Amino Acid Sequence of Rubber Elongation Factor Protein Associated with Rubber Particles in Hevea Latex*

Mark S. Dennis‡, William J. Henzel§, John Bell¶, William Kohs, and David R. Light¶

From the Departments of ‡Medicinal and Biomolecular Chemistry and ¶Developmental Biology, Genentech, Inc., South San Francisco, California 94080 and ¶Neurex, Inc., Menlo Park, California 94025

The amino acid sequence of rubber elongation factor, a recently discovered protein tightly bound to rubber particles isolated from the commercial rubber tree Hevea brasiliensis, is presented. The role of this protein in rubber elongation and its interaction with prenyltransferase and rubber particles have been discussed in the preceding paper in this series (Dennis, M. S., and Light, D. R. (1989) J. Biol. Chem. 264, 18608–18617). Trypsin, Staphylococcus protease, chymotrypsin, acetic acid, and hydroxylamine cleavage were used to generate peptide fragments that were isolated by reverse phase high pressure liquid chromatography and analyzed by amino acid composition and automated Edman degradation. Each digest contained one blocked peptide identified as the amino terminus. The blocked amino-terminal peptide from the tryptic digest was analyzed by amino acid composition, fast atom bombardment mass spectrometry (molecular ion 1659.9), subdigested with Staphylococcus protease for partial sequence analysis, and finally deblocked with bovine liver acyl-peptide hydrolase removing an acetylation to allow analysis by Edman degradation.

Rubber elongation factor is 137 amino acids long, has a molecular mass of 14,600 daltons, and lacks four amino acids: cysteine, methionine, histidine, and tryptophan. The NH₂ terminus is highly charged and contains only acidic residues (5 of the first 12 amino acids). The first four amino acids are highly represented in other known NH₂-terminally acetylated proteins.

Comparison of the sequence of rubber elongation factor with other known sequences does not reveal significant sequence similarities that would suggest an evolutionary relationship.

In earlier communications we described experiments designed to elucidate the biosynthesis of rubber in Hevea brasiliensis (1-3). Rubber transferase described by Archer et al. (4, 5) was purified in our laboratory from the latex of the commercial rubber tree, H. brasiliensis, based on its ability to elongate rubber molecules by cis additions of isopentenyl pyrophosphate (IPP). After purification, this enzyme was found to be a typical FPP synthase capable of making all-trans-farnesyl pyrophosphate from IPP and dimethylallyl pyrophosphate (DMAPP). This led us to investigate the properties of rubber particles that enable prenyltransferase to make the stereochemical switch of trans additions of IPP to cis and override the usual termination after two trans additions to DMAPP. During the course of these studies we discovered a new protein, rubber elongation factor (REF), which is found tightly associated to rubber particles from the latex of H. brasiliensis (2, 3).

REF is present in stoichiometric amounts relative to the number of growing rubber molecules. Elongation ceases upon removal of REF and is inhibited upon binding of specific antibodies raised to REF. REF probably plays a role in rubber elongation by docking and positioning prenyltransferase on the rubber molecule. Such docking may involve a reorientation of the binding sites for IPP and the allylic primer. Additionally REF may position and protect the growing pyrophosphate ends on the rubber molecule. In two previous papers (2, 3), the identification, purification, and characterization of this protein is described in detail. In this report, we describe the complete amino acid sequence of REF and discuss its role in rubber biosynthesis.

MATERIALS AND METHODS

Purification of REF

Purification of REF was performed as described previously (3). Rubber particles were isolated containing greater than 95% pure REF, extracted with 1% SDS and the protein was acetone-precipitated after removal of the rubber fraction by centrifugation. Unless otherwise specified, REF was then further purified by SDS-PAGE followed by electroelution from the gel.

Sequence Similarity Search

We searched for sequence similarity between REF and other known sequences using FASTP, a rapid search program based on the Lipman-Pearson method (6) and tested the quality of the alignments using a global alignment program, HOMOLOBAL, based on an accepted algorithm (7-9). Both programs were written by Colin Watanabe, Genentech, Inc. The data base consists of 7400 sequences, Release 16, March, 1988, translations of significant coding regions (4000 sequences) from the GenBank Nucleotide Database (Release 16, March, 1988) and EMBL (Release 13, October, 1987), and around 700 in-house sequences.

Secondary Structure Prediction

In an attempt to predict regions of secondary structure in REF the sequence was analyzed by the program HYDRO which uses a Fourier pro

* Portions of this paper (including part of "Materials and Methods," Figs. 3-11, Footnote 3, and Tables 1-21) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
transform amphipathic analysis algorithms and which was written by Janet Finer-Moore and Robert M. Stroud, University of California, San Francisco (10). We also used secondary structure prediction algorithms for globular proteins using the Chou-Fasman method (11) and the Robson-Garnier method (12).

