Резюмета научните трудове на д-р инж Валентин Стойчев Лозанов за участие в конкурса за доцент в област на висше образование 4. Природни науки, математика и информатика по професионално направление 4.2. Химически науки и научна специалност „Биоорганична химия, химия на природните и биологически физиологично активни вещества“ за нуждите на Катедрата по медицинска химия и биохимия за сектор „Медицинска химия“, Медицински факултет на Медицински университет – София, обявен в ДВ № 91 от 04.11.2014 г.


**ABSTRACT:** Polyamines are essential polycations, playing important roles in mammalian physiology. Theoretically, the involvement of homocysteine in polyamine synthesis via S-adenosylmethionine is possible; however, to our knowledge, it has not been established experimentally. Here, we propose an original approach for investigation of homocysteine metabolites in an animal model. The method is based on the combination of isotope-labeled homocysteine supplementation and high-resolution accurate mass spectrometry analysis. Structural identity of the isotope-labeled metabolites was confirmed by accurate mass measurements of molecular and fragment ions and comparison of the retention times and tandem mass spectrometry fragmentation patterns. Isotope-labeled methionine, spermidine, and spermine were detected in all investigated plasma and tissue samples. The induction of moderate hyperhomocysteinemia leads to an alteration in polyamine levels in a different manner. The involvement of homocysteine in polyamine synthesis and modulation of polyamine levels could contribute to a better understanding of the mechanisms connected with homocysteine toxicity.


**ABSTRACT:** Liquid chromatography Orbitrap mass spectrometry method for quantification of biological aminothiols (cysteine, homocysteine, and glutathione) in cereal products has been developed. The assay is based on preliminary derivatization with N-(2-acridonyl)maleimide and high resolution accurate mass spectrometry with utilization of dl-Homocystine-3,3′,3′,4,4,4′,4′-d8 (homocystine-d8) as internal standard. The limits of quantification for homocysteine, cysteine, and glutathione are 19.44, 40.78, and 338.93 pg, respectively, per 10 µl injection. Intra- and inter-day precision expressed as relative standard deviations are in the range of 1.76 to 2.94 % and 1.06 to 4.13 %, respectively. The average recoveries were 98 % for Hcy, 87 % for Cys, and 92 % for GSH. Wheat, maize, and bakery products with different origin were analyzed. The content of Hcy in the investigated samples was found to be in range of 9-436 µg/100 g, Cys in range of 29-6,895 µg/100 g and GSH in range of 259-14,795 µg/100 g.

ABSTRACT: Melatonin is involved in the control of circadian and seasonal rhythmicity, possesses potent antioxidant activity, and exerts a neuroprotective and anticonvulsant effect. Spontaneously hypertensive rats (SHRs) are widely accepted as an experimental model of essential hypertension with hyperactivity, deficient sustained attention, and alterations in circadian autonomic profiles. The purpose of the present study was to determine whether melatonin treatment during epileptogenesis can prevent the deleterious consequences of status epilepticus (SE) in SHRs in the kainate (KA) model of temporal lobe epilepsy (TLE). Spontaneous recurrent seizures (SRSs) were EEG- and video-recorded during and after the treatment protocol. Melatonin (10 mg/kg diluted in drinking water, 8 weeks) increased the seizure-latent period, decreased the frequency of SRSs, and attenuated the circadian rhythm of seizure activity in SHRs. However, melatonin was unable to affect the disturbed diurnal rhythms and behavioral changes associated with epilepsy, including the decreased anxiety level, depression, and impaired spatial memory. Melatonin reduced neuronal damage specifically in the CA1 area of the hippocampus and piriform cortex and decreased hippocampal serotonin (5-HT) levels both in control and epileptic SHRs. Although long-term melatonin treatment after SE shows a potential to attenuate seizure activity and neuronal loss, it is unable to restore epilepsy-associated behavioral abnormalities in SHRs.


