Birth Defects Res A Clin Mol Teratol. 2004 Aug;70(8):519-27.

Activation of the hexosamine pathway causes oxidative stress and abnormal embryo gene expression: involvement in diabetic teratogenesis.

Horal M, Zhang Z, Stanton R, Virkamäki A, Loeken MR.

Section on Developmental and Stem Cell Biology, Joslin Diabetes Center, Boston, Massachusetts 02215, USA.

Abstract

BACKGROUND: Oxidative stress is critical to the teratogenic effects of diabetic pregnancy, yet the specific biochemical pathways responsible for oxidative stress have not been fully elucidated. The hexosamine pathway is activated in many tissues during diabetes and could contribute to oxidative stress by inhibiting the pentose shunt pathway, thereby diminishing production of the cellular antioxidant, reduced glutathione (GSH).

METHODS: To test the hypothesis that activation of the hexosamine pathway might contribute to the teratogenic effects of diabetic pregnancy, pregnant mice were injected with glucose, to induce hyperglycemia, or glucosamine, to directly activate the hexosamine pathway. Embryo tissue fragments were also cultured in physiological glucose, high glucose, or physiological glucose plus glucosamine, to test effects on oxidative stress and embryo gene expression.

RESULTS: Glucosamine increased hexosamine synthesis and inhibited pentose shunt activity. There was a trend for transient hyperglycemia to have the same effects, but they did not reach statistical significance. However, both glucose and glucosamine significantly decreased GSH, and increased oxidative stress, as indicated by 2',7'-dichloro-dihydrofluorescein fluorescence. Glucose and glucosamine inhibited expression of Pax-3, a gene required for neural tube closure both in vivo and in vitro, and increased neural tube defects (NTDs) in vivo; these effects were prevented by GSH ethyl ester. High glucose and glucosamine inhibited Pax-3 expression by embryo culture, but culture in glutamine-free media to block the hexosamine pathway prevented the inhibition of Pax-3 expression by high glucose.

CONCLUSIONS: Activation of the hexosamine pathway causes oxidative stress through depletion of GSH and consequent disruption of embryo gene expression. Activation of this pathway may contribute to diabetic teratogenesis.

PMID: 15329829 [PubMed - indexed for MEDLINE]

Biochim Biophys Acta. 2004 Jul 6;1673(1-2):13-28.

O-GlcNAc a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress.

Zachara NE, Hart GW.

Department of Biological Chemistry, The Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205-2185, USA.

Abstract

Myriad nuclear and cytoplasmic proteins in metazoans are modified on Ser and Thr residues by the monosaccharide O-linked beta-N-acetylglucosamine (O-GlcNAc). The rapid and dynamic change in O-GlcNAc levels in response to extracellular stimuli, morphogens, the cell cycle and development suggests a key role for O-GlcNAc in signal transduction pathways. Modulation of O-GlcNAc levels has profound effects on the functioning of cells, in part mediated through a complex interplay between O-GlcNAc and O-phosphate. In many well-studied proteins, the O-GlcNAc modification and phosphorylation are reciprocal. That is, they occur on different subsets of the protein population, as the site of attachment occurs on the same or adjacent Ser/Thr residues. Recently, O-GlcNAc has been implicated in the etiology of type II diabetes, the regulation of stress response pathways, and in the regulation of the proteasome.

PMID: 15238246 [PubMed - indexed for MEDLINE]

Invest Ophthalmol Vis Sci. 2003 Sep;44(9):3802-9.

Elevated expression of O-GlcNAc-modified proteins and O-GlcNAc transferase in corneas of diabetic Goto-Kakizaki rats.

Akimoto Y, Kawakami H, Yamamoto K, Munetomo E, Hida T, Hirano H.

Department of Anatomy, Kyorin University School of Medicine, Mitaka, Tokyo, Japan. yakimoto@kyorin-u.ac.jp

Abstract

PURPOSE: The hexosamine biosynthetic pathway is one of the possible mechanisms involved in diabetic keratopathy. The purpose of this study was to examine the role of O-glycoside-linked N-acetylglucosamine (O-GlcNAc) modification of proteins in the pathogenesis of diabetic keratopathy in the Goto-Kakizaki (GK) rat, which has spontaneous development of diabetes.