**Peptide Nomenclature**

The peptides were named according to the digestion procedure used, and are numbered from the amino terminus. For double digests of fragments, the same from the first digest is given followed by a name derived from the digestion procedure employed starting from the amino terminus of the parent peptide. Where more than one fragment for a given digest was obtained over the same region owing to alternate cleavage sites, the peptides are distinguished by the letters a or b. Specific designations follow.

**Tryptic Peptides of Intact REF**—T1 (1-15), T2 (16-39), T3 (40-41), T4 (42-57), T5 (58-66), T6 (67-76), T7 (77-91), T8 (92-98), T9 (99-109), T10 (110-116), T11 (117-127), T12 (128-131), T13 (132-137).

**Acetic Acid Peptides of Intact REF**—HA1 (1-22), HA2 (23-40), HA3 (41-50), HA4 (51-79), HA5 (80-90), HA6 (91-99), HA7 (100-137).

**Hydroxylamine Peptides of Intact REF**—NG1 (1-72), NG2 (73-137).

**Staphylococcus Protease Peptides of Intact REF**—(only one observed) V1 (113-137).

**Staphylococcus Protease Peptides of NG1**—NG1-V1 (12-22), NG1-V2 (23-40), NG1-Y3 (41-53), NG1-V4 (54-62).

**Chymotrypsin Peptides of NG1**—NG1-CY1a (1-16), NG1-CY1b (1-14), NG1-CY2 (17-26), NG1-CY3 (27-31), NG1-CY4 (32-35), NG1-CY5 (36-37), NG1-CY6 (38-46), NG1-CY7 (47-54), NG1-CY8 (55-64), NG1-CY9 (65-72).

**Tryptic Peptides of NG1-T2**—NG1-T2 (16-38).

**Staphylococcus Protease Peptides of T1**—T1-Vla (1-4), T1-Vlb (1-12), T1-V2 (5-15).

**T1 Peptide Digested with Acyl-peptide Hydrolase**—T1-ACE (2-15).

**RESULTS**

**Purification and Amino Acid Analysis**—REF was purified from the commercial rubber tree H. brasiliensis grown in Costa Rica as previously described (3). When washed rubber particles are extracted with 1% SDS, only one protein of 14.6 kDa is observed by SDS-PAGE (3). SDS was used to release REF from particles, and then the SDS was removed by acetone precipitation. Following resuspension, REF is suitable for hydrolysis by trypsin, Staphylococcus protease, acetic acid, hydroxylamine, and chymotrypsin. CNBr digestion was ruled out after amino acid composition analysis (Table 1) which revealed a lack of methionine as well as cysteine and histidine in the sequence of the protein. After completing the sequence reported in Fig. 1, the lack of these amino acids and, additionally, the lack of tryptophan (not detected by amino acid analysis) was confirmed.

**Sequencing REF**—When 3 nmol (42 μg) of REF was loaded on the sequencer, no sequence could be detected (less than 1 pmol of phenylthiodyantoin-derivative released) indicating that the amino terminus of the protein is blocked. The determination of the NH2-terminal modification of REF is presented in a separate section below.

Initial digestion of REF with trypsin allowed isolation of 10 tryptic peptides (Fig. 3) identified as T4 (position 42-57), T5 (position 58-66), T6 (position 67-76), T7 (position 92-98), T9 (position 99-109), T10 (position 110-116), T11 (position 117-127), T12 (position 128-131), T13 (position 132-137), and a peptide, T1 (position 1-15), which was blocked and did not yield a sequence in the analyzer. Peptides T10 and T12 were not sequenced but were later identified by amino acid composition. Peptides T2 (position 16-39), T3 (position 40-41), and T7 (position 77-91) were not observed. Peptide T2 was later obtained by subdigestion of the hydroxylamine-cleaved peptide NG2.

Intact REF was almost completely resistant to digestion with Staphylococcus protease at pH 8 in ammonium bicarbonate, although one peptide V1 (position 114-137) was isolated and the sequence determined. This sequence allowed the alignment of tryptic peptides T10-T13. Since both V1 and T13 end with an asparagine at position 137 there is strong evidence that this is the carboxyl terminus. Compositions of V1, HA7, and T13 are also consistent with Asn-137 being the carboxyl terminus of REF (Tables 1-3). Additional evidence was provided by the lack of a Staphylococcus protease cut at Glu-136. Austen and Smith (13) have shown that this protease will not cut at glutamic or aspartic acids if these residues are within 2-3 residues from the amino or carboxyl terminus. A potential cleavage site for hydroxylamine (Asn-Gly) at position 72 was observed when the sequence of peptide T6 was determined; subsequent digestion of REF with hydroxylamine (Fig. 4) and identification of the unblocked peptide NG2 (73-137) by sequence analysis provided overlap for T6-T9. Positions 81 and 88 were not identifiable in the first sequencing run. These positions were later identified as threonine in a second sequence analysis with more material.