ABSTRACT: Recently, we have shown that the blockade of AT1 receptor might be useful as an adjuvant treatment strategy for the prevention of oxidative stress and neurotoxicity caused by status epilepticus (SE) in rats. The purpose of the present study was to further assess the efficacy of long-term treatment with losartan (10 mg/kg), the selective AT1 receptor antagonist, during kainate (KA)-induced epileptogenesis in Wistar rats. Losartan treatment started after onset of SE and continued for 4 weeks. The rats were video- and EEG-recorded for 3 months. Locomotor activity, anxiety and depressive-like behavior were evaluated 9 weeks after SE, when all rats had developed chronic epileptic state. Neuronal damage in hippocampus was analyzed by hematoxylin while serotonin (5-HT) levels in hippocampus by HPLC. AT1 receptor antagonism increased the latent seizure-free period and decreased the frequency of spontaneous motor seizures. Losartan positively affected epilepsy-provoked behavioral changes, including impulsivity, low anxiety level and depression in a phase-dependent manner and restored the changes in diurnal fluctuation of motor activity. Losartan exerted neuroprotection selectively in the CA1 area of the hippocampus in the KA-treated rats and lowered the 5-HT levels both in normal and abnormal conditions. Our findings suggest that the AT1 receptor antagonist exerts disease-modifying effects during KA-induced epileptogenesis and neuronal damage in CA1.
hippocampal area, attenuated some of the behavioral changes and restored diurnal variability in locomotor activity.


ABSTRACT: Introduction: Polyamines - putrescine, spermidine and spermine are polycationic compounds ubiquitous for all living organisms. They are essential for the cell growth and differentiation, the control of cell cycle progress, apoptosis, and cancerogenesis. Accumulated scientific evidence suggests the central role of polyamines in the process of keratinocytic proliferation, differentiation, and regulation. Objective: To elucidate the polyamine metabolic changes that occur in benign keratinocytic proliferation. Fifty eight patients were enrolled in the study, 31 with plaque-form of psoriasis vulgaris, which had been referred to as a model of benign keratinocytic proliferation, and 27-healthy controls. Materials and Methods: An original, innovative chromatographic method was used to detect the levels of putrescine, spermidine, and spermine in all skin samples. Results: Were significantly proven (P < 0.05). No difference was found between the polyamines levels of non-lesional psoriatic skin and healthy controls. Psoriatic lesions showed a two-time higher concentration of all polyamines in lesional, compared to non-lesional skin. Spermine had the highest concentration and highest proliferation trend, which demonstrated the importance of propylamine synthesis in the pathogenesis of psoriasis. Spermine highest concentrations suggested the leading role of adenosine methionine decarboxylase (AMDC) in the pathogenesis of benign keratinocytic proliferations. Conclusions: Non-lesional skin in psoriatic patients did not show latent changes in polyamine metabolism. Psoriatic lesions demonstrated two-time higher levels of the most essential biogenic polyamines compared to healthy controls. The highest level of spermine proved the crucial role of AMDC in the polyamine metabolism changes in psoriasis. Future therapeutic approaches should be focused on reduction of exogenic spermine intake, utilizing new spermine blockers, and synthesis of AMDC inhibitors.


ABSTRACT: Medicinal plants have been intensively studied as a source of antitumor compounds. Due to the beneficial climate conditions Bulgarian herbs have high pharmacological potential. Currently, the antitumor effect of the Bulgarian medicinal plant Tribulus terrestris L. on human cancer cell lines is not studied. The main active compounds of the plant are the steroid saponins. The present study aims to analyze the effect on cell viability and apoptotic activity of total extract and saponin fraction of Bulgarian Tribulus terrestris L. On human breast cancer (MCF7) and normal (MCF10A) cell lines. Antitumor effect was established by MTT cell viability assay and assessment of apoptotic potential was done through analysis of genomic integrity (DNA fragmentation assay) and analysis of morphological cell changes (Fluorescence microscopy). The results showed that total extract of the herb has a marked dose-dependent inhibitory effect on viability of MCF7 cells (half maximal inhibitory concentration is 15 μg/ml). Cell viability of MCF10A was moderately decreased without visible dose-dependent effect. The
saponin fraction has increased inhibitory effect on breast cancer cells compared to total extract. Morphological changes and DNA fragmentation were observed as markers for early and late apoptosis predominantly in tumor cells after treatment. Apoptotic processes were intensified with the increase of treatment duration. The obtained results are the first showing selective antitumor activity of Bulgarian *Tribulus terrestris* L. on human cancer cells *in vitro*. Apoptotic processes are involved in the antitumor mechanisms induced by the herb. This results give directions for future investigations concerning detailed assessment of its pharmacological potentia.


