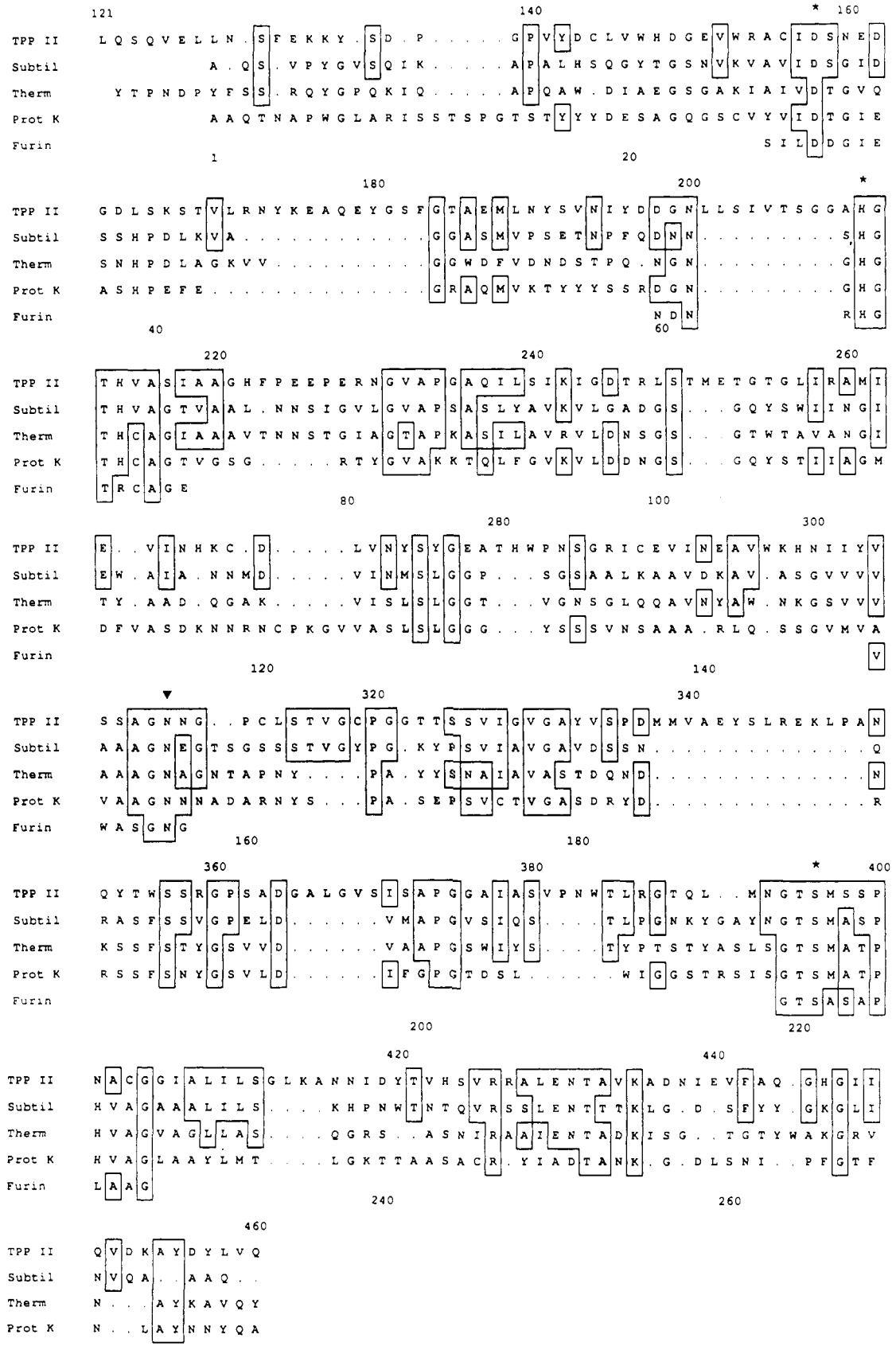

FIGURE 4: Comparison between the deduced amino acid sequence of human TPP II and other serine peptidases of the subtilisin type. Identical amino acids are blocked. Asterisks indicate amino acids corresponding to the catalytic triad in subtilisin. (▼) indicates stabilizing Asn. The numbering above the sequences refers to TPP II and that below the sequences to subtilisin BPN′. Sequence data for subtilisin BPN′ were obtained from Wells et al. (1983), for thermitase from Meloun et al. (1985), for proteinase K from Jany et al. (1986), and for furin from Roebroek et al. (1986) and Fuller et al. (1989) (incomplete data).

the D2 clone and 55% of the E3 clone. The strategy for sequencing clones A2 and F5 is outlined in Figure 2.