Acetic acid digestion of REF resulted in poor yields and peptides were difficult to resolve on HPLC (Fig. 5). However, HA7 (positions 100-137) provided overlap between NG2 and V1, and these peptides along with the tryptic peptides T6-T13 provided contiguous sequence from position 67 through the carboxyl terminus. HA4 (position 51-79) was used to align tryptic peptides T4-T6, and HA3 (position 41-50) extended the sequence of T4 back to position 41.

In order to connect the carboxyl-terminal side of HA2 at position 40, a chymotryptic peptide was required. Digestion of REF with chymotrypsin provided a complicated mixture of peptides; however, subdigestion of NG1 by chymotrypsin (Fig. 6) allowed identification of peptide NG1-CY6 (position 38-46) which provided the needed overlap to extend the sequence back to position 23 with HA2. An additional chymotryptic peptide NG1-CY2 (position 17-26) enabled extension of the sequence back to position 17.

Since the composition of T1 (Table 4) was known to be different than the NH2-terminal tryptic contained in HA2, it was suspected that a tryptic peptide was missing from the digest in this region of REF. At this point peptide T2 which was previously not observed was obtained in low yield (Fig. 3) confirming the sequence back to position 17. This peptide was later obtained from a tryptic digestion of NG1 (NG1-T2) where it was recovered with much higher yield (Fig. 7), allowing identification of a tyrosine at position 16. The only remaining piece to insert the sequence of T1 is preceding Tyr-16. Based on the composition of the entire protein, this accounted for all the tryptic peptides (Lys = 7, Arg = 4).

**Sequence of T1**—In order to elucidate the primary sequence of T1, first atom bombardment mass spectrometry, amino acid composition, subdigestion with Staphylococcus protease, and acyl-peptide hydrolase were used. The amino acid composition of T1 indicated 3 Asp, 3 Gly, 1 Ala, 1 Leu, 1 Lys, and 6 Gln residues, when normalized to 1 Lys (Table 4). To verify the amino acid composition especially the high level of glutamic acid and to identify the amino-terminal blocking group, fast atom bombardment mass spectrometry was used to determine an accurate molecular weight for T1. In the positive ion mode, a molecular ion of 1660.895 was observed (Fig. 8a), whereas in the negative ion mode a molecular ion of 1658.98 was observed (Fig. 8b), suggesting a molecular mass of approximately 1659.9 Da. No other major peaks in either mode were detected. The fragment molecular weight was then compared with the calculated core molecular weight from the
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**Fig. 1. Peptide map and complete amino acid sequence of REF.** Bars indicate the total length of the peptide. Striped bars indicate peptides verified by amino acid analysis as containing the NH₂-terminal alanine which was not obtained by sequence analysis. Shaded bars indicate amino acid sequence determined by Edman degradation. The dark-shaded bar indicates the peptide T1 which was analyzed by fast atom bombardment mass spectrometry. Peptides are numbered from the NH₂ terminus and labeled based on their derivation from trypsin (T), acetic acid (HA), hydroxylamine (NG), Staphylococcus protease (V), acyl-peptide hydrolase (ACE), or combinations of digestive methods.

The amino acid composition. Several combinations of acids and amides of Glx and Asx and the molecular weights of potential NH₂-terminal blocking groups were fit to the measured data. The analysis yielded a single solution: there had to be four amides and five acids with an acetylated NH₂ terminus (Fig. 8C); this analysis confirmed the existence of 6 Glx residues. The composition of chymotryptic peptides NG1-CYla and NG1-CYlb, strong evidence was provided that T1 indeed preceded peptide T2: NG1-CYla has the same composition as T1 with an additional tyrosine, while NG1-CYlb lacked only the lysine when compared to the T1 composition. In addition, subdigestion with *Staphylococcus* protease of the NG1 peptide in phosphate, pH 7.4 (Fig. 11), provided several peptides in the amino-terminal region of the protein, including NG1-V1, providing an overlap between T1 and T2. This peptide fixed the positions of Gly-13, Leu-14, and Lys-15, and thus, with the sequence from T1-ACE and T1-V2, completed the entire sequence of REF.

**Discussion**

In the previous two papers in this series (2, 3), we reported the identification and characterization of REF. The presence of this protein on rubber particles is required for rubber elongation. We proposed that REF interacts with *Hevea* prenyltransferase to alter the stereochemistry of IPP addition from the normal trans addition to cis and overrides the normal termination after two trans additions to affect the formation of cis-polyisoprene. This protein is only associated with rubber particles and is not detected in latex serum. Enzymatic, chemical, or physical removal of REF from the rubber particles renders them incapable to accept further IPP additions during incubation with a prenyltransferase. Removal of REF from rubber particles and reconstitution of a rubber biosynthetic system has not been successful to date. However, affinity-
purified antibodies raised specifically to REF inhibit rubber biosynthesis in vitro.