METHODS: An anti-O-GlcNAc antibody, an anti-O-GlcNAc transferase antibody, and digoxigenin (DIG)-labeled cRNA probes were used to examine the localization of O-GlcNAc-modified proteins, O-GlcNAc transferase protein and mRNA in the corneas of diabetic GK rats and in those of nondiabetic Wistar rats. The corneas from Wistar rats were organ cultured in control medium or in medium containing 100 micro M O-(2-acetamide-2-deoxy-D-glucopyranosylidene) amino-N-phenyl-carbamate (PUGNAc), an inhibitor of O-GlcNAcase, the enzyme that removes O-GlcNAc from proteins. The morphologic changes were examined by electron microscopy.

RESULTS: In normal corneas, immunoreactive O-GlcNAc and O-GlcNAc transferase were observed in the epithelial, endothelial, and stromal cells. In the diabetic corneas, their immunoreactivities in the epithelium were increased in intensity. Morphologically, the number of hemidesmosomes in the epithelial basal cells was lower than that in those cells from the nondiabetic rats. In some areas, the basement membrane had detached from the epithelial basal cells. The PUGNAc treatment of Wistar rat corneas increased the level of O-GlcNAc immunoreactivity and caused a decrease in the number of hemidesmosomes and the detachment of corneal epithelial cells from the basement membrane.

CONCLUSIONS: The elevated expression of O-GlcNAc-modified proteins and O-GlcNAc transferase may play a causative role in the corneal epithelial disorders of diabetic GK rats.

PMID: 12939295 [PubMed - indexed for MEDLINE]

Curr Opin Struct Biol. 2003 Oct;13(5):631-6.

Dynamic interplay between O-GlcNAc and O-phosphate: the sweet side of protein regulation.

Slawson C, Hart GW.

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205-2185, USA.

Abstract

Beta-O-linked N-acetylglucosamine (O-GlcNAc) is an abundant modification of cytosolic and nuclear proteins that occurs in metazoans. O-GlcNAc is dynamically processed by a unique set of enzymes that actively add and remove the modification. Functionally, O-GlcNAc appears to regulate protein stability, subcellular localization and protein-protein interactions. The modification often acts in a reciprocal manner to O-phosphate modifications of proteins and together they can synergistically control the activity of many cellular processes. Recently, O-GlcNAc has been demonstrated to play a significant role in diseases such as diabetes, cancer and neurodegeneration. For example, the increased levels of O-GlcNAc that occur in diabetes are associated with decreased insulin responsiveness in adipocytes.

PMID: 14568619 [PubMed - indexed for MEDLINE]

Acta Biochim Pol. 2002;49(1):77-86.

Facilitated diffusion of glucosamine-6-phosphate synthase inhibitors enhances their antifungal activity.

Janiak A, Cybulska B, Szlinder-Richert J, Borowski E, Milewski S.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Poland.

Abstract

N3-(4-Methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) and 2-amino-2-deoxy-D-glucitol-6-phosphate (ADGP) are strong inhibitors of the essential fungal enzyme, glucosamine-6-phosphate synthase, but their antifungal activity is poor, due to slow penetration of these agents through the cytoplasmic membrane. In the present studies we have exploited the possibility of enhancement of ADGP and FMDP antifungal activity by improving their transport properties. It has been found that membrane-permeabilising polyene macrolides amphotericin B (AMB) and its N-methyl-N-fructosyl methyl ester derivative (MF-AME), at subinhibitory concentrations, facilitate diffusion of ADGP through the fungal cell membrane, thus allowing a decrease of its minimal inhibitory concentration (MIC). Synergistic effects have been observed for combinations of ADGP with AMB or MF-AME. Fractional inhibitory concentration (FIC) indexes, determined against a number of Candida spp., have been in the 0.18-0.81 range. Weak antifungal synergistic effects have been found for combinations of FMDP with AMB or MF-AME. ADGP can be easily encapsulated into unilamellar lipid vesicles. Liposomal preparations of ADGP demonstrated stronger antifungal activity against some fungal strains than free ADGP.