ABSTRACT: Melatonin is a potent antioxidant which showed anticonvulsant activities both in experimental and clinical studies. In the present study, we examined the effect of melatonin treatment (10 mg/kg/day, diluted in drinking water, 8 weeks) during epileptogenesis on the consequences of a kainate (KA)-induced status epilepticus (SE) in rats. Melatonin increased the latency in the appearance of spontaneous recurrent seizures (SRSs) and decreased their frequency only during the treatment period. The behavioral alterations associated with hyperactivity, depression-like behavior during the light phase, and deficits in hippocampus-dependent working memory were positively affected by melatonin treatment in rats with epilepsy. Melatonin reduced the neuronal damage in the CA1 area of the hippocampus and piriform cortex and recovered the decrease of hippocampal serotonin (5-HT) level in rats with epilepsy. Taken together, long-term melatonin treatment after SE was unable to suppress the development of epileptogenesis. However, it showed a potential in reducing some of the deleterious alterations that develop during the chronic epileptic state in a diurnal phase-dependent mode.


ABSTRACT: The development of red solid-state fluorochromes is important for different applications. The influence of the electronic effects of substituents on the chemical shifts in the 1H NMR spectra and solid-state fluorescent properties of aryl hydrazones of 4-hydrazino-N-hexyl-1,8-naphthalimide is evaluated. The main fragmentation pathway of hydrazones is determined using electrospray ionization mass spectrometry and high resolution MS/MS. A possible application of these fluorochromes for the in situ imaging of enzyme activities is presented.
9) Broshtilova, V., Lozanov, V., Miteva, L. Comparative analysis of polyamine metabolism in benign and neoplastic keratinocytic proliferations (2012) Acta Dermatovenerologica Alpina, Pannonica et Adriatica, 21 (1), pp. 3-5; IF=0.16

ABSTRACT: Introduction: Polyamines (putrescine, spermidine, and spermine) are polycationic compounds that play a central role in keratinocytic proliferation, differentiation, and regulation. The objective was to elucidate the polyamine metabolic changes that occur in various benign and neoplastic skin proliferations. Methods: The study included 58 patients: 31 with the plaque form of psoriasis vulgaris and 27 with non-melanoma skin tumors. The levels of putrescine, spermidine, and spermine were detected in lesional and non-lesional skin samples. Results: Findings were representative (p < 0.05). Psoriatic lesions showed a twofold elevation of all polyamines in lesional skin compared to non-lesional skin. Spermine had the highest concentration, which suggested a leading position of propylamine synthesis in psoriatic pathogenesis. Results on the polyamine metabolism of basal cell carcinoma represented basic characteristics similar to those of psoriasis. Conversely, squamous-cell carcinoma lesions showed the highest concentration of putrescine, suggesting a crucial role of spermidine-spermine acetyltransferase in their pathogenesis. Discussion: Our findings showed different polyamine metabolic changes in lesions from benign and neoplastic keratinocytic proliferations. Basal-cell carcinoma polyamine metabolism revealed a closer relationship to psoriasis than to squamous-cell carcinoma, which might explain its long-term benign course and non-metastatic nature.