Sequence Analysis of REF—The complete protein sequence of REF is shown in Fig. 1. REF was digested with trypsin, acetic acid, hydroxylamine, Staphylococcus protease, and chymotrypsin to yield overlapping fragments that resulted in sequence determination. All peptides from each digest were recovered with the exception of T7 and H8.5 which were never observed, suggesting that this region of the protein may present recovery problems due to its hydrophobic nature. The sequence provided by NG2 eliminated the need to pursue this problem, however. One peptide from each digest was observed to be blocked to Edman degradation, and the NHz-terminal sequence of REF is compared to other NHz-termini tryptic fragments. From the fast atom bombardment data and the known composition of T1, an acetyl group was predicted to be the blocking group. Acyl-peptide hydrolase digestion of the NHz-terminal tryptic T1 was performed to yield a peptide of which the sequence could be determined. REF is 137 amino acids long with a molecular weight of 14,604 Da and does not contain four of the amino acids: cysteine, methionine, histidine, and tryptophan.

Acetylated NH2 Terminus—NH2-terminal acetylation is a common post-translational modification of eukaryotic proteins. The function of acetylation is unknown, although protection against proteolysis has been suggested (14). We note that both REF and the prenyltransferase we purified from H. brasiliensis are predicted to be blocked at NH2 termini, although the blocking group of the latter protein was not determined. When the NH2-terminal sequence of REF is compared to other NH2-terminally acetylated proteins it appears to be very typical of this class of proteins. Thus, acetylated alanine is a very common first amino acid, a close second to acetylserine, and the glutamic acids at positions two and four are consistent with the observation that negatively charged residues predominate these positions (14). However, the acidic amino acid content of the NH2 terminus of REF is noteworthy even in the class of acetylated proteins. Thus, Lys-15 is the first positive charge in the sequence and the first 5 of 12 amino acids are negatively charged and 9 of the first 12 are from the group aspartic acid, asparagine, glutamic acid, and glutamine.

Search for Similar Sequences—We searched extensively for similar sequences to REF. We note that the sequence from amino acid 48-73 shows a 42% sequence similarity to a region in the major chlorophyll a/b binding polypeptide from the light harvesting complex. These plant proteins bind chlorophyll and its isoprenyl side chain (15, 16). However, REF is not likely to be related evolutionarily to these proteins, even by this relatively short sequence. There is no compelling functional similarity between the two proteins, and there are too many evolutionarily disfavored pairings in the alignment. If the nonidentical residues in this region are evaluated by a similarity search using the Chou-Fasman (11) and the Robson-Garnier methods. Programs used are described under “Materials and Methods.” B, helix net for the predicted helix IV in A. This long stretch of predicted helix begins with Pro-94-Pro-95 and terminates with Pro-123-Gly-124. This treatment readily enables the visualization of the extensive hydrophobic surface this predicted helix would contain (18).

Secondary Structure Predictions—The NH2 terminus of REF is one of four regions predicted to be predominately a-helix by both the Chou-Fasman method (11) and the Robson-Garnier method (12). These regions are shown graphically in Fig. 24 in relation to the two-dimensional plot of the Fourier-transform of the hydrophobicity of the individual amino acid side chains. Such plots can be used to identify amphipathic secondary structural elements (10). Overall REF is predicted to have a high amount of amphipathic a-helix, since many of the contours throughout the length of the sequence in Fig. 24 fall on the 1/3.6 line, the frequency of an a-helix. The a-helices I and III predicted by the Chou-Fasman (11) and Robson-Garnier (12) methods do not match regions of amphipathic a-helix, which are highly predicted by both the Chou-Fasman (10) and the Robson-Garnier (11) methods. Programs used are described under “Materials and Methods.” B, helix net for the predicted helix IV in A. This long stretch of predicted helix begins with Pro-94-Pro-95 and terminates with Pro-123-Gly-124. This treatment readily enables the visualization of the extensive hydrophobic surface this predicted helix would contain (18).

The hydrophobic face that would be an important secondary structural element of an amphipathic a-helix formed in this region is outlined in Fig. 2B. REF contains rubber particles at the interface between the aqueous serum and the extremely hydrophobic polysisoprene molecules in a manner that is at least superficially similar the protein coating of the lipid and cholesterol ester core of high density lipoprotein. Amphipathic a-helices are important elements of models of self-association and surface binding of apolipoprotein E (18) and apolipoprotein C-1 (19) in this latter example of a protein-lipid interface. As more of the structural elements of REF are determined, we can begin to create a better picture of the role of REF, phospholipids, and cis-polyisoprene on the surface of the growing rubber particle.
Attempts to Clone REF—Isolation of quality mRNA suitable for making a cDNA library from *H. brasiliensis* has been unsuccessful in our laboratory owing to time delay in obtaining fresh tissue samples. Further, the lack of methionine and tryptophan and the high content of serine, leucine, and arginine in the protein sequence lead to redundancy in any possible DNA probe to locate the gene. It is not known in which part of the *Hevea* plant REF is synthesized. It is present in latex that is derived from the cytosol of the cells fused to form the laticifers (5), and Western analysis (25) demonstrates its presence in leaves (data not shown). Whether REF is present in leaves in newly formed laticifers or due to secretion into the latex is not known. A synthetic gene for REF has been constructed using high frequency codons from known plant genes. The gene product is expressed at low levels in *Escherichia coli* and the low expression levels impede further characterization of the recombinant product. However, this DNA probe will be a useful tool for ongoing studies in *H. brasiliensis*.