PMID: 12136959 [PubMed - indexed for MEDLINE]Free Article

Microbiology. 2001 Jul;147(Pt 7):1955-9.

A diffusible analogue of N(3)-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid with antifungal activity.

Zgódka D, Milewski S, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, 11/12 Narutowicza St, 80-952 Gdansk, Poland.

Abstract

N(3)-(4-Methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), a specific and potent inactivator of glucosamine-6-phosphate (GlcN-6-P) synthase from Candida albicans, exhibits relatively poor anticandidal activity, with an MIC value amounting to 50 microg ml(-1) (200 microM). Uptake of FMDP into C. albicans cells follows saturation kinetics and is sensitive to the action of metabolic inhibitors, thus indicating the active transport mechanism. However, the acetoxymethyl ester of FMDP penetrates the fungal cell membrane by free diffusion and is rapidly hydrolysed by C. albicans cytoplasmic enzymes to release the free FMDP. This mechanism gives rise to continuous accumulation of the enzyme inhibitor and results in higher antifungal activity of the FMDP ester (MIC=3.1 microg ml(-1), 10 microM). These results show that the 'pro-drug' approach can be successfully applied for the enhancement of antifungal activity of glutamine analogues that inhibit GlcN-6-P synthase.

PMID: 11429472 [PubMed - indexed for MEDLINE]

Antimicrob Agents Chemother. 2001 Jan;45(1):223-8.

Unusual susceptibility of a multidrug-resistant yeast strain to peptidic antifungals.

Milewski S, Mignini F, Prasad R, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Gdańsk, Poland. milewski@altis.chem.pg.gda.pl

Abstract

The susceptibility of Saccharomyces cerevisiae JG436 multidrug transporter deletion mutant, Deltapdr5, to several antifungal agents was compared to that of JG436-derived JGCDR1 and JGCaMDR1 transformants, harboring the CDR1 and CaMDR1 genes, encoding the main drug-extruding membrane proteins of Candida albicans. The JGCDR1 and JGCaMDR1 yeasts demonstrated markedly diminished susceptibility to the azole antifungals, terbinafine and cycloheximide, while that to amphotericin B was unchanged. Surprisingly, JGCDR1 but not JGCaMDR1 cells showed enhanced susceptibility to peptidic antifungals, rationally designed compounds containing inhibitors of glucosamine-6-phosphate synthase. It was found that these antifungal oligopeptides, as well as model oligopeptides built of proteinogenic amino acids, were not effluxed from JGCDR1 cells. Moreover, they were taken up by these cells at rates two to three times higher than by JG436. The tested oligopeptides were rapidly cleaved to constitutive amino acids by cytoplasmic peptidases. Studies on the mechanism of the observed phenomenon suggested that an additive proton motive force generated by Cdr1p stimulated uptake of oligopeptides into JGCDR1 cells, thus giving rise to the higher antifungal activity of FMDP [N(3)-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid]-peptides.

PMID: 11120970 [PubMed - indexed for MEDLINE]

Bioorg Med Chem. 2001 Apr;9(4):931-8.

Amide and ester derivatives of N3-(4-methoxyfumaroyl)-(S)-2,3-diaminopropanoic acid: the selective inhibitor of glucosamine-6-phosphate synthase.

Zgódka D, Jedrzejczak R, Milewski S, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdañsk, Poland. dzgodka@altis.chem.pg.gda.pl

Abstract

Several amide and ester derivatives of a glutamine analogue, N3-(4-methoxyfumaroyl)-(S)-2,3-diaminopropanoic acid (FMDP) (1-8), were synthesized and evaluated for the inhibitory activity in regard to glucosamine-6-phosphate synthase from Candida albicans. The syntheses were accomplished by the reaction of N2-tert-butoxycarbonyl-N3-(4-methoxyfumaroyl)-(S)-2,3-diaminopropanoic acid (BocFMDP) with the corresponding amines to give the FMDP amides (1-4) or with alkyl halides to give corresponding esters of FMDP (5-8). Among the synthesized compounds, the acetoxymethyl ester of FMDP was the most active inhibitor of the enzyme. Its IC50 value compared to that of FMDP (4 microM) was equal to 11.5 microM. The methyl and allyl esters and the N-hexyl-N-methyl-amide of FMDP exhibited a moderate enzyme inhibitory activity.