ABSTRACT: In the present paper, we present our results from studies where the electrode solutions were separated from the carrier with the polytetrafluoroethylene-based membranes (Nafion®). We achieved a 40-fold decrease of the average ionic strength in the gel and a twofold lowering of the current already during the first 30 min from the start of IEF, as compared to the routinely employed method. The change of these parameters made it possible to carry out the electrophoresis under conditions considerably closer to steady state, and to achieve a sharp protein separation and shortening of the duration of the process. The comparative analysis of the electrophoretic parameters in question proved that the basis for this newly developed improvement of the method is the selective restriction of the processes of migration and diffusion in the whole electrophoretic system, due to the specific properties of the semi-permeable membrane Nafion®.


ABSTRACT: The purpose of this study was to explore whether the kainate (KA) model of temporal lobe epilepsy (TLE) can be used as a model of comorbid epilepsy and depression to study diurnal behavioral variations in rats. Development of chronic epilepsy was confirmed by the detection of spontaneous motor seizures (SMS) with video monitoring (24 hours/3-5 months after status epilepticus [SE]). KA-treated spontaneously hypertensive rats (SHRs) exhibited
higher seizure frequency than Wistar rats during the light phase in the fourth and fifth months after SE. Although epileptic Wistar rats showed depression-like behavior and reduced anxiety mostly during the light phase, there were no diurnal variations in depression-like patterns in SHR. Anxiety levels of control and epileptic SHR were similar. Decreases in serotonin, tryptophan, and dopamine concentrations in the hippocampus were detected in epileptic Wistar rats compared with naive controls. However, monoamine levels of epileptic SHR were close to those of their controls. Wistar rats and SHR develop stable depression-like behavior during the chronic epileptic phase with strain-dependent diurnal differences.


ABSTRACT: While studying the effect of electrochemical reactions occurring at the electrodes on achievement of steady state in isoelectric focusing (IEF) we observed an abnormal increase of the current. Because the magnitude of the current determines the progress of IEF, knowledge gained from studies of its nature and generation may enable this effect to be controlled. We observed that addition of gelatin to the electrode solutions suppresses the magnitude of the current flowing through the system; this enables IEF to be performed under conditions closer to steady state, and steady state is achieved more quickly.


ABSTRACT: The phosphodiesterase (PDE) enzymes catalyze the breakdown of 3’5’-cyclic monophosphate (cAMP) and guanosine 3’5’-cyclic monophosphate (cGMP) and thus are important regulators of cell function. Among these isoenzymes the cAMP-specific PDE4 has been shown to be the predominant form of PDE expressed in immune and inflammatory cells. Mast cells appear to play an important role in the pathogenesis of airway inflammation as they release many mediators such as histamine. We were interested to investigate the production of histamine from P-815 mastocytoma cells. Pretreatment of cells with TPA (10 ng/ml) induced histamine synthesis approximately 5 times greater than the controls. Addition of rolipram (10 μM) completely abolished TPA stimulated histamine production. A potent PKA activator forskolin (10 μM) had similar effect and selective increase of PKA activity led to inhibition of TPA stimulated histamine synthesis. We also studied the efficacy of rolipram on proliferation of P-815 cells. Rolipram (10 μM) reduced more than 50% [3H] thymidine incorporation and TPA enhanced the inhibitory effect of rolipram. In contrast that effect was not observed for forskolin alone or in combination with phorbol ester. Obviously, rolipram mediated inhibition of P-815 cell proliferation via PKA independent manner. Combined effect of TPA and rolipram indicated that protein kinase C could take part in rolipram triggered cellular signals.

ABSTRACT: A series of new low molecular weight peptide inhibitors, antistasin and ghilantens fragment analogues was designed and synthesized by manual solid phase peptide synthesis. These compounds only differ either by the amino acid placed in position 109 (different basic amino acids) and 115 position (Val or Ile) or 116 position Pro (as free acid or as amide). The anticoagulant activity of the different synthesized peptide mimetics was measured. Further the IC50 was obtained by means of Activated Partial Thromboplastin Time measurement. Using Mihaelis-Menthen equation the mixed type of inhibition toward thrombin and Factor Xa is determined.