The insert of the A2 clone, with an internal *Eco*RI site, proved to consist of an open reading frame of 810 bp and a 3′-untranslated region of 864 bp (Figure 3, bp 2780–4453). The latter region includes two possible polyadenylation signals, AATAAA, starting at bp 4171 and 4420, respectively, and a short poly(A) tail. Since the polyadenylation signal usually is 10–30 nucleotides upstream from the poly(A) (Proudfoot & Brownlee, 1976), the signal at bp 4420 was probably the one utilized in this case. The F5 clone contained a 2887 bp insert with an open reading frame covering the entire insert.

The differences between the clones are discussed below. However, a 4453 bp nucleotide sequence of the cDNA for TPP II could be assembled (Figure 3), since the nucleotide sequence from bp 2780 to 2990 was represented in at least two different clones, even though it differed in one clone (Figure 1).

Residues 387–397 and 1046–1065 of the deduced amino acid sequence are in complete agreement with the previously determined amino acid sequence for the N-terminus of two chymotryptic TPP II fragments (Tomkinson & Zetterqvist, 1990). The lysine residue at position 1064 could, however, not be identified at the previous occasion. Furthermore, the amino acid sequence of the N-terminus of a 30-kDa TPP II fragment, obtained from chymotryptic digestion of TPP II in the presence of Ca-EGTA (B. Tomkinson and Ö. Zetterqvist, unpublished results), corresponds to residues 939–946. This shows that the isolated cDNA clones code for TPP II.

Differences between Clones. The cause of the minor discrepancies between the different clones, as indicated in Figures 1 and 3, is not known. There is a single mismatch between E3 and A2 at bp 3397, which does not alter the deduced amino acid. The poly(A) stretch at the 3′ end of the F5 and E3 clones might tentatively be explained as a cloning artifact, since there are only three and seven mismatches, respectively, between these and the corresponding A-rich parts of the A2 clone. The mismatching 3′ end of the D2 clone is identical with the antisense strand of bp 2655–2612, and is thus a cloning artifact.

The differences between the F5 clone and the 5′ end of the A2 clone were investigated by means of PCR, performed with cDNA as the template, and with clone-specific oligonucleotide primers together with primers binding to common parts of the clones. Fragments of the expected sizes could be obtained both when primers specific for the F5 clone and for the 5′ end of the A2 clone were used (data not shown). It cannot, however, be determined whether this is an example of alternative splicing or whether the mismatching 5′ end of the A2 clone represents an intron sequence left by incorrect splicing.

Sequence Comparison. The deduced amino acid sequence of TPP II was compared to that of the subtilisin type of serine peptidases by use of the command "Gap" of the GCG program (Devereux et al., 1984) (Figure 4). Residues 121–460 in the N-terminal part of TPP II represent a sequence with 56% similarity (37% identity) to subtilisin. While Ser-396 of the catalytic triad of TPP II was identified earlier (Tomkinson et al., 1987), Asp-158 and His-211 can be deduced by sequence homologies to subtilisin. In subtilisin, Asn-155 is believed to stabilize the tetrahedral transition state (Robertus et al., 1972a). This residue also seems to be conserved in TPP II (Asn-309, Figure 4).