PMID: 11354676 [PubMed - indexed for MEDLINE]

J Enzyme Inhib. 2000;15(5):429-41.

N3-oxoacyl derivatives of L-2,3-diaminopropanoic acid and their peptides; novel inhibitors of glucosamine-6-phosphate synthase.

Andruszkiewicz R, Jedrzejczak R, Zieniawa T, Wojciechowski M, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Poland.

Abstract

Novel inhibitors 1-4 of glucosamine-6-phosphate synthase from Candida albicans have been designed based on acylation of the N3 amino group of L-2,3-diaminopropanoic acid with the corresponding ketoacids. These inhibitors have been shown to alkylate the fungal enzyme in a time-dependent manner. Compound 3 containing trans-beta-benzoyl acrylic acid as an acyl residue was found to be the most potent inhibitor in the series. Dipeptides composed of the active inhibitors and norvaline demonstrated potent antifungal activity against selected strains of Candida spp. and Saccharomyces cerevisiae. Their activity was reversed upon addition of N-acetylglucosamine to the medium.

PMID: 11030083 [PubMed - indexed for MEDLINE]

Med Mycol. 1998 Jun;36(3):177-80.

Antihistoplasmal in vitro and in vivo effect of Lys-Nva-FMDP.

Milewski S, Mignini F, Micossi L, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Poland.milewski@altis.chem.pg.gda.pl

Abstract

The new synthetic antifungal agent, L-Lysyl-L-Norvalyl-FMDP, inhibits growth of the yeast form of Histoplasma capsulatum. The compound is transported into the fungal cells by peptide permeases, cleaved intracellularly to constitutive amino acids, and the released C-terminal amino acid inhibits glucosamine-6-phosphate synthase. Promising antihistoplasmal in vivo activity of the FMDP-peptide was observed in an organ load test in mice.

PMID: 9776831 [PubMed - indexed for MEDLINE]

Med Mycol. 1998 Jun;36(3):177-80.

Antihistoplasmal in vitro and in vivo effect of Lys-Nva-FMDP.

Milewski S, Mignini F, Micossi L, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Poland.milewski@altis.chem.pg.gda.pl

Abstract

The new synthetic antifungal agent, L-Lysyl-L-Norvalyl-FMDP, inhibits growth of the yeast form of Histoplasma capsulatum. The compound is transported into the fungal cells by peptide permeases, cleaved intracellularly to constitutive amino acids, and the released C-terminal amino acid inhibits glucosamine-6-phosphate synthase. Promising antihistoplasmal in vivo activity of the FMDP-peptide was observed in an organ load test in mice.

PMID: 9776831 [PubMed - indexed for MEDLINE]

Metabolism. 1998 May;47(5):573-7.

Glucosamine infusion in rats mimics the beta-cell dysfunction of non-insulin-dependent diabetes mellitus.

Shankar RR, Zhu JS, Baron AD.

Department of Pediatrics, Indiana University School of Medicine, Indianapolis, USA.