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REFERENCES

1. Light, D. R., and Dennis, M. S. (1989) *J. Biol. Chem.* 264, 18598–18607
2. Light, D. R., Lazarus, R. A., and Dennis, M. S. (1989) *J. Biol. Chem.* 264, 18598–18607
3. Dennis, M. S., and Light, D. R. (1989) *J. Biol. Chem.* 264, 18608–18617
4. Archer, B. L., and Audley, B. G. (1969) *Methods Enzymol.* 15, 467–480
5. Archer, B. L., and Audley, B. G. (1967) in Advances in Enzymology (Nord, F. E., ed) Vol. 29, pp. 221–257, Interscience, New York
6. Lipman, D. J., and Pearson, W. R. (1988) *Science* 227, 1455–1441
7. Needleman, S. J., and Wunsch, C. D. (1970) *J. Mol. Biol.* 48, 443–453
8. Fitch, W. M., and Smith, T. F. (1983) *Proc. Natl. Acad. Sci. U. S. A.* 80, 1382–1386
9. Gotow, O. (1982) *J. Mol. Biol.* 162, 705–708
10. Finer-Moore, J., Bazan, F., Rubin, R., and Stroud, R. M. (1989) in Prediction of Protein Structure and Principles of Protein Conformation (Fasan, J. D., ed) Plenum Publishing Corp., New York, in press
11. Chou, P. Y., and Fasman, G. D. (1974) *Biochemistry* 13, 222–245
12. Garnier, J., Osguthorpe, D. J., and Robson, B. (1978) *J. Mol. Biol. 120*, 97–120
13. Austen, B. M., and Smith, E. L. (1976) *Biochim. Biophys. Res. Commun.* 72, 411–417
14. Persson, B., Flinta, C., Heijne, G. V., and Jörnvall, H. (1985) *Eur. J. Biochem.* 152, 523–527
15. Coruzzi, G., Broglio, R., Cashmore, A., and Chua, N.-H. (1983) *J. Biol. Chem.* 258, 1399–1402
16. Fichersky, E., Bernatsky, R., Tankesley, S. D., Breidenbach, R. B., Kraush, A. P., and Cashmore, A. R. (1985) *Gene (Amst.*) 45, 247–258
17. Dunnill, P. (1968) *Bioch. J.* 85, 253–875
18. Yokoyama, S., Kawai, Y., Tajima, S., and Yamamoto, A. (1985) *J. Biol. Chem.* 260, 16375–16382
19. Rall, S., Jr., Weisgraber, K. H., Mahley, R. W., Ogawa, Y., Fielding, C. J., Utermann, G., Haas, J., Steinmetz, A., Menzel, H.-J., and Assman, G. (1984) *J. Biol. Chem.* 259, 10063–10070
20. Inglis, A. S. (1983) *Methods Enzymol.* 91, 324–332
21. Sarris, C. M., Van Ensenbergen, J., Jenks, B. G., and Bloomers, P. J. (1988) *Anal. Biochem.* 158, 54–67
22. Rodrigues, H., Kohr, W. J., and Harkins, R. N. (1984) *Anal. Biochem.* 140, 538–547
23. Kohr, W. J., Keck, R., and Harkins, R. N. (1982) *Anal. Biochem.* 122, 348–359
24. Barker, W. C., and Dayhoff, M. O. (1972) in Atlas of Protein Sequence and Structure (Dayhoff, M. O., ed) Vol. 5, pp. 101–110, National Biomedical Research Foundation, Silver Spring, MD
25. Burnette, W. N. (1981) *Anal. Biochem.* 112, 195–203
**Primary Structure of Rubber Elongation Factor**

Amino acid composition analysis. Proteins were hydrolyzed for 24 h with constant heating in 6 N HCl in evacuated sealed tubes at 110°C. The hydrolysates were reduced and alkylation with Cys-SH and analyzed on an amino acid analyzer equipped with a multiple detector using a 4% non-ascending program.