ABSTRACT: The aim of the present study was to evaluate the plasma endothelin-1 (ET-1) and total homocysteine (tHcy) levels as biochemical markers of endothelial dysfunction and atherosclerosis in patients with active and cured acromegaly in order to assess the relationship between the secretory status of growth hormone (GH)/insulin-like growth factor I (IGF-I) and ET-1/tHcy levels. The patients were divided in two subgroups: 1) patients with active disease (n = 30); and 2) patients with nonactive cured acromegaly (n = 21). Plasma ET-1 levels were directly determined by a highly sensitive enzyme immunoassay and plasma tHcy concentrations were measured by a fluorescence polarization immunoassay. In active acromegaly subjects, plasma ET-1 levels were 1.24 ± 0.2 pmol/L, significantly higher than in both nonactive acromegalics (0.39 ± 0.1 pmol/L) and age-matched healthy controls (0.49 ± 0.2 pmol/L) (P < 0.001). Plasma tHcy concentrations, however, did not differ significantly in all studied groups: nonactive acromegalics: 9.54 ± 4.42 μmol/L; active acromegalics: 9.0 ± 3.14 μmol/L; and control subjects: 9.96 ± 2.95 μmol/L (P > 0.05). In conclusion, our study demonstrated that elevated ET-1 levels probably contributed to premature atherosclerosis and cardiovascular disease and represent a new risk factor for endothelial dysfunction and early vascular complications in acromegaly. We propose that GH and IGF-I secretory status are important determinants of plasma ET-1 but not tHcy levels


ABSTRACT: The specificity of 10 recombinant caspases was investigated using a set of competitive substrates. The caspase activity was determined by high-performance liquid chromatography using highly fluorescent peptides containing 2-aminoacridone (AMAC) as reporting group. The sequences of the used substrates were designed according to literature data for being specific for 10 of the caspases. The described approach allows the concentration
changes of several substrates to be monitored simultaneously in a single sample. Because the substrates are in competitive conditions, the preferences of particular caspases to given peptide sequences are most clearly demonstrated. In the studied competitive assay conditions, all tested caspases except caspase 2 exhibit activity toward more than one substrate. None of the used peptide sequences was found to be highly specific for a defined caspase. The results obtained indicate that there is well-expressed group specificity among the caspases.


ABSTRACT: Alcohol consumption has been previously shown to correlate with elevated plasma homocysteine levels, but investigations have not been carried out on the possible availability of this compound in alcoholic beverages such as wine or spirits. Therefore, in this study we investigated the levels of homocysteine in various Bulgarian wines. A total of 36 different Bulgarian wines with known origins were studied. The measured values were in the range of 0.09-0.64 mg l-1 for the tested white wines and in the range of 0.10-1.37 mg l-1 for the red wines. The method used for homocysteine determination was based on RP-HPLC with fluorescent detection after derivatization with N-(2-acridonyl)maleimide. The method was linear in the range of 0.0070-1.35 mg l-1 homocysteine and showed low limits of detection and quantification (LOD = 6 fmol, LOQ = 68 fmol). The within-run precision expressed as relative standard deviation (RSD, %) was 2.2-2.4% and the between-run precision was 2.6-3.9%. Enzyme immunoassay and LC-MSn analyses were used for confirmation of presence of homocysteine in wine.


ABSTRACT: The process occurring as the background to IEF is electrolysis of water. During IEF the yield of water ions decreases, following a non-linear relationship similar to that of the current. Different extents of acidification and basification of the electrode solutions, causing a drift of the pH gradient, were observed. We derived a relationship underlying this process which enables calculation of the electrode current. By using a novel approach we showed that the sum of the pH of distilled water electrode solutions at the end of the IEF process tends to the ionic product of water.