Abstract

Sustained hyperglycemia can cause peripheral insulin resistance and pancreatic beta-cell dysfunction and has been termed glucose toxicity or glucose-induced desensitization. Glucosamine, a product of glucose flux through the hexosamine biosynthetic pathway (HBP), causes insulin resistance in peripheral tissues and has been shown to cause abnormal glucose-insulin secretion coupling, and thus has been implicated in the pathogenesis of glucose toxicity. Here, we investigate whether glucosamine-induced insulin secretory dysfunction is specific to glucose or also extends to nonglucose secretagogues such as arginine. Two groups of 12 weight-matched Sprague-Dawley rats underwent hyperglycemic clamp studies (steady-state blood glucose, approximately 220 mg x dL(-1)) during infusion of normal saline or glucosamine 3.5 mg x kg(-1) x min(-1) over a 100-minute period. Insulin levels were measured at baseline and between 90 and 100 minutes. One hundred minutes into the hyperglycemic clamp, subgroups of seven rats each (saline- and glucosamine-infused rats) received a bolus of arginine (100 mg x kg(-1)) while the glucose infusion rate was unaltered. Glucose and insulin levels were measured at 1, 3, 5, 10, 15, and 30 minutes after the arginine bolus. Both groups had similar fasting glucose and insulin levels. At steady state (60 to 100 minutes), glucose levels were almost identical in both groups (223.58+/-3.94 v 224.58+/-4.34 mg x dL(-1)), but the glucose infusion rate (26.55+/-1.60 v 8.83+/-1.35 mg x kg(-1) x min(-1), P < .0001) and insulin level (41.36+/-6.47 v 18.04+/-2.95 mU x mL(-1), P < .0001) were markedly reduced in animals receiving glucosamine. Peak insulin levels 1 minute after the arginine bolus were lower in rats infused with glucosamine versus saline (274.00+/-30.38 v 176.25+/-20.12 microU x ml(-1), P=.0319). Total insulin secretion in response to arginine was significantly lower in the glucosamine group as determined by the area under the curve (1,268.09+/-142.27 v 706.77+/-84.79 microU x mL(-1) x min, P=.0054). In conclusion, glucosamine causes severe impairment in glucose-induced insulin secretion. Further, glucosamine-induced beta-cell secretory dysfunction extends to nonglycemic stimuli like arginine. This pattern of insulin secretory dysfunction is similar to that observed in patients with non-insulin-dependent diabetes mellitus (NIDDM).

These data suggest that glucosamine may participate in the pathogenesis of glucose toxicity at the level of the beta cell in NIDDM patients.

PMID: 9591749 [PubMed - indexed for MEDLINE]

Microbiology. 1998 May;144 (Pt 5):1349-58.

Antibacterial action of dipeptides containing an inhibitor of glucosamine-6-phosphate isomerase.

Chmara H, Milewski S, Andruszkiewicz R, Mignini F, Borowski E.

Department of Pharmaceutical Technology & Biochemistry, Technical University of Gdańsk, Poland.

Abstract

Several dipeptides, containing the N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) moiety linked to protein and non-protein amino acids, exhibited a strong growth-inhibitory and bactericidal effect against Bacillus subtilis. FMDP-dipeptides were efficiently transported into bacterial cells by a di-tripeptide permease and subsequently cleaved by intracellular Mn2+/Co2+-dependent peptidases. Cleavage rates [0.1-5.6 micromol min-1 (mg protein)-1] were about two orders of magnitude lower than transport rates [40-200 micromol min-1 (mg dry wt)-1]. The released FMDP inactivated glucosamine-6-phosphate (GlcN-6-P) isomerase, an enzyme catalysing the first committed step in a biosynthetic pathway leading to amino sugar-nucleotide precursors of bacterial peptidoglycan. Inhibition of GlcN-6-P isomerase precluded peptidoglycan biosynthesis and resulted in a strong bacteriolytic effect. Results of the studies on consequences of GlcN-6-P isomerase inhibition upon the action of FMDP-dipeptides provided evidence demonstrating that the lack of endogenous GlcN-6-P could be a reason for the triggering of bacterial autolysis. Peptides containing the inhibitors of GlcN-6-P isomerase are one of the very few antimicrobial agents known that exhibit both bactericidal and fungicidal effects.

PMID: 9660640 [PubMed - indexed for MEDLINE]

J Enzyme Inhib. 1996;10(1):17-26.

Constrained search of conformational hyperspace of inactivators of glucosamine-6-phosphate synthase.

Wojciechowski M, Mazerski J, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Poland.

Abstract

Glucosamine-6-phosphate (GlcN-6-P) synthase (EC 2.6.1.16) is a key enzyme in amino sugar metabolism in micro-organisms and its selective and irreversible inhibitors can become valuable antifungal drugs. We performed a constrained search of the conformational hyperspace of glutamine and of the set of specific inactivators of the enzyme, as well as of some non-specific inhibitors of many cysteine containing enzymes. From these calculations we obtained spatial relationships of functional groups, the presence and specific orientation of which in the active site of the enzyme is important for effective and selective action of the inhibitor. Subsequent quantum chemical calculations confirmed the correctness of the pharmacophore conformation we obtained. Pharmacophore conformation of FMDP molecule, the most potent inhibitor in the selective inhibitors group, is placed close to the energy minimum on the conformational energy map.