In subtilisin, S_1, the binding pocket for P_1, i.e., the amino acid on the N-terminal side of the scissile peptide bond, is sufficiently large to harbor practically any amino acid residue. This pocket is formed by the loop Ala-152 to Gly-169, of which Ala-152, Glu-156, Gly-166, and Gly-169 are in direct or in-

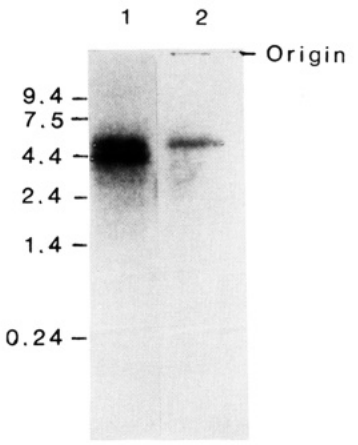

FIGURE 5: Northern blot analysis of mRNA from EBV-transformed human B-lymphocytes. Lane 1, hybridization with the ^{32}P-labeled 956 bp *Eco*RI fragment of clone A2; lane 2, hybridization with the ^{32}P-labeled 718 bp *Eco*RI fragment. The sizes of the RNA ladder markers (Bethesda Research Laboratory) are shown in kilobases.

direct contact with the P_1 residue (Robertus et al., 1972b). The corresponding area in TPP II seems to be fairly well conserved (Figure 4), which may explain the broad substrate specificity observed for TPP II (Bålöw et al., 1983, 1986).

It is also evident from Figure 4 that the similarity between TPP II and subtilisin is greater than between TPP II and the thiol-dependent serine peptidases, like thermitase and proteinase K, indicating a different mechanism for the thiol dependence of TPP II. It is interesting to note that cysteine residues exist in the tentative S_1 region (Cys-313, Cys-319), as well as close to the catalytic Asp (Cys-156) and near the catalytic Ser (Cys-403). Some of these cysteine residues may therefore explain the observed thiol dependence (Bålöw et al., 1986).

TPP II also shows some similarity to furin, the *fur* gene product (Roebroek et al., 1986). This gene product was identified as a potential human subtilisin-like serine peptidase due to its similarity to the *KEX2* gene product, a prohormone processing endoprotease of the yeast *Saccharomyces cerevisiae* (Fuller et al., 1989). Recently, a cDNA (PC2) encoding another KEX2-like protein was isolated from a human insulinoma cDNA library (Smeekens & Steiner, 1990). However, to our knowledge, no peptidase activity has been demonstrated so far for furin or PC2.

As an immunological similarity between TPP II and fibronectin has been found (Tomkinson & Zetterqvist, 1990), the available sequence information on TPP II was compared with that of plasma fibronectin, using the "Compare", "Bestfit", and LFASTA commands (Devereux et al., 1984; Pearson & Lipman, 1988). However, no sequence similarity was evident, and the immunological similarity remains to be explained.

Sequence similarities between the deduced amino acid sequence of TPP II and sequences of current sequence databases (Swiss Protein, release 13.0; NBRF-Protein, release 21.0) were sought by means of the search program FASTA (Pearson & Lipman, 1988). No entries showing an overall similarity to TPP II could be detected though, which seems to confirm the unique nature of the enzyme.

Evidence for the Existence of Two Different Tripeptidyl Peptidase II mRNAs. In order to obtain an estimate of the size of the complete mRNA coding for TPP II, a Northern blot analysis was performed (Figure 5). Two bands representing mRNA of 4.2 ± 0.1 and 5.0 ± 0.2 kilobases, respectively, could be detected when the 956 bp *Eco*RI fragment of

