SFTI-1 ANALOGS OF RESTRICTED INHIBITORY SPECIFICITIES AS LIGANDS FOR THE AFFINITY CHROMATOGRAPHIC ISOLATION OF SERINE PROTEASES

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SFTI-1 is a 14-residue cyclic peptide isolated from sunflower seeds as a potent trypsin inhibitor, whose structure resembles the active site loop of the Bowman-Birk inhibitors. SFTI-1 is amenable to chemical synthesis, allowing for the preparation of acyclic variants displaying novel specificities. Here we describe the syntheses of SFTI-1 analogs having either Lys, Arg, Phe or Leu at position 5 and a handle for attaching them to Sepharose-hydrazide to create the affinity supports Sepharose-Lys-5-SFTI-1, Sepharose-Arg-5-SFTI-1, Sepharose-Phe-5-SFTI-1 and Sepharose-Leu-5-SFTI-1, respectively. Chymotrypsin and trypsin were selectively purified from pancreatin by using Sepharose-Phe-5-SFTI-1 and Sepharose-Lys-5-SFTI-1 columns, respectively; the latter column yielded approximately 28 mg of trypsin per ml of packed gel. Chromatography of *Bothrops moojeni* venom over Sepharose-Arg-5-SFTI-1 column yielded a mixture of proteins of approximately 35 kDa capable of hydrolyzing the substrate DL-BApNA, indicating that this resin discriminates between the various venom arginyl-amidases since the well-known thrombin-like enzyme passed unretarded through the column. Rat angiotensin II-forming elastase-2 was isolated from crude mesenteric arterial bed perfusate by chromatography over Sepharose-Leu-5-SFTI-1 but contaminated with a hitherto unknown angiotensinase. In conclusion, the relative easiness with which SFTI-1-based affinity resins can be prepared indicate that further development of these supports may find application as novel protease-discovery approach in different areas of biological investigation.

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