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ABSTRACTS

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number of peptidomimetics based on the N-terminal were synthesized, tested and investigated as potential lead compounds for the treatment of osteoporosis. On the basis of the shortest PTH analogue displaying nanomolar potency, the undecapeptide H-Aib-Val-Aib-Glu-Ile-Gln-Leu-Nle-His-Gln-Har-NH2 that contains two helix-stabilizing residues (Aib1,3), we present the synthetia approach and SAR analysis carried out on various positions to evaluated the effect of substitution of unnatural aminoacids [3, 4, 5] or introduction of peptide-peptoid hybrids [6], containing N-substituted glycines, or conformational constrained such as with a bridge to increase the helical character [7]. To clarify the relationship between the structure and activity, the structural data were used to generate a pharmacophoric model, obtained overlapping all the analogues [5]. This model underlines the fundamental functional role of the side chain of Val2 and, at the same time, reveals that the introduction of conformationally constrained C-tetrasubstituted -amino acids in the peptides increases their helical content, but does not necessarily ensure significant biological activity. These data are then validated by literature informations [8].

Bicyclic peptides based on dithiol amino acids

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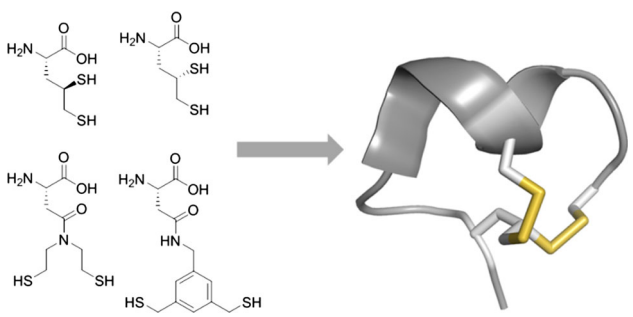
Disulfide bonds forming between pairs of cysteines are important for the structures of proteins and peptides. We have development of a novel type of amino acid, termed 'dithiol amino acid' (Dtaa) that can form two disulfide bridges at a single amino acid site. Application of these Dtaas to a serine protease inhibitor[a] and a nicotinic acetylcholine receptor inhibitor[b] that contain each two disulfide bridges improved their inhibitory activities 40- and 7.6-fold, respectively. Structural analysis revealed that the peptide ligands have retained their native structures. In addition, we show that substitution of two cysteines by Dtaas can reduce the formation of disulfide bond isomers. With these qualities, Dtaas are highly useful for the rational design or directed evolution of peptides and proteins with high activity and stability.[c]



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A role for antizyme inhibitor 2 in the biosynthesis and content of serotonin and histamine in mouse mast cells

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Polyamines (putrescine, spermidine and spermine; PAs) are essential for the majority of living cells. Antizymes and antizyme inhibitors are key regulatory proteins of PA levels by affecting ornithine decarboxylase and PA uptake. In addition to PAs, mast cells (MC) synthesize and store in their granules histamine (Hia) and serotonin (5-HT), which are critical for their function. Our previous studies have indicated a metabolic interplay among PAs, Hia and 5-HT in this cell type. For instance, we showed that PAs affect Hia synthesis during early stages of IL-3-induced bone marrow cell differentiation into bone marrow derived MCs (BMMCs) and demonstrated that PAs are present in MC secretory granules and are important for granule homeostasis, including Hia storage and 5-HT levels. A few years ago, a novel antizyme inhibitor (AZIN2) was described whose expression is restricted to a few tissues and cell types including brain, testis and MCs. In MCs, it was recently proposed that AZIN2 could act as a local regulator of PA biosynthesis in association with 5-HT-containing granules and with 5-HT release following MC activation. To gain insight into the role of AZIN2 in the biosynthesis and storage of 5-HT and also Hia, we have generated BMMCs from both wild-type and transgenic mice with severe Azin2 hypomorphism, and have analyzed the content of PAs, 5-HT and Hia, and some elements of their metabolisms. Spermine and 5-HT levels were reduced in Azin2 hypomorphic BMMCs compared with wild-type controls, whereas the amount of Hia was increased. Accordingly, the level of tryptophan hydroxylase 1 (the key enzyme for 5-HT biosynthesis) was reduced and the amount of enzymatic activity of histidine decarboxylase (the enzyme responsible for Hia biosynthesis) was increased in Azin2 hypomorphic BMMCs. Taken together, our results show evidence that AZIN2 has an important role in the regulation of 5-HT and Hia biosynthesis and storage in MCs.

