虻虫中的生物活性肽的高分辨质谱快速鉴定与生物活性预测研究

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摘要: 本研究旨在从虻虫中筛选并鉴定具有潜在生物活性的肽类分子, 解析其结构特征与功能属性, 为新型天然肽类药物的研发提供理论基础。首先使用磷酸缓冲盐溶液 (phosphate buffered saline, PBS) 提取虻虫中生物活性肽, 采用冷乙醇沉淀蛋白质并进行固相萃取纯化 (solid-phase extraction, SPE)。经LC-MS/MS获得原始质谱数据后, 利用PEAKS Studio软件进行肽段识别和序列分析。随后使用Peptide Ranker预测肽段活性潜力, 进一步结合多种生物信息学工具, 对肽段功能特性进行分析, 包括活性位点、水溶性、稳定性、毒性及肠道吸收能力等指标。最终, 结合肽段结构信息开展分子对接分析。本研究共鉴定出10 378条虻虫肽段, 长度集中于6～26个氨基酸, 大部分肽段相对分子质量在1 500 Da以下, 低于2 500 Da的肽段占97.49%。通过Peptide Ranker筛选出95条评分 ≥ 0.80的候选活性肽段, 进一步借助BIOPEP-UWM确定了59条具有神经调节作用关键残基的肽段。生物信息学预测显示, 这59条肽段中29条水溶性良好、22条稳定性较高、43条具备较好的肠道吸收潜力, 且均无毒, 为后续功能验证及活性肽开发提供了理论依据。在此基础上, Peptide Ranker评分最高的多肽FPPRDF与二肽基肽酶III (dipeptidyl peptidase III, DPP III) 进行分子对接时稳定结合, 提示其可能具有神经调节与抗肿瘤作用。本研究构建了一套系统、高效的活性肽筛选与功能预测流程, 能够从天然资源中快速识别具有药用潜力的生物活性肽。该策略为后续的功能验证、作用机制解析及新型肽类药物开发提供了技术支撑。

关键词: 虻虫; 生物活性肽; 肽段功能预测; 分子对接

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Rapid identification of bioactive peptides in *Tabanus* by high-resolution mass spectrometry and prediction of their biological activities

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Abstract: This study aimed to screen and identify bioactive peptides from *Tabanus*, characterize their

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structural and functional features, and provide a theoretical basis for the development of novel natural peptide-based therapeutics. Initially, bioactive peptides were extracted using phosphate buffered saline (PBS), followed by protein precipitation with cold ethanol and purification *via* solid-phase extraction (SPE). Peptide identification and sequence analysis were performed based on LC-MS/MS data using PEAKS Studio software. Peptide activity potential was then predicted using Peptide Ranker, and a comprehensive bioinformatics analysis was conducted to evaluate key functional characteristics, including active sites, solubility, stability, toxicity, and intestinal absorption capacity. A total of 10 378 peptide sequences were identified, with lengths predominantly ranging from 6 to 26 amino acids. Most peptides had a molecular weight below 1 500 Da, and 97.49% were below 2 500 Da. Based on Peptide Ranker score (≥ 0.80), 95 candidate bioactive peptides were selected. Among them, 59 peptides were identified as having neuroregulatory potential *via* the BIOPEP-UWM database, due to the presence of key functional residues. Bioinformatics prediction further revealed that among these 59 peptides, 29 exhibited good water solubility, 22 were predicted to be stable, 43 had favorable intestinal absorption potential, and none were toxic, highlighting their suitability for further functional studies and therapeutic development. Molecular docking analysis demonstrated that FPPRDF, the highest-ranking peptide according to Peptide Ranker, formed stable interactions with dipeptidyl peptidase III (DPP III), suggesting its potential neuroregulatory and antitumor activities. Overall, this study established a systematic and efficient workflow for the screening and functional prediction of bioactive peptides from natural sources. The proposed strategy provides robust technical support for subsequent experimental validation, mechanistic studies, and the development of novel peptide-based drugs.

Key words: *Tabanus*; bioactive peptide; peptide function prediction; molecular docking

生物活性肽 (bioactive peptides, BAPs) 是由两个至数十个氨基酸通过肽键连接而成的多肽, 分子质量通常小于6 000 Da[1]。近年来, BAPs因其结构多样、生物利用度高、毒性低及靶向性强的特点, 受到生物医药领域的广泛关注[2]。

按照功能分类, BAPs主要包括抗氧化肽、免疫调节肽、抗菌肽、神经调节肽及抗肿瘤肽等[3]。其中, 神经调节肽和抗肿瘤肽因其在调节神经递质平衡、促进神经再生、抑制肿瘤生长与转移等方面的潜力, 成为当前神经系统疾病与肿瘤治疗研究的重要方向。BAPs的吸收特性也使其在人体健康中发挥重要作用, 3～10个氨基酸残基的短肽可轻松穿过肠道上皮细胞, 具有高效吸收性[4]。此外, 与氨基酸和完整蛋白质相比, 短肽还具有低过敏性、稳定性好等特点[5]。

在中医药领域, 虫类药被认为具有活血化瘀、通经活络的功效。近年来, 其在治疗脑病 (如缺血性脑卒中)[6,7]中展现出多靶点、多途径等作用特点, 现代研究表明, 虫类药治疗缺血性脑卒中的作用机制包括抗血栓与改善微循环、神经保护、抗炎与免疫调节、促进血管新生与修复等。