PMID: 8835927 [PubMed - indexed for MEDLINE]

J Med Vet Mycol. 1994;32(1):1-11.

Specific inhibition of acid proteinase secretion in Candida albicans by Lys-Nva-FMDP.

Milewski S, Mignini F, Covelli I, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, Poland.

Abstract

Secretion of aspartic (acid) proteinase by Candida albicans is inhibited by the action of a new anticandidal agent, L-lysyl-L-norvalyl-[N3-(4-methoxyfumaroyl)]-L-2,3-diamino pro panoic acid (Lys-Nva-FMDP), at low, even sub-minimum inhibitory concentrations. The observed phenomenon is a direct consequence of inhibition of the enzyme, glucosamine-6-phosphate synthase. As a result of this inhibition, biosynthesis of candidal mannoproteins is markedly reduced. A possible correlation between general inhibition of mannoprotein biosynthesis and acid proteinase secretion is suggested. The reported inhibition of acid proteinase secretion by Lys-Nva-FMDP is more specific than the previously described effects of methyl patricin, 5-fluorocytosine and fenticonazole.

PMID: 8207618 [PubMed - indexed for MEDLINE]

J Chemother. 1992 Apr;4(2):88-94.

The influence of serum proteins on biological activity of anticandidal peptides containing N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid.

Kasprzak L, Milewski S, Gumieniak J, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, Poland.

Abstract

The binding of several anticandidal peptides containing N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) to serum proteins was studied using equilibrium dialysis. The affinity of these FMDP-peptides for serum albumin was low and well correlated with their biological activity against Candica albicans ATCC 26278 in serum albumin solution. This binding did not affect the biological activity of FMDP-peptides. On the other hand, substantial raising of MIC values was observed when anticandidal activity of FMDP peptides was assayed in the presence of complete serum proteins. This effect was likely to be a result of interaction with non-albumin components of serum proteins. Preliminary evidence points to the possibility of non-specific interaction with components containing sulfhydryl groups. In this study Nva-FMDP-Nva peptide was shown to be the most active compound in the serum protein solution. Moreover Nva-FMDP-Nva was most resistant to inactivation by serum components in comparison to other FMDP-peptides.

PMID: 1629751 [PubMed - indexed for MEDLINE]

Eur Biophys J. 1992;21(4):273-80.

Investigation of the inhibition pathway of glucosamine synthase by N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid by semiempirical quantum mechanical and molecular mechanics methods.

Tarnowska M, Oldziej S, Liwo A, Grzonka Z, Borowski E.

Department of Chemistry, University of Gdańsk, Poland.

Abstract

Glucosamine synthase (E.C. 2.6.1.16) is a promising target in antifungal drug design. It has been reported that its potent inhibitor, N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), inactivates the enzyme by the Michael addition of the S-H group to the FMDP molecule followed by cyclisation reactions. In this study we have investigated, by means of semiempirical MNDO, PM3 and molecular mechanics methods, the energetics and kinetic possibility of the formation of various stereoisomers of the products of cyclisation of the Michael addition products detected experimentally. It was found that the substituted 1,4-thiazin-3-one can be formed in one step under alkaline conditions; the stereoisomers of this compound predicted to be the most stable on the basis of theoretical calculations are also the dominant ones in reality.

PMID: 1425480 [PubMed - indexed for MEDLINE]

Biochim Biophys Acta. 1992 Jan 23;1115(3):225-9.

N3-haloacetyl derivatives of L-2,3-diaminopropanoic acid: novel inactivators of glucosamine-6-phosphate synthase.

Milewski S, Chmara H, Andruszkiewicz R, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Poland.

