BIOCHEMICAL LETTERS

Elder (*Sambucus nigra* L.)-fruit lectin (SNA-IV) occurs in monomeric, dimeric and oligomeric isoforms

In recent years, numerous carbohydrate-binding proteins have been isolated from the tissues of *Sambucus nigra*, the common elder tree. Two distinct haemagglutinins (SNA-I, SNA-II) have been obtained from bark extracts [1,2]. Elder seeds contain a lectin which is immunologically related to SNA-II, but these two proteins differ in their physicochemical and carbohydrate-binding properties [3]. In a previous report we have described a novel lectin which occurs in the fruit of the elder [4]. This glycoprotein was originally named SNA-III, as was independently the elder-seed lectin. Since these two proteins appear not to be identical, we later suggested that the fruit haemagglutinin be called SNA-IV [5]. The diversity of carbohydrate-binding proteins in *S. nigra* tissues is further documented by the existence of lectin-like protein translation inhibitors belonging to the family of non-toxic two-chain ribosome-inactivating proteins (RIPs). So far, two homologous RIPs, nigrin b and nigrin s, have been identified in bark and seed extracts respectively [6,7].

SNA IV occurs mainly as a monomeric glycoprotein, but tends to form aggregates of various complexities. We have proposed that this aggregation is a prerequisite for the lectin to agglutinate red blood cells [4]. Using a more general approach for the purification of plant haemagglutinins, Girbés et al. have now found that elderberries contain, besides elder-fruit lectin, a second carbohydrate-binding protein, which appears to be a novel RIP [8]. This 58 kDa protein, named nigrin f, is composed of two subunits and has been reported to agglutinate red blood cells. It was most likely these properties which led the authors to postulate that the putative dimer of SNA-IV actually represents nigrin f, implying that the latter, rather than the former, protein accounts for the haemagglutinating capacity of elderberries. Indeed, a polypeptide with an apparent molecular mass similar to nigrin f is present in our preparations of dimeric as well as oligomeric elderberry lectin aggregates. However, it appears unlikely that this protein represents the (major) elder-fruit haemagglutinin, since it is less abundant in the fraction with the highest specific agglutinating activity than in others (see Figure 2 and the accompanying text of reference [4]). Furthermore, the 58 kDa polypeptide exhibits a novel N-terminal amino acid sequence:

D-G-E-P-F-T-R-H-I-I-G-...

which rules out its identity with nigrin f (L. Mach, H. Schwihla and J. Glössl, unpublished work). Therefore we conclude that SNA-IV still represents the best candidate for the (major)

haemagglutinin in elderberry extracts, whereas the biological properties of nigrin f have yet to be elucidated.

Girbés et al. proposed a close relationship between *S. nigra* lectins and RIPs, mainly on the basis of preliminary amino-acid-sequence information. Further evidence for this hypothesis was derived from immunoblotting experiments, which revealed an immunological cross-reactivity of polyclonal antibodies raised against nigrin b with the various elder lectins [7,8]. However, we have found that polyclonal antibodies against SNA-I would bind to elder-fruit lectin on immunoblots ([5]; L. Mach, R. Kerschbaumer and J. Glössl, unpublished work), although double-immunodiffusion experiments showed that these two proteins are immunologically distinct [4]. We have already previously pointed out that anti-carbohydrate antibodies which recognize an immunogenic epitope common to (most) plant glycoprotein N-glycans account for this cross-reaction [5]. Therefore the assessment of the relationship between two plant glycoproteins by means of immunoblotting appears to be prone to error unless appropriate precautions, e.g. preadsorption of antisera with heterologous plant glycoproteins, are undertaken.

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