Isolasi dan pemurnian inhibitor tripsin kacang kedelai = The isolation and purification of soybean trypsin inhibitor

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Abstrak

Ruang Lingkup dan Cara Penelitian: Peneliti di Indonesia sering mengalami kesulitan memperoleh bahan baku seperti petanda protein untuk menentukan 'berat molekul'. Salah satu petanda protein adalah inhibitor tripsin kacang kedelai (ITK). Penelitian ini bertujuan mengisolasi dan memurnikan ITK dari biji kacang kedelai (Glycine max), Isolasi dilakukan dengan cara ekstraksi asam, diikuti 'salting out, dialisis dan pengendapan aseton. Pemurnian dilakukan dengan kromatografi kolom pertukaran ion. Aktivitas ITK fraksi 'kasar' dan 'murni' diperiksa dengan mengamati pengaruh hambatan terhadap aktivitas enzimatik tripsin, dengan substrat azokasein (proteolitik) dan BAPA/BAPNA (amidase). Azokasein yang dipakai sebagai substrat reaksi tripsin disintesis sendiri. Penilaian kemurnian ITK ?kasar? dan 'murni', juga tripsin dilakukan dengan elektroforesis gel 'slab' SDS-poliakrilamid. Sebagai pembanding pada penelitian ini digunakan inhibitor tripsin produk Sigma.

Hasil dan Kesimpulan : Dari 100 g biji kacang kedelai kering panen, diperoleh 1,16 g isolat (ITK 'kasar') setara dengan 418,46 mg protein. Pemurnian lewat kolom pertukaran ion menghasilkan 2 fraksi dengan 'recovery' protein total minimal 63,7 %. Azokasein yang disintesis hasilnya berbeda bila jenis alkohol yang digunakan juga berbeda. Pada penelitian ini baik ITK 'kasar` maupun 'murni' memperlihatkan hambatan terhadap reaksi enzimatik tripsin. Hambatan 50 % terjadi pads rasio inhibitor/enzim yang bervariasi; ITK `murni' 1 memperlihatkan angka yang paling tinggi. Elektroforetogram menunjukkan bercak ITK 'murni' II identik dengan SBTI (Sigma).
<hr><i>Scope and Method of Study: The chemicals required for laboratory investigations are quite often difficult to obtain, e. g. the standard protein markers for molecular weight determination. Among the markers used for that purpose is the soybean trypsin inhibitor. This work was carried out to isolate and purify trypsin inhibitor from soybean (Glycine max) seeds (SBTI). The procedure included an acid extraction, followed by salting-out, dialysis and an acetone precipitation. Purification was carried out by ion-exchange column chromatography. The protein content of the isolate and of the purified substance was determined by spectrophotometer. The activities of the crude and of the purified soybean trypsin inhibitor were tested against trypsin activity. The trypsin used was obtained commercially. Trypsin's proteolytic activity was performed on azo-casein while its amidase activity was tested on BAPA/BAPNA. The azo-casein was synthesized in the laboratory. The purity of the crude and of the purified soybean trypsin inhibitors, and of the trypsin itself was examined on a slab SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Findings and Conclusions: From 100 g of dried soy-bean seeds, 1.16 of product was isolated (= 418.46 mg protein). Further purification on an ion-exchange column yielded two fractions, with a minimum of 63.7% protein recovered. The azo-casein synthesis revealed two different products depending on the grade of

alcohol used in the process. The crude and the purified soybean trypsin inhibitors showed inhibitory effects towards trypsin. The fifty percent inhibition occurred at varied inhibitor/enzyme ratios, the highest was shown by the purified SBTI I. The electrophoretogram showed that the purified SBTI II was identical to the SBTI (Sigma).</i>