BIOCHEMICAL LETTERS

A non-toxic two-chain ribosome-inactivating protein co-exists with a structure-related monomeric lectin (SNA III) in elder (*Sambucus nigra*) fruits

In the last few years there has been an important effort in the research on plant lectins. In elder (*Sambucus nigra*), the haem-agglutinins SNA-I, SNA-II and seed-SNA have been isolated. SNA-I displays specificity for α 2,6-linked sialic acid residues [1]. SNA-II and seed SNA display a D-galactose specificity [2,3]. A recent paper from Mach et al. [4], dealing with the finding of a new D-galactose-binding lectin, reported that elder fruits contain a new lectin, named SNA III, and they suggested that SNA III forms dimeric aggregates. However, the authors did not sequence the putative dimers, and neither did they investigate biological effects other than the agglutination capacity. Using an affinity-chromatographic procedure we have confirmed the presence of SNA III in elder fruits. However, in our hands elder-fruit SNA III behaves like a monomeric D-galactose-binding lectin with the properties described previously [4]. Furthermore, we have found that the putative dimeric aggregate of SNA III is in fact a protein translation inhibitor which binds D-galactose residues like SNA III and strongly inhibits protein synthesis in rabbit reticulocyte lysates (IC_{50} 1.8 ng/ml). We have named this protein 'nigrin f'. Ribosome-inactivating proteins (RIPs) display an N-glycosidase activity on the large ribosomal RNA of mammalian, plant, yeast and bacterial-sensitive ribosomes, thus promoting an irreversible

impairment of protein synthesis (for a recent review, see [5]). The molecular action of plant RIPs involves the hydrolysis of one [5,6] or more adenine residues from the rRNA [7,8] or the DNA [9].

Nigrin f contains two different polypeptide chains of M_r 26 200 (catalytic chain) and 31 600 (D-galactose-binding lectin chain). Nigrin f fulfils all the requirements (data on protein synthesis, electrophoretic analysis, 28 S RNA N-glycosidase activity, amino acid sequence and red-blood-cell-agglutinating activity) to be classified as a non-toxic type 2 RIP that strongly resembles, but is clearly different from, the other two non-toxic type 2 RIPs reported to date: nigrin b [10] and ebulin 1 [11]. The small changes in the amino acid sequence of nigrin f with respect to nigrin b and ebulin 1 indicate that all such type 2 RIPs are derived from a common ancestor, also probably related to the lectins SNA II [2] and SNA III [4] (Figure 1). It is noteworthy that there is a close correlation between the nigrin f A chain and some type 1 RIPs which display potent anti-viral, in particular anti-HIV-1, effects [12–14]. We have also shown (T. Gírbés, L. Citores, F. M. de Benito, R. Inglesias and J. M. Ferreras, unpublished work) that anti-(nigrin b) rabbit polyclonal antibodies react in Western-blot analysis with SNA I, SNA II and SNA III, in agreement with the structural data known to date. This could suggest a functional correlation between the elder type 2 RIPs and the elder lectins not yet ascertained.

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Type 1 RIPs and type 2 RIP-A chains

Nigrin f A-chain	I D Y P S V S F N L A G A X S A T Y R D F L S N
Nigrin b A-chain	I D Y P S V S F N L D G A V S A T Y R D F L S N
Ebulin 1 A-chain	I D Y P S V S F N L A G A K S T T Y R D F L K N
Trichosanthin R	D V S F R L S G A T S S S Y G V F I S N
TAP 29	D V S F R L S G A T S K K K V Y F I S N

Type 2 RIP-B chains and lectins

Nigrin f B-chain	D G E T X P I P A S F T R X I V G R D G L X V D
Nigrin b B chain	D G E T X T L X T S F T R N I V G R D G L X V D
Ebulin 1 B chain	D G E T X A I P A P F T R R I V G X D G L E V D
SNA III	D G E - - - - - P I T G N I I G R
SNA II	T S F T R N I V G R D G L N V D

Figure 1 N-terminal amino-acid sequences of nigrin f and other RIPs and lectins

N-terminal amino acid sequence analysis was carried out as described in [11]. Identical residues are boxed. The broken line has been introduced for optimal alignment. Abbreviation: TAP 29, *Trichosanthes* antiviral protein [14].

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