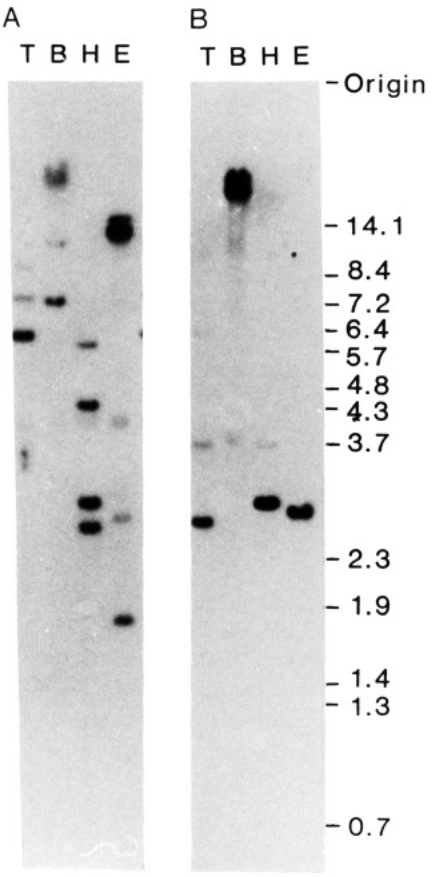

FIGURE 6: Southern blot analysis. Human DNA was digested with the different restriction endonucleases indicated (T, *Taq*I; B, *Bam*HI; H, *Hin*dIII; E, *Eco*RI). (A) Hybridization with the ^{32}P-labeled 956 bp *Eco*RI fragment of the A2 clone; (B) hybridization with the ^{32}P-labeled 718 bp *Eco*RI fragment. The sizes of the λ-phage *Bst*EII markers are shown in kilobases.

the A2 clone was used as a probe (Figure 5, lane 1). In contrast, with the 718 bp *Eco*RI fragment of the A2 clone as the probe, only the 5.0-kilobase band could be discerned (Figure 5, lane 2). Two bands were also seen when a 147 bp *Eco*RI–*Bgl*II fragment, a 637 bp *Eco*RI–*Nsi*I fragment, a 271 bp *Nsi*I fragment, or a 299 bp *Stu*I–*Eco*RI fragment of the A2 clone (cf. Figure 2A) were used as probes in Northern blot analysis, whereas a 259 bp *Eco*RI–*Stu*I fragment could only detect the larger mRNA. Interestingly enough, a closer examination of the noncoding region reveals an additional possible adenylation signal, ATTAAA (Nevins, 1983), starting at bp 3710 (Figure 3), followed by the sequence CACTG (starting at bp 3740), which has been found close to the polyadenylation site in many genes (Berget, 1984). Thus, the two mRNAs could tentatively be explained by the use of two different polyadenylation signals. The observed size difference between the two mRNAs and the hybridization pattern in Northern blots are compatible with this interpretation.

Two different mRNAs may also be products of two different genes, coding for TPP II. To elucidate this possibility, human DNA was hybridized to the 718 bp *Eco*RI fragment of the A2 clone in a Southern blot analysis. The faint bands at 3.7 kb in several lanes in Figure 6B, and at about 4 kb in Figure 6A, are probably due to contaminating plasmid DNA. The pattern obtained (Figure 6B) is consistent with the restriction map of the 718 bp fragment, assuming that it is encoded in a single exon. When the 956 bp *Eco*RI fragment was used as a probe, three to four bands were seen in each lane (Figure 6A). Although this pattern may be due to the existence of several genes, the location of exon–intron boundaries in a single gene is the more likely explanation, since smaller probes, like the 271 bp *Nsi*I fragment or the 147 bp *Eco*RI–*Bgl*II fragment, displayed only one band in each lane (data not shown).

It is clear that TPP II is represented by two mRNAs and that the isolated clones account for 90% of the larger mRNA.

Cross-Hybridization between Species. TPP II activity has been found in liver homogenates and red blood cells from a number of other species (Bålöw et al., 1986; Bålöw & Eriksson, 1987). Therefore, it was interesting to investigate the simi-