ABSTRACT: Synthesis and properties of a new fluorescent/fluorogenic substrate Ac-DEVD-AMAC for caspase-3 are reported. The substrate is obtained by conventional Fmoc-based solid phase peptide synthesis and its properties are investigated with regard to fluorescence, sensitivity, applicability and kinetic constants. A non-traditional approach to assay the proteases activity using 2-aminoacridone labeled peptides is proposed. This approach utilizes the decrease of fluorescence intensity of a sample as a measure for the enzyme activity.
Benkova, B., Lozanov, V., Ivanov, I.P., Todorova, A., Milanov, I., Mitev, V. 
Determination of plasma aminothiols by high performance liquid chromatography after 
precolumn derivatization with N-(2-acridonyl)maleimide (2008) Journal of 
Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 870 (1), 
pp. 103-108; IF 2,694; цитирания 25.

ABSTRACT: Design, synthesis and properties of new derivatization reagent N-(2-acridonyl)-
maleimide (MIAC) for thiol groups is presented. The reaction of MIAC with aminothiols is 
specific, very fast and yield highly fluorescent products. The HPLC method for determination of 
homocysteine, cysteine and glutathione based on utilization of MIAC is developed. A baseline 
separation of derivatives is achieved by isocratic elution on reverse phase column within 6 min. 
The method is linear in the range of 0.5-25 μM for homocysteine and glutathione, and in the 
range of 0.5-200 μM for cysteine. The limits of detection for homocysteine, cysteine and 
glutathione are 1.2, 1.4 and 2.0 pmol, respectively, per 20 μl injection. Within and between-run 
precision expressed as relative standard deviations are in the range of 1.35-4.38% and 0.89-
4.13%, respectively.

Mateva, L., Petrov St., Lozanov, V., Elenkova, A., & Zacharieva, S., Mitev V 
Simultaneous Determination of Free Polyamines, Catecholamines and Metanephrines in 
Technologies, 31(14), 2128-2140; IF 0,638; цитирания 4.

ABSTRACT: We describe a new, rapid, and sensitive HPLC method with fluorescent detection 
for simultaneous analysis of plasma and urine free catecholamines, metanephrines, and 
polyamines without prior treatment procedures via precolumn derivatization with Fmoc-Cl. The 
separation gradient was 40 minutes. The results showed good linearity across the calibration 
range 0.05-20 μM and linearity coefficient (r2) higher than 0.990, except for epinephrine, 
r2 = 0.985. The LOQ was in range 680-1300 fmol and LOD 204-390 fmol of these analytes per 
injection 20 microlitres. The reproducibility of the method for the analyzed samples, expressed as 
R.S.D. was from 1.2 to 4.5%. Isoproterenol was used as internal standard.

Zacharieva, S., Kirilov, G., Orbetzova, M., Elenkova, A., Shigarminova, R., Lozanov, V., 
Mitev, V. Homocysteine, renin and aldosterone in patients with Cushing's syndrome 
(2008) Methods and Findings in Experimental and Clinical Pharmacology, 30 (3), pp. 221-
224; IF 1,037; цитирания 1.

ABSTRACT: In the present study, we assessed the levels of fasting homocysteine in patients with 
active Cushing's syndrome using two different assay methods. To determine a possible link 
between homocysteine and renin-angiotensin-aldosterone system (RAAS), nine patients with 
Cushing's syndrome and nine patients with metabolic syndrome were given a 1-month treatment 
with angiotensin II (All) receptor blocker valsartan. Plasma homocysteine, active renin and 
aldosterone did not differ significantly among patients with Cushing's syndrome, patients with 
metabolic syndrome and controls. As expected, active renin increased significantly during 
valsartan treatment in patients with Cushing's syndrome as well as in patients with metabolic 
syndrome. Plasma homocysteine did not change after valsartan treatment, suggesting a lack of 
direct relationship between homocysteine and RAAS. Our data suggest that homocysteine might
not serve as a reliable marker of endogenous hypercortisolism or of cardiovascular risk associated with Cushing's syndrome and metabolic syndrome.