Abstract

N3-Haloacetyl derivatives of L-2,3-diaminopropanoic acid, novel glutamine analogs, were shown to be strong inhibitors of glucosamine-6-phosphate synthase from bacteria and Candida albicans. The inhibition was competitive with respect to glutamine and non-competitive with respect to D-fructose-6-phosphate. In the absence of glutamine, the tested compounds inactivated glucosamine-6-phosphate synthase from C. albicans with Kinact = 0.5 microM, 0.55 microM and 18.5 microM for bromoacetyl-, iodoacetyl- and chloroacetyl derivatives of L-2,3-diaminopropanoic acid, respectively. The inactivation obeyed the criteria for active site-directed modification.

PMID: 1739736 [PubMed - indexed for MEDLINE]

Antimicrob Agents Chemother. 1991 Jan;35(1):36-43.

Mechanism of action of anticandidal dipeptides containing inhibitors of glucosamine-6-phosphate synthase.

Milewski S, Andruszkiewicz R, Kasprzak L, Mazerski J, Mignini F, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Poland.

Abstract

The mechanism of anticandidal action of novel synthetic dipeptides containing N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) residues was shown to be consistent with the "warhead delivery" concept. FMDP dipeptides were shown to be transported into Candida albicans cells by the di-tripeptide permease and subsequently hydrolyzed by intracellular peptidases, especially aminopeptidase. The anticandidal activity of the particular FMDP dipeptide was influenced by the rate of its transport and, to a lower extent, by the intracellular cleavage rate. A high transport rate accompanied by a high cleavage rate resulted in the high anticandidal activity of L-norvalyl-FMDP. The strong growth-inhibitory effect of this compound was the consequence of inhibition of the enzyme glucosamine-6-phosphate synthase by the released FMDP. The action of L-norvalyl-FMDP on exponentially growing C. albicans cells resulted in a sharp decrease of incorporation of 14C label from [14C]glucose into chitin, mannoprotein, and glucan. This effect, as well as the growth-inhibitory effect, was fully reversed by exogenous N-acetyl-D-glucosamine. Glucosamine-6-phosphate synthase was proved to be the only essential target for FMDP dipeptides. Scanning electron microscopy of C. albicans cells treated with L-norvalyl-FMDP revealed highly distorted, wrinkled, and collapsed forms. Cells formed long, bulbous chains, and partial lysis occurred.

PMID: 1901701 [PubMed - indexed for MEDLINE]PMCID: PMC244938

J Med Chem. 1990 Oct;33(10):2755-9.

Antimicrobial properties of N3-(iodoacetyl)-L-2,3-diaminopropanoic acid-peptide conjugates.

Andruszkiewicz R, Chmara H, Milewski S, Zieniawa T, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, Poland.

Abstract

Six peptide conjugates consisting of either norvaline, methionine, or lysine and N3-(iodoacetyl)-L-2,3-diaminopropanoic acid--a strong, irreversible inactivator of bacterial and fungal glucosamine-6-phosphate synthase--were synthesized and their antibacterial and antifungal activities were evaluated. Antimicrobial potencies of these peptides were correlated with their transport and cleavage rates inside the cells. Bacteriolysis of Bacillus pumilus cells and inhibition of [14C]glucose incorporation into cell-wall polysaccharides of Candida albicans as a result of glucosamine 6-phosphate inactivation were also observed. Reversal of growth inhibitory effect of these peptides by N-acetylglucosamine in bacteria and fungi suggests the effective delivery of N3-iodoacetyl-L-2,3-diaminopropanoic acid into the cell by a peptide-transport system.

PMID: 2120441 [PubMed - indexed for MEDLINE]

J Med Chem. 1990 Jan;33(1):132-5.

Anticandidal properties of N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid oligopeptides.

Andruszkiewicz R, Milewski S, Zieniawa T, Borowski E.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, Poland.

Abstract

Tri-, tetra-, and pentapeptides containing N3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP), an inactivator of glucosamine 6-phosphate synthase of fungal origin (a key enzyme in the biosynthesis of macromolecular components of the fungal cell wall) have been synthesized and investigated as anticandidal agents. Structure-activity relationships of a series of peptides revealed that tripeptides were generally more active than the other peptides examined. In this study, the lysyl peptide, Lys-Nva-FMDP has been found to be the most active compound in the series.

PMID: 2104933 [PubMed - indexed for MEDLINE]

Biochemistry. 1990 Apr 17;29(15):3668-76.