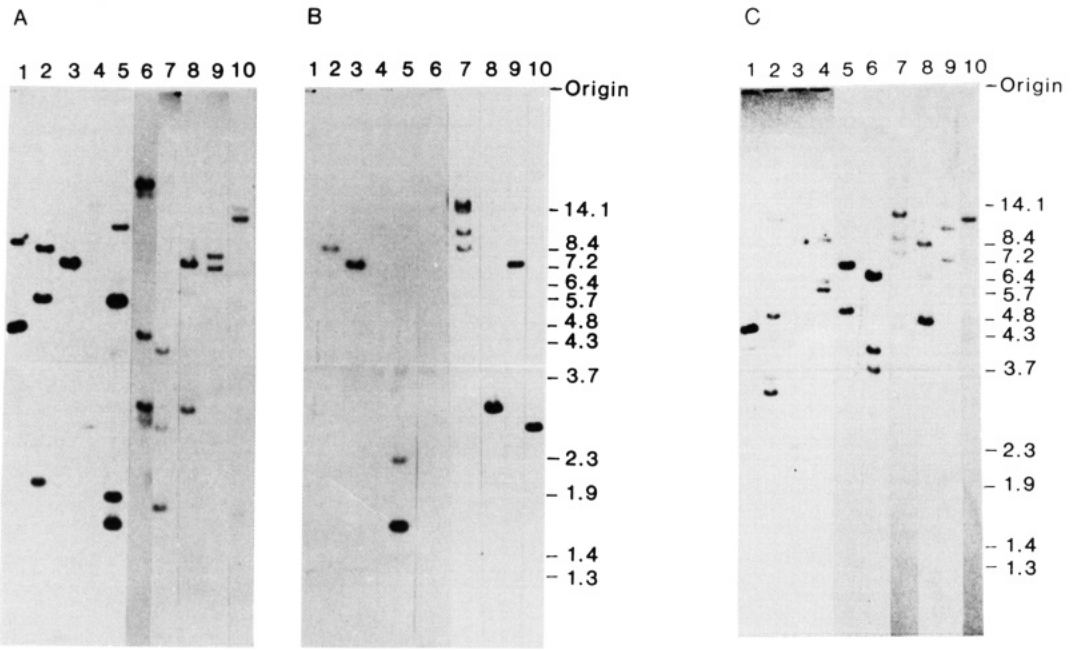

FIGURE 7: Southern blot analysis showing cross-hybridization between DNA from different species and probes from human TPP II cDNA. DNA was digested with *Pvu*II (lanes 1–5) or *Eco*RI (lanes 6–10), and the Southern blot analysis, under low stringency conditions, was performed as described under Materials and Methods. (A) Hybridization with ^{32}P-labeled 956 bp *Eco*RI fragment of the A2 clone; (B) hybridization with ^{32}P-labeled 718 bp *Eco*RI fragment of the A2 clone. (C) Hybridization with ^{32}P-labeled 1955 bp *Eco*RI fragment of the F5 clone. Lanes 1 and 6, chicken DNA; lanes 2 and 7, equine DNA; lanes 3 and 8, bovine DNA; lanes 4 and 9, mouse DNA; lanes 5 and 10, human DNA. The sizes of the λ-phage *Bst*EII markers are shown in kilobases.

larities of TPP II between the species also at the DNA level.

It is evident that probes corresponding to both the N-terminal part of TPP II (Figure 7C) and the C-terminal part (Figure 7A) hybridize with DNA from all species tested under the low stringency conditions, which have been shown to detect a similarity of 60% or higher (Andersson et al., 1987; Bourlet et al., 1988). Under high stringency conditions, hybridization to the 5-kb band of *Pvu*II-digested hen DNA (Figure 7A, lane 1), and the 25- and 4.8-kb bands of *Eco*RI-digested hen DNA (Figure 7A, lane 6), was markedly reduced (data not shown), whereas all other bands remain, indicating that the observed cross-hybridization was caused by a high degree of similarity. Hybridization with smaller probes originating from the 956 bp *Eco*RI fragment, like the 147 bp *Eco*RI–*Bgl*II or the 271 bp *Nsi*I fragments, detected only one to two of the bands observed with the larger probe (data not shown). This is compatible with the existence of only one gene coding for TPP II in these species. In contrast, when a probe corresponding to the noncoding region was used for hybridizing, no band was seen with hen DNA even under low stringency conditions (Figure 7B), nor could a band be detected in *Pvu*II-digested mouse DNA, possibly due to unfavorable placing of *Pvu*II sites in the mouse genome.

Thus, it can be concluded that the structure of TPP II is highly conserved, not only in the subtilisin-like N-terminal part but also in the C-terminal part. Although this finding indicates that the C-terminal part of the enzyme is of functional importance, no clues as to its function could be obtained from comparisons with current sequence databases. However, one possible role to consider may be in the stabilization of the complicated oligomeric structure of the enzyme, which has been demonstrated to be a prerequisite for enzymatic activity (Macpherson et al., 1987).

