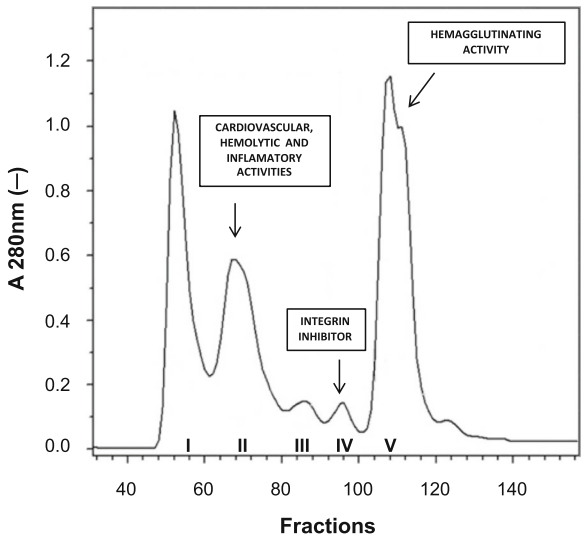

Fig. 3 Elution profile of the gel filtration fractionation of the *Scorpaena plumieri* extract (SpV). A sample of SpV (approximately 83 mg of protein) was applied on a Sephacryl S-200 HR column (2.0 cm × 120 cm) previously equilibrated and eluted with 0.01 M phosphate buffer at pH 7.6 with 0.4 M NaCl at 4 °C. Flow rate, 5.25 mL/h, fractions of 1.75 mL. Figure adapted from [27]

optimal pH value for its activity was found to be within the range of 7–8 [20]. Although many fish venoms were found to perform proteolytic activity, the only other isolated fish venom proteases comprise a group of five toxins termed natterins (5.9–41.4 kDa) found in the venom of the toadfish *Thalassophryne nattereri*. These proteases cleave human kininogen and degrade type I and type IV collagen in vitro. The latter leads to direct induction of necrosis, stimulating an inflammatory response, which, in turn, correlates with the edema-inducing effects of the toxin [53, 54].

Lectins

Extracts from vegetable or animal sources — venoms, for instance — have the ability to induce the agglutination of hemocytes and to disrupt cell-ECM interactions [48, 55]. These abilities are related to the activity of molecules with carbohydrate-binding properties: the lectins.

Two lectins — (i) plumieribetin, a lectin homologous to monocot mannose-binding B-type lectin and (ii) a group of five isolectins (Sp-CL 1–5) homologous to fish C-type lectins — were purified from *S. plumieri* venom [27, 28].

Plumieribetin was purified with a high degree of homogeneity by gel filtration chromatography — from both SpV (Fig. 3) and skin mucus — as a 14 kDa band in SDS-PAGE. Analytical gel filtration on a calibrated size exclusion column provided several peaks, most of which contained this same protein in different oligomeric states (mainly as a tetramer). Cross-linkage studies confirmed the oligomeric nature of this integrin-inhibiting factor. Plumieribetin is characterized by an abundance of anti-parallel beta strands, just as the aforementioned B-type lectins. The primary structure of plumieribetin is highly similar to those of homologous proteins isolated from other fishes, namely *Platycephalus indicus* (71.5%), the green puffer fish *Tetraodon nigroviridis* (63.7%) and the Japanese pufferfish *T. rubripes* (56.8%) [27].

Plumieribetin binds to α1β1 integrin irrespective of N-glycosilation — indicating direct protein-protein interaction — supressing α1β1 integrin binding to basement membrane collagen IV. It could not fully detach hepatocarcinoma HepG2 cells or primary arterial smooth muscle cells from collagen IV fragment CB3. It did, however, attenuate cell-collagen contacts and cell spreading, changing the actin cytoskeleton after blocking the compensating α2β1 integrin as well [27].

In addition to the hemagglutinating fraction (FV) (Fig. 3), five main absorbance peaks were detected by reverse phase high performance liquid chromatography (RP-HPLC) (RP1, 2, 3, 4 and 5). Mass spectrometry analysis of these fractions on matrix-assisted laser desorption/ionization — time of flight (MALDI-TOF) revealed a high degree of homogeneity with m/z signals and molecular masses of 16.981, 16.982, 16.975, 16.841 and 16.842 kDa. The amino acid sequence of RP4 revealed homology (24–32% of identity) with various fish C-type lectins. Finally, the presence of the glycan moiety galactose-β(1 → 4)-N-acetylglucosamine was also revealed in the FV structure [28].

The similar chemical characteristics exhibited by RP fractions (elution in RP-HPLC and MALDI-TOF) — together with the similarities found among amino acid sequences — strongly suggest that RP1-5 are C-type lectin isoforms (isolectins) [28].

Scorpaena plumieri cytolytictoxin (SP-Ctx)

Considerable evidence suggests that the cardiovascular, inflammatory and cytolytic effects attributed to Scorpaenidae fish venoms are due to the action of a single labile "lethal protein factor" [1, 5, 56].

A cytolysin denominated Sp-CTx — a glycoprotein with two subunits (of ≈ 65 kDa each) — was purified from the venom of *S. plumieri* [24]. Next, an improved purification approach was established, which reduced the time and the number of chromatography steps needed to obtain the pure toxin [25]. Due to the lability of Sp-CTx, such a time reduction is crucial to the success of its isolation and functional characterization.

Orbitrap-MS analyses revealed thirty-seven Sp-CTx internal amino acid sequences after proteolytic fragmentation with trypsin. Through the protein database NCBInr, 29 tryptic peptide fragments were found to have identity