ABSTRACT: α-Amylase inhibitor (AAI), a 32-residue miniprotein from the Mexican crop plant amaranth (Amaranthus hypochondriacus), is the smallest known α-amylase inhibitor and is specific for insect α-amylases (Chagolla-Lopez, A., Blanco-Labra, A., Patthy, A., Sanchez, R., and Pongor, S. (1994) J. Biol. Chem. 269, 23675-23680). Its disulfide topology was confirmed by Edman degradation, and its three-dimensional solution structure was determined by two-dimensional 1H NMR spectroscopy at 500 MHz. Structural constraints (consisting of 348 nuclear Overhauser effect interproton distances, 8 backbone dihedral constraints, and 9 disulfide distance constraints) were used as an input to the X-PLOR program for simulated annealing and energy minimization calculations. The final set of 10 structures had a mean pairwise root mean square deviation of 0.32 Å for the backbone atoms and 1.04 Å for all heavy atoms. The structure of AAI consists of a short triple-stranded β-sheet stabilized by three disulfide bonds, forming a typical knottin or inhibitor cystine knot fold found in miniproteins, which binds various macromolecular ligands. When the first intercystine segment of AAI (sequence IPKWNR) was inserted into a homologous position of the spider toxin Huwentoxin I, the resulting chimera showed a significant inhibitory activity, suggesting that this segment takes part in enzyme binding.

ABSTRACT: Background: α-Amylases constitute a family of enzymes that catalyze the hydrolysis of α-D-(1,4)-glucan linkages in starch and related polysaccharides. The Amaranth α-amylase inhibitor (AAI) specifically inhibits α-amylases from insects, but not from mammalian sources. AAI is the smallest proteinaceous α-amylase inhibitor described so far and has no known homologs in the sequence databases. Its mode of inhibition of α-amylases was unknown until now. Results: The crystal structure of yellow meal worm α-amylase (TMA) in complex with AAI was determined at 2.0 Å resolution. The overall fold of AAI, its three-stranded twisted β-sheet and the topology of its disulfide bonds identify it as a knottin-like protein. The inhibitor binds into the active-site groove of TMA, blocking the central four sugar-binding subsites. Residues from two AAI segments target the active-site residues of TMA. A comparison of the TMA-AAI complex with a modeled complex between porcine pancreatic α-amylase (PPA) and AAI identified six hydrogen bonds that can be formed only in the TMA-AAI complex. Conclusions: The binding of AAI to TMA presents a new inhibition mode for α-amylases. Due to its unique specificity towards insect α-amylases, AAI might represent a valuable tool for protecting crop plants from predatory insects. The close structural homology between AAI and ‘knottins’ opens new perspectives for the engineering of various novel activities onto the small scaffold of this group of proteins.

ABSTRACT: We report here the total synthesis of the α-amylase inhibitor (AAI), a 32-residue-long peptide with three disulfide bridges, isolated from amaranth seeds (Chagolla-Lopez, A., Blanco-Labra, A., Pathy, A. Sanchez, R. and Pongor S. (1994) J. Biol. Chem. 269, 23675-23680). The synthesis was carried out using a stepwise solid-phase approach based on the Fmoc/t-Bu chemistry, combined with the S-acetamidomethyl protection for cysteines. The linear, reduced peptide was obtained after two reduction steps, using 1,4-dithio-DL-threitol and tri(2-carboxyethyl)phosphine hydrochloride in basic and acidic conditions, respectively. Disulfide bridges were formed by oxidative folding in a cystine/cysteine redox buffer, these conditions were found superior to air oxidation and to glutathione-catalyzed oxidative folding. The physicochemical and enzyme inhibitory properties of synthetic AAI were found identical with those of the natural product. Several orthogonal protection schemes proved unsuccessful in obtaining a biologically active product.


ABSTRACT: An enzymic synthesis of aspartame (H-Asp-Phe-OMe) has been designed and realized based on the structure-activity study of thermolysin and penicillin amidase hydrolysis of its p-substituted phenylacetyl derivatives. These compounds meet the structural and energetic requirements of two enzymic binding sites. The peptide sweetener has been prepared by thermolysin-catalyzed condensation of the p-substituted phenylacetyl-Asp-OH and H-Phe-OMe followed by penicillin amidase-catalyzed deprotection of the resulted aspartame precursors.