The physiological role of the enzyme remains to be determined, although the apparently conserved structure indicates a fundamental role in protein degradation. Considering the broad substrate specificity (Bålöw et al., 1983, 1986), a tentative role for TPP II may be to act as one among several exopeptidases required for the efficiency of the final steps of intracellular proteolysis.

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Registry No. TPP II, 127497-93-2; BPN, 9014-01-1; thermitase, 69772-87-8; proteinase K, 39450-01-6.

References

Andersson, L., Lundberg, C., Rask, L., Gissel-Nielsen, B., & Simonsen, M. (1987) *Immunogenetics 26*, 79–84.

Bålöw, R.-M., & Eriksson, I. (1987) *Biochem. J. 241*, 75–80.

Bålöw, R.-M., Ragnarsson, U., & Zetterqvist, Ö. (1983) *J. Biol. Chem. 258*, 11622–11628.

Bålöw, R.-M., Tomkinson, B., Ragnarsson, U., & Zetterqvist, Ö. (1986) *J. Biol. Chem. 261*, 2409–2417.

Berget, S. M. (1984) *Nature 309*, 179–182.

Bourlet, Y., Bêhar, G., Guillemot, F., Frêchin, N., Billault, A., Chaussê, A.-M., Zoorob, R., & Auffray, C. (1988) *EMBO J. 7*, 1031–1039.

Devereux, J., Haeberli, P., & Smithies, O. (1984) *Nucleic Acids Res. 12*, 387–395.

Feinberg, A. P., & Vogelstein, B. (1984) *Anal. Biochem. 137*, 266–267.

Fuller, R. S., Brake, A. J., & Thorner, J. (1989) *Science 246*, 482–486.

Jany, K.-D., Lederer, G., & Mayer, B. (1986) *Biol. Chem. Hoppe-Seyler 367*, 87.

Jonsson, A.-K., Andersson, L., & Rask, L. (1989) *Immunogenetics 29*, 308–316.

Macpherson, E., Tomkinson, B., Bålöw, R.-M., Höglund, S., & Zetterqvist, Ö. (1987) *Biochem. J. 248*, 259–263.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) in *Molecular Cloning: a laboratory manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mason, P. J., & Williams, J. G. (1985) in *Nucleic acid hybridization: a practical approach* (Hames, B. D., & Higgins, S. J., Eds.) pp 113–137, IRL Press, Oxford and Washington D.C.

Meloun, B., Baudys, M., Kostka, V., Hausdorf, G., Frömmel, C., & Höhne, W. E. (1985) *FEBS Lett. 183*, 195–200.

Neurath, H. (1984) *Science 224*, 350–357.

Nevins, J. R. (1983) *Annu. Rev. Biochem. 52*, 441–466.

Pearson, W. R. and Lipman D. J. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 2444–2448.

Proudfoot, N. J., & Brownlee, G. G. (1976) *Nature 263*, 211–214.

Robertus, J. D., Kraut, J., Alden, R. A., & Birktoft, J. J. (1972a) *Biochemistry 11*, 4293–4303.

Robertus, J. D., Alden, R. A., Birktoft, J. J., Kraut, J., Powers, J. C., & Wilcox, P. E. (1972b) *Biochemistry 11*, 2439–2449.

Roebroek, A. J. M., Schalken, J. A., Leunissen, J. A. M., Onnekink, C., Bloemers, H. P. J., & Van de Ven, W. J. M. (1986) *EMBO J. 5*, 2197–2202.

Smeekens, S. P., & Steiner, D. F. (1990) *J. Biol. Chem. 265*, 2997–3000.

Tabor, S., & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 4767–4771.

Tomkinson, B., & Zetterqvist, Ö. (1990) *Biochem. J. 267*, 149–154.

Tomkinson, B., Wernstedt, C., Hellman, U., & Zetterqvist, Ö. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7508–7512.

Vieira, J., & Messing, J. (1982) *Gene 19*, 259–268.

Wells, J. A., Ferrari, E., Henner, D. J., Estell, D. A., & Chen, E. Y. (1983) *Nucleic Acids Res. 11*, 7911–7925.