Database normalization is crucial for reliable protein identification in mass spectrometry-based proteomics

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Research in proteomics is driven by mass spectrometry, especially the identification of proteins from complex samples. Computational analysis of the resulting data determines the peptide sequences of the recorded spectra and integrates identifications into proteins. For this, database search algorithms can be employed, but they need a list of amino acid sequences that are expected to exist in the sample. Many algorithms have been proposed and consensus scoring has been performed. While the comparison/integration among results from different algorithms is important, there has been no attempt to integrate the results from searching multiple databases. This is, however, important since it poses technical problems when all databases, needed for a study, are simply concatenated. Unfortunately, it has been shown that databases of different size influence scoring and prohibit the direct comparison of results.

Here we analyzed seven algorithms on databases of the same size and of increasing difference in size as well as in respect to the sequence redundancy within the databases using about twenty thousand high quality MS/MS spectra from directly infused synthetic peptides, measured with an LTQ mass spectrometer.

We performed three different data integration approaches, one which employs false discovery rate, another which collects the results from the first database search on individual database and combine results into a meta-database which is again searched. The final approach modifies the databases such that they have similar size and complexity. The last approach turned out to be the most successful and will be indispensable for future proteogenomic analyses.

Preparing sequence databases for application in proteogenomics

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Proteomics involves the identification of proteins from complex mixtures which is performed using mass spectrometry (MS) followed by computational data analysis. MS/MS spectra can either be sequenced de novo if no sequence is available for the proteins in the mixture, or by using database search algorithms such as OMSSA, X!Tandem, and MSGF+.

We identified three problems in respect to the use of database search algorithms:

1. Some of the currently used database search tools cannot utilize large databases like the non-redundant protein database or even as small databases as the human chromosome one.
2. Therefore, databases need to be run independently in smaller chunks, but results from database having different sequence redundancy cannot easily be compared.
3. This is even more complicated for different sized databases.

Here we present a methodology, overcoming these problems, providing proper integration of results from databases different in size and sequence redundancy. Large, medium and small sized six-frame translation databases, their split versions, and their split and merged versions were created and were tested on benchmark data derived from synthetic peptides measured on an LTQ mass spectrometer. Comparison of the results showed that the split-merge approach is simple and powerful and facilitates the search of arbitrarily large databases and the seamless integration of results for the eight database search tools we tested. This enables the integrative analysis of MS results using protein, EST, known and predicted gene models, as well as the six frame translation of large eukaryotic genomes and, thus facilitates proteogenomic studies.

Dietary branched-chain amino acids supplementation improves growth performance and nitrogen retention of piglets: association with increased feed intake and direct skeletal muscle growth-promoting effec

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Leucine has been shown to be as a major regulator of animal growth. However, supplementing leucine alone dramatically decreases plasma concentrations of isoleucine and valine due to antagonism among BCAA. In the present study, we studied effect of supplementing all the BCAA to a reduced-protein diet on muscle growth in piglets and the signaling pathways involved. 28 Large White × Landrace barrows were fed the positive control (PC) diet or the reduced-protein negative control diet supplemented with 0, 1 or twofold doses of each BCAA (NC, T1, and T2, respectively) ($n = 7$/group). Pigs consumed their feed ad libitum for 28 days. On day 14 of the trial, whole-body protein turnover was determined by using the end-product method after a single oral dose of 15N-glycine. All date were analyzed using the One-way ANOVA Duncan t test of SAS appropriate for a randomized complete block design. The results showed PC, T1 and T2 groups had increased ($P < 0.05$) weights of muscles particularly in the hindquarter compared with NC group. Cross-sectional areas of longissimus dorsi (LD) muscle, protein accretion and protein synthesis rates were greater ($P < 0.05$) in T1 group than NC group, but no difference ($P > 0.05$) in protein degradation was detected among four groups. Supplementation with 1- or twofold doses of each BCAA increased ($P < 0.05$) Akt, FoxO1, S6K1 and mTOR phosphorylation while decreasing ($P < 0.05$) ubiquitin-protein conjugates, atrogin-1 and MuRF1 proteins expression in muscle compare to NC group, but there were no differences ($P > 0.05$) in these proteins phosphorylation or expression between PC and NC groups except for a higher ($P < 0.05$) S6K1 phosphorylation in PC group. LC3-II to total LC3 ratio was lower ($P < 0.05$) in PC, T1, and T2 groups than in NC group. However, no differences ($P > 0.05$) were observed between T1 and T2 groups except for lower ($P < 0.05$) cross-sectional areas of LD muscle in T2 group. In conclusion, supplementing BCAA to the reduced-protein diet increases mTOR activity while decreases atrogenes expression both in the ubiquitin–proteasome and autophagy-lysosome systems probably through PI3K/Akt pathway activation, but high protein diet induces S6K1 phosphorylation through PI3K/Akt-independent pathway.

Keywords: Branched-chain amino acids; Piglets; Skeletal muscle; Protein turnover; PI3K/Akt signaling pathway

Prevalence of childhood overweight and obesity among school children (6–12 years) in Enugu state Nigeria

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