虻虫为虻科昆虫复带虻 *Tabanus bivittatus* Mats或其他同属昆虫的雌性全虫[8], 其药用最早记载于《神农本草经》: “主逐瘀血, 破下血积, 坚痞症瘕”。历代医家均重视其破血祛瘀的作用, 并在《伤寒论》《金匮要略》中留下记载, 奠定了其在破血逐瘀领域的经典地位。此外,《备急千金要方》记载“肿满气逆, 腹胀瘕痛”,《本草纲目》则总结:“逐瘀血, 破血积, 坚痞瘕, 寒热, 通利血脉及九窍”。《别录》中有“主女子月水不通, 积聚, 除贼血在胸腹五脏者, 及喉痹结塞”的记载, 而《日华子本草》则写道“破症结, 消积脓, 堕胎”。现代研究显示, 虻虫在抗凝、改善微循环、神经保护等方面表现出一定的药理活性, 提示其体内天然肽段可能具有抗氧化、免疫调节以及神经保护等功能[9]。

虻虫具有多方面药效, 然而其药效物质基础尚未完全明晰。目前对虻虫成分的研究多集中于脂溶性成分和粗提物, 而其内源性多肽, 尤其是具有神经调节或抗肿瘤潜力的BAPs, 尚缺乏系统挖掘与功能预测。内源性BAPs作为体内天然存在的活性成分, 可能具有更高的稳定性和生物相容性。考虑到BAPs在神经保护、肿瘤抑制等方面的独特优势, 深入解析虻虫中的内源性多肽成分, 识别其潜在功能肽段, 对于阐明虫类药“搜剔通络”功效的分子机制具有重要意义。

材料与方法

样品 虻虫购买于中国药材公司, 由北京中医药大学石晋丽教授鉴定为虻科昆虫复带虻 *Tabanus bivittatus* Mats或其他同属昆虫的雌性全虫。

仪器 Easy-nano LC II纳流液相色谱系统、Orbitrap Fusion Lumos三合一高分辨质谱仪和Sorvall ST8R台式离心机 (美国Thermo Scientific公司), 十万分之一电子分析天平 (德国Sartorius公司), Millipore Synergy UV型超纯水机 (美国Millipore公司), BILON-FD50AE

冷冻干燥机 (上海比朗仪器制造有限公司)。

试剂 石油醚 (20230601415, 天津市致远化学试剂有限公司); 三氟乙酸 [trifluoroacetic acid, TFA, 20210220, 福晨 (天津) 化学试剂有限公司]; 磷酸缓冲盐溶液 (GA24040093224, 上海尚宝生物科技有限公司); 乙醇 (210957)、乙腈 (221156)、甲酸 (201260), 美国 Thermo Scientific 公司; 固相萃取小柱(046533212A, 德国 MilliporeSigma 公司); Nano 色谱柱 (164718, 德国 Dr. Maisch 公司); 实验用水为纳米纯水。

虻虫 BAPs 的提取 称取 1 g 干燥的虻虫, 研磨粉碎。使用石油醚按 1∶3 的比例对粉末进行三轮次脱脂处理, 每轮次 20 min, 不断搅拌直至石油醚澄清。离心弃去上清, 将脱脂的虻虫粉末风干。超声 (100 W) 提取 4 h, 前 2 h 按 1∶11 的比例向脱脂虻虫粉末中加入 40 ℃磷酸缓冲盐溶液 (phosphate buffered saline, PBS), 搅拌均匀; 后 2 h 再按 1∶11 的比例加入相同的提取溶剂。提取溶液 4 000 ×*g* 离心 25 min, 收集上清液。将上清液与二倍量乙醇混合, 并在 4 ℃下孵育 6 h 以进行蛋白质沉淀。充分沉淀后, 13 000 ×*g*、4 ℃离心 30 min, 上清液冷冻干燥, 即得样本一[10]。沉淀溶于等体积纳米纯水, 13 000 ×*g*、4 ℃离心 20 min, 重复 3 次, 合并上清液, 冷冻干燥, 即得样本二[11]。通过分步提取步骤, 以极性差异分离虻虫中的多肽组分, 有助于全面获取不同性质的活性物质, 为后续功能解析提供基础。

SPE 纯化 样品一和样品二分别与等体积的 0.2% TFA 溶液预混后, 加载到已使用 5 mL 乙腈和 5 mL 0.1% TFA 溶液预处理的 SPE 小柱中。用 6 mL 0.1% TFA 溶液洗涤 SPE 小柱以消除盐分和极性干扰, 并用 6 mL 含 0.1% TFA 的 80% 乙腈溶液洗脱 BAPs。洗脱液于 4 ℃冷冻干燥, 复溶后用于 LC-MS/MS 分析。

LC-MS/MS 分析 在 Easy-nano LC II-Orbitrap Fusion Lumos 三合一高分辨质谱上进行 LC-MS/MS 分析。样品复溶于 0.1% 甲酸, 采集 2.0 μL 样品进入配有 Nano 色谱柱 (150 mm × 0.3 mm) 的系统。色谱分离采用梯度洗脱, 流动相 A 为 0.1% 甲酸水溶液, 流动相 B 为 80% 乙腈。梯度设置: 前 2 min 内从 2% B 线性增加至 8% B, 随后在 110 min 内继续线性升至 35% B, 接着以 95% B 冲洗 8 min, 最后使用 100% A 平衡 15 min 以备下