Amino acid sequence analysis. Peptide sequences were determined by using sequential Edman degradation with modified Buchta's sequence separator (models 700 and 760, Buchta) equipped with on-line mass spectrometric equipment. Polypeptide (1.3 kg) was subjected to a new name in the order. Modifications include automatic conversion of the amide bonds to the corresponding amides, and in line detection onto an reverse phase HPLC column (240). Each PTH analysis and was identified as previously described (29).

| REF | Arg | Asp | Cys | Gln | Glu | His | Ile | Leu | Lys | Met | Phe | Pro | Ser | Thr | Val |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |
| 2   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |
| 3   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |

**Table 1. Amino Acid Composition of REF and Peptides Derived from It by Hydrolysis and Steady-State Protease Digestion**

- The numbers in parentheses represent the number of residues determined by sequence analysis.
- Sequence indicates peptides analyzed by automated Edman degradation and A indicates peptides analyzed by amino acid composition only.

| REF | Arg | Asp | Cys | Gln | Glu | His | Ile | Leu | Lys | Met | Phe | Pro | Ser | Thr | Val |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |
| 2   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |
| 3   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |

**Table 2. Amino Acid Composition of Amino Acid Peptides of REF**

- The numbers in parentheses represent the number of residues determined by sequence analysis.
- Sequence indicates peptides analyzed by automated Edman degradation and A indicates peptides analyzed by amino acid composition only.

| REF | Arg | Asp | Cys | Gln | Glu | His | Ile | Leu | Lys | Met | Phe | Pro | Ser | Thr | Val |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |
| 2   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |
| 3   | 2.0 | 3.0 | 2.2 | 1.4 | 2.6 | 4.2 | 3.3 | 2.1 | 1.0 | 2.0 | 2.3 | 1.7 | 2.0 | 1.6 | 1.0 |

**Table 3. Amino Acid Composition of Steady-State and Compartment Protease Peptides Derived from Thiopeptide and Steady-State Proteases Digestion**

- The numbers in parentheses represent the number of residues determined by sequence analysis.
- Sequence indicates peptides analyzed by automated Edman degradation and A indicates peptides analyzed by amino acid composition only.

- Not determined by amino acid analysis.

**Table 4. Amino Acid Composition of Steady-State and Compartment Protease Peptides Derived from Thiopeptide and Steady-State Proteases Digestion**

- The numbers in parentheses represent the number of residues determined by sequence analysis.
- Sequence indicates peptides analyzed by automated Edman degradation and A indicates peptides analyzed by amino acid composition only.

- Not determined by amino acid analysis.

**Table 5. Sequence of Peptide T**

| Cycle | Thiopeptide | Yield (mol) |
|-------|-------------|------------|
| 1     | Tyr         | 0.4        |
| 2     | Lys         | 0.8        |
| 3     | Ser         | 0.6        |
| 4     | Phe         | 0.7        |
| 5     | His         | 0.8        |
| 6     | Glu         | 2.0        |
| 7     | Thr         | 0.4        |
| 8     | Ala         | 1.0        |
| 9     | Val         | 2.0        |
| 10    | Pro         | 0.8        |
| 11    | Cys         | 3.0        |
| 12    | Val         | 1.0        |
| 13    | Cys         | 1.0        |
| 14    | Tyr         | 0.4        |
| 15    | Lys         | 0.8        |
| 16    | Ser         | 0.4        |

**Table 6. Sequence of Peptide H**

- Not determined by amino acid analysis.

| Cycle | Thiopeptide | Yield (mol) |
|-------|-------------|------------|
| 1     | Tyr         | 0.4        |
| 2     | Lys         | 0.8        |
| 3     | Ser         | 0.6        |
| 4     | Phe         | 0.7        |
| 5     | His         | 0.8        |
| 6     | Glu         | 2.0        |
| 7     | Thr         | 0.4        |
| 8     | Ala         | 1.0        |
| 9     | Val         | 2.0        |
| 10    | Pro         | 0.8        |
| 11    | Cys         | 3.0        |
| 12    | Val         | 1.0        |
| 13    | Cys         | 1.0        |
| 14    | Tyr         | 0.4        |
| 15    | Lys         | 0.8        |
| 16    | Ser         | 0.4        |

**Table 7. Sequence of Peptide H**

- Not determined by amino acid analysis.

| Cycle | Thiopeptide | Yield (mol) |
|-------|-------------|------------|
| 1     | Tyr         | 0.4        |
| 2     | Lys         | 0.8        |
| 3     | Ser         | 0.6        |
| 4     | Phe         | 0.7        |
| 5     | His         | 0.8        |
| 6     | Glu         | 2.0        |
| 7     | Thr         | 0.4        |
| 8     | Ala         | 1.0        |
| 9     | Val         | 2.0        |
| 10    | Pro         | 0.8        |
| 11    | Cys         | 3.0        |
| 12    | Val         | 1.0        |
| 13    | Cys         | 1.0        |
| 14    | Tyr         | 0.4        |
| 15    | Lys         | 0.8        |
| 16    | Ser         | 0.4        |

- Not determined by amino acid analysis.
### Table 1. Sequence Analysis of Peptide T5

| Cycle | Amino Acid | Yield (pmol) |
|-------|------------|--------------|
| 1     | Asp        | 970          |
| 2     | Val        | 780          |
| 3     | Ala        | 630          |
| 4     | Pro        | 630          |
| 5     | Leu        | 135          |
| 6     | Tyr        | 123          |
| 7     | Arg        | 88           |
| 8     | Ser        | 20           |

Residues: 54-68
Amount Applied: 2.4 nmol
Initial Yield: 52%
Average Repetitive Yield: 86%

### Table 2. Sequence Analysis of Peptide T6

| Cycle | Amino Acid | Yield (pmol) |
|-------|------------|--------------|
| 1     | Ser        | 1000         |
| 2     | Tyr        | 7100         |
| 3     | Leu        | 120          |
| 4     | Pro        | 1600         |
| 5     | Arg        | 270          |
| 6     | Gly        | 190          |
| 7     | Leu        | 330          |
| 8     | Gly        | 240          |

Residues: 67-76
Amount Applied: 2.4 nmol
Initial Yield: 52%
Average Repetitive Yield: 86%

### Table 3. Sequence Analysis of Peptide T7

| Cycle | Amino Acid | Yield (pmol) |
|-------|------------|--------------|
| 1     | Ser        | 168          |
| 2     | Ala        | 197          |
| 3     | Ser        | 74           |
| 4     | Ile        | 123          |
| 5     | Gly        | 38           |
| 6     | Val        | 191          |
| 7     | Val        | 50           |

Residues: 93-108
Amount Applied: 2.0 nmol
Initial Yield: 42%
Average Repetitive Yield: 86%

### Table 4. Sequence Analysis of Peptide T8

| Cycle | Amino Acid | Yield (pmol) |
|-------|------------|--------------|
| 1     | Ser        | 1034         |
| 2     | Leu        | 171          |
| 3     | Pro        | 35           |
| 4     | Thr        | 60           |
| 5     | Ile        | 33           |
| 6     | His        | 28           |
| 7     | Lys        | 11           |

Residues: 55-68
Amount Applied: 1.0 nmol
Initial Yield: 35%
Average Repetitive Yield: 86%

### Table 5. Sequence Analysis of Peptide T9

| Cycle | Amino Acid | Yield (pmol) |
|-------|------------|--------------|
| 1     | Ser        | 131          |
| 2     | Ala        | 128          |
| 3     | Ser        | 40           |
| 4     | Leu        | 21           |
| 5     | Pro        | 71           |
| 6     | Gly        | 10           |

Residues: 110-122
Amount Applied: 0.25 nmol
Initial Yield: 52%
Average Repetitive Yield: 86%

### Table 6. Sequence Analysis of Peptide T10

| Cycle | Amino Acid | Yield (pmol) |
|-------|------------|--------------|
| 1     | Ser        | 50           |
| 2     | Leu        | 131          |
| 3     | Ala        | 126          |
| 4     | Ser        | 41           |
| 5     | Leu        | 291          |
| 6     | Pro        | 71           |
| 7     | Gly        | 34           |

Residues: 117-129
Amount Applied: 0.23 nmol
Initial Yield: 52%
Average Repetitive Yield: 86%
### Table 12. Sequence Analysis of Peptide NG2-CN

| Cycle | Amino Acid | Yield (mmol) |
|-------|------------|--------------|
| 1     | Ala        | 346          |
| 2     | Lys        | 288          |
| 3     | Arg        | 330          |
| 4     | Ser        | 264          |
| 5     | Gly        | 182          |
| 6     | Pro        | 53           |
| 7     | Val        | 22           |
| 8     | Ser        | 21           |
| 9     | Lys        | 21           |

Residues: 80-96
Amount Applied: 0.5 mmol
Initial Yield: 52%
Average Repetitive Yield: 50%

### Table 13. Sequence Analysis of Peptide NG2

| Cycle | Amino Acid | Yield (mmol) |
|-------|------------|--------------|
| 1     | Gly        | 421          |
| 2     | Ala        | 762          |
| 3     | Lys        | 772          |
| 4     | Ser        | 622          |
| 5     | Pro        | 252          |
| 6     | Val        | 213          |
| 7     | Arg        | 500          |
| 8     | Thr        | 213          |
| 9     | Lys        | 344          |
| 10    | Thr        | 322          |
| 11    | Leu        | 213          |
| 12    | Ser        | 115          |
| 13    | Val        | 115          |
| 14    | Lys        | 110          |
| 15    | Ala        | 118          |
| 16    | Ile        | 115          |
| 17    | Thr        | 113          |
| 18    | Leu        | 114          |
| 19    | Ser        | 3           |
| 20    | Lys        | 4           |
| 21    | Arg        | 4           |

Residues: 73-127
Amount Applied: 0.1 mmol
Initial Yield: 93%
Average Repetitive Yield: 50%