Glucosamine-6-phosphate synthase from Escherichia coli: determination of the mechanism of inactivation by N3-fumaroyl-L-2,3-diaminopropionic derivatives.

Kucharczyk N, Denisot MA, Le Goffic F, Badet B.

Laboratoire de Bioorganique et Biotechnologies, UA CNRS 1389, ENSCP, Paris, France.

Abstract

A mechanistic investigation of the inactivation of Escherichia coli glucosamine-6-phosphate synthase by N3-(4-methoxyfumaroyl)-L-2,3-diaminopropionate (FMDP) was undertaken. On the basis of the known participation of the N-terminal cysteine residue in this process [Chmara et al. (1986) Biochim. Biophys. Acta 870, 357; Badet et al. (1988) Biochemistry 27, 2282], the model reactions between FMDP and L-cysteine and between FMDP and the synthetic decapeptide Cys-Gly-Ile-Val-Gly-Ala-Ile-Ala-Gln-Arg, corresponding to the amino-terminal protein sequence, were studied. The results allowed us to propose a pathway that is in perfect agreement with the biochemical results: enzyme inactivation arose from Michael addition of glutamine binding site cysteine-1 on the fumaroyl double bond at the beta-position of the ester group. Upon denaturation under slightly alkaline conditions, this adduct underwent cyclization to a transient succinimide adduct, which rearranged into the stable 2-substituted 1,4-thiazin-3-one-5-carboxylate involving participation of the cysteine amino group. The tryptic radiolabeled peptides purified from [3H]FMDP-treated enzyme and resistant to Edman degradation coeluted with the products resulting from the model reaction between the synthetic decapeptide and the inhibitor.

PMID: 2111163 [PubMed - indexed for MEDLINE]

Drugs Exp Clin Res. 1988;14(7):461-5.

Antifungal peptides with novel specific inhibitors of glucosamine 6-phosphate synthase.

Milewski S, Chmara H, Andruszkiewicz R, Borowski E, Zaremba M, Borowski J.

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, Poland.

Abstract

N3-4-Methoxyfumaroyl-L-2,3-diaminopropanoic acid (FMDP) has been found to be a strong and selective inhibitor of glucosamine 6-phosphate synthase from Candida albicans. Incorporation of FMDP into a dipeptide structure has produced effective antifungal agents (portage transport). A number of dipeptides containing FMDP have been synthesized, with Nva-FMDP showing the highest in vitro activity against different fungi, including Candida albicans (MIC90 = 2.2 micrograms/ml for 50 clinical strains), Cryptococcus neoformans and Aspergillus spp. This compound, when tested in a general candidiosis model infection in mice, gave PD50/10 and CD50/10 values of 5.0 and 1.63 mg/kg, respectively. Meanwhile, the LD50 value after i.v. administration was higher than 300 mg/kg.

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Synthetic derivatives of N3-fumaroyl-L-2,3-diaminopropanoic acid inactivate glucosamine synthetase from Candida albicans.

Milewski S, Chmara H, Andruszkiewicz R, Borowski E.

Abstract

Synthetic derivatives of N3-fumaroyl-L-2,3-diaminopropanoic acid constitute the novel group of glutamine analogs. They are powerful, competitive inhibitors of the glucosamine synthetase (2-amino-2-deoxy-D-glucose-6-phosphate ketol-isomerase (amino-transferring), EC 5.3.1.19) from Candida albicans with respect to glutamine and uncompetitive with respect to D-fructose 6-phosphate. Some of the compounds tested irreversibly inactivate glucosamine synthetase with Kinact values of 10(-4) to 10(-6) M. The addition of glutamine protects enzyme from the inactivation, while the absence of D-fructose 6-phosphate lowers the rate of inactivation. An ordered, sequential mechanism is suggested for binding of the inhibitors to the glutamine-binding site. A number of tested compounds act as active-site-directed, irreversible inhibitors. It is suggested that derivatives of N3-fumaroyl-L-2,3-diaminopropanoic acid should be classified as mechanism-based enzyme inactivators. Structural requirements for an effective inactivator containing N3-fumaroyl-L-2,3-diaminopropanoic acid moiety are discussed.

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