Short Communication

Complete Amino Acid Sequence of Luffin-a, a Ribosome-inactivating Protein from the Seeds of Luffa cylindrica*

Mohammad Rafiqul Islam, Hiromi Nishida and Gunki Funatsu†

Laboratory of Biochemistry and Protein Chemistry Engineering, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan

Received February 13, 1990

Luffin is a ribosome-inactivating protein (RIP) present in the seeds of the sponge gourd (Luffa cylindrica) that inactivates eucaryotic ribosomes by cleaving the N-glycosidic bond of A4324 of 28S rRNA, inhibiting protein biosynthesis.1 When assayed using rabbit reticulocyte lysates, luffin is the strongest protein biosynthesis inhibitor among the RIPs isolated so far.2) Previously we isolated two isoforms of luffin (a and b) from seeds produced in Tottori Prefecture of Japan, and characterized both of them.3) Here we describe the complete amino acid sequence of luffin-a, which is approximately 5.5-fold more active in protein biosynthesis inhibition than ricin A-chain and compare it with that of the latter.

Tryptic and chymotryptic digestions were done in aqueous solution at pH 8.0 and 37°C for 7 to 10 hr and 1 to 1.5 hr, respectively, and digestion with Staphylococcus aureus V8 protease in 50 mM ammonium bicarbonate solution at 37°C for 24 hr. Cyanogen bromide (CNBr) cleavage was done in 80% trifluoroacetic acid (TFA) at room temperature for 24 hr by the method of Schroeder et al.4) Peptides were separated by reverse-phase HPLC (RP-HPLC) with a Finepak C_4 or C_8 column (4.6 x 250 mm) or an Asahipak C_8 column (5.5 x 150 mm) using either a 5 mM phosphate buffer (pH 6.0)-acetonitrile (MeCN) system, a 0.1% TFA-MeCN system, a 0.1% TFA-2-propanol system, or a 5 mM ammonium bicarbonate solution-2-propanol system. The fragments obtained by CNBr-cleavage were separated by gel filtration through a Bio-Gel P-30 column (2.1 x 98 cm) in 50% formic acid. Peptides were sequenced by the 4-N,N-dimethylaminoazobenzene 4'-isothiocyanate/phenylisothiocyanate (DABITC/PITC) double coupling method.5) Carbohydrate in peptides was analysed by gas liquid chromatography of trimethylsilylated derivatives by the procedure of Chambers and Clamp.6)

All the twenty-two tryptic peptides (designated T-1–T-22) were isolated from the tryptic digest of luffin-a and sequenced directly or after fragmentation with chymotrypsin by the DABITC/PITC method. Sequence analysis of the eight lysine- or arginine-containing peptides (designated CH-1–CH-8) obtained from chymotryptic digestion of luffin-a gave the linkages of (T-1)-(T-2), (T-2)-(T-3), (T-3)-(T-4), (T-4)-(T-5), (T-7)-(T-8), (T-8)-(T-9), (T-16)-(T-17), and (T-17)-(T-18)-(T-19).

Luffin-a was cleaved with CNBr into three fragments (designated as CB-I, -II, and -III). Amino acid sequences of CB-I and CB-II were established by sequencing up to the 18th amino acid residue and analyzing the tryptic peptides from them. CB-III was further fragmented into nine peptides (designated V-1–V-9) by V8 protease digestion. The sequences of seven peptides (V-1–V-7) were identified by their amino acid compositions and N-terminal sequences, and those of V-8 and V-9 were by sequencing the chymotryptic peptides from them in addition to direct sequencing. From these results and the sequences of the tryptic peptides, the sequence of CB-III was established. Since the N- and C-terminal sequences of intact luffin-a were Asp-Val-Arg-Phe-Ser-3) and -Val-Ala, respectively, and the arrangement of the CNBr-fragments was (CB-I)-(CB-

---

* This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.
† To whom correspondence should be addressed.
**Fig. 1. Complete Amino Acid Sequence of Luffin-a.**

T, CH, and CB: tryptic peptides, chymotryptic peptides, and cyanogen bromide fragments, respectively, from luffin-a; ST: tryptic peptides from succinylated luffin-a; V: *S. aureus* V8 protease peptides of CB-III fragment; C: chymotryptic peptides from the tryptic peptides T-4, T-6, and T-12, and the V8 protease peptides V-8 and V-9; CPY: amino acid released by carboxypeptidase Y digestion of luffin-a. The solid lines indicate the sequenced parts, and broken lines indicate those parts of the peptides in which the amino acid residues were not identified.
II)-(CB-III), the complete amino acid sequence of luffin-a was established as illustrated in Fig. 1. The linkages of (T-2)-(T-3), (T-8)-(T-9)-(T-10), (T-11)-(T-12), (T-13)-(T-14) and (T-19)-(T-20)-(T-21)-(T-22) were also confirmed by analyzing five arginine-containing peptides isolated from the tryptic digest of partially succinylated luffin-a.

Luffin-a consists of 248 amino acid residues: Asp₉Asn₁₄Thr₂₃Ser₃₀Glu₈Gln₁₂Pro₆Gly₁₃Ala₂₂Val₁₇Met₁₁le₁₈Leu₂₆Tyr₁₃Phe₁₀Lys₁₆His₁Arg₁Trp₁, which correspond to the values obtained by direct amino acid analysis of luffin-a. The occurrence of 24 basic and 17 acidic residues is compatible with the basic isoelectric point (pI > 10) of this protein. Six Asn-X-Thr/Ser sequences are observed at positions Asn-28, Asn-33, Asn-77, Asn-84, Asn-206 and Asn-227, and each of these Asn residues carries a single residue of N-acetylg glucosamine. The relative molecular mass, including the six sugar residues, is calculated to be 28,242 daltons. Recently, Lys-231 has been demonstrated to be at or near the active site of luffin-a.⁷)

Seventy-five identical residues are observed when luffin-a is aligned with the ricin A-chain consisting of 267 amino acid residues. The identical residues comprise 33% of the sequence of luffin-a, indicating these proteins are homologous.

Details of the amino acid sequencing, carbohydrate analysis, and sequence comparison with other RIPs will be described elsewhere.

References