### Table 20. Sequence Analysis of Peptide NG1

| Cycle | Amino Acid | Yield (mmol) |
|-------|------------|--------------|
| 1     | Ala        | 366          |
| 2     | Ala        | 200          |
| 3     | Lys        | 127          |
| 4     | Arg        | 37           |
| 5     | Ser        | 17           |
| 6     | Ala        | 225          |
| 7     | Ser        | 34           |
| 8     | Lys        | 22           |
| 9     | Gly        | 76           |
| 10    | Val        | 48           |
| 11    | Arg        | 40           |
| 12    | Thr        | 29           |
| 13    | Ser        | 29           |
| 14    | Lys        | 15           |
| 15    | Ala        | 15           |
| 16    | Lys        | 15           |
| 17    | Ala        | 15           |
| 18    | Ile        | 15           |
| 19    | Thr        | 14           |
| 20    | Val        | 14           |
| 21    | Lys        | 4           |
| 22    | Arg        | 4           |
| 23    | Thr        | 3           |

Residues: 114-127
Amount Applied: 0.43 mmol
Initial Yield: 96%
Average Repetitive Yield: 50%

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*In cycle 9 and 15 was not identified during this analysis.

### Fig. 3. Tryptic Digest of REF. After a tryptic digest of REF was made on a Vydac C18 column (4.6 mm x 25 cm), the digest was loaded on a 0-60% linear gradient of acetonitrile. 0.1% TFA, 60 minutes at a flow rate of 1 ml/min.

### Fig. 4. Hydroxylamine Digest of REF. After a hydroxylamine digest of REF was made on a Supelco 4.6 mm column, the digest was loaded on a Vydac C18 column (4.6 mm x 25 cm) equilibrated in 1% TFA. The digest was loaded on a 0-60% linear gradient of acetonitrile. 0.1% TFA, 60 minutes at a flow rate of 1 ml/min.
Primary Structure of Rubber Elongation Factor

Fig. 5. Acetic Acid Digest of REF. Peptides from an acetic acid digest of REF were loaded on a Synchronis RP4 column (4.6 mm x 10 cm) equilibrated in 98% TFE-containing 0.1% TFA (v/v), and eluted in a 0 to 50% linear TFE gradient in 30 minutes at a flow rate of 1 ml/min.

Fig. 6. Chromatographic Dijst of NG1. Peptides from a chromatographic digest of NG1 were separated on a Vydac C18 column (4.6 mm x 25 cm) equilibrated in 0.1% TFA (v/v) with a linear gradient of 0 to 66% acetonitrile for 60 minutes at a flow rate of 1 ml/min.

Fig. 7. Tryptic Digest of NG1. Peptides from a tryptic digest of NG1 were separated on a Synochrom RP4 column (4.6 mm x 10 cm) equilibrated in 0.1% TFA (v/v) with a linear gradient of 0 to 30% TFE containing 0.1% TFA (v/v) for 50 minutes at a flow rate of 1 ml/min.

Fig. 8. FAB Mass Spectrometry of T1. Samples were prepared in a 5% acetic acid matrix and scanned from 1500 m/z to 2000 m/z. Numbers show observed mass values. A. FAB of T1, Positive ion mode +1 ion. B. FAB of T1, Negative ion mode -1 ion. C. Possible Compositions for T1.

Fig. 9. Saphylococcus V8 Digest of T1. Peptides from a Saphylococcus primum digest of T1 were loaded on a Vydac C18 column (4 mm x 25 cm) equilibrated in 0.1% TFA (v/v), and eluted in a 55 to 30% linear acetonitrile gradient in 30 minutes at a flow rate of 1 ml/min.

Fig. 10. Acetyl peptide hydrazine Digest of T1. Peptides from an acetyl peptide hydrazine digest of T1 were loaded on a Vydac C18 column (4 mm x 25 cm) equilibrated in 0.1% TFA (v/v), and eluted in a 5 to 30% linear acetonitrile gradient in 30 minutes at a flow rate of 1 ml/min.

Fig. 11. Saphylococcus V8 Digest of NG1. Peptides from a Saphylococcus primum digest of NG1 in phosphate buffer pH 7.4 (see methods) were eluted from a Vydac C18 column (4 mm x 25 cm) equilibrated in 0.1% TFA (v/v), eluted in a 5 to 40% linear acetonitrile gradient in 30 minutes at a flow rate of 1 ml/min.

Possible Compositions for T1

| Amino Acid | Analysis | Mass (a) | Possibilities |
|------------|----------|----------|---------------|
| Asn        | Asx      | 114.104  | 3 2 1 0       |
| Asp        | 115.089  | 0 1 2 3  |
| Gin        | Glx      | 126.131  | 1 2 3 4       |
| Glu        | 129.116  | 4 3 2 1  |
| Gly        | 171.156  | 1        |
| Ala        | 113.157  | 1        |
| Leu        | 112.157  | 1        |
| C-term -OH | 17.007   | 1        |

(c) is the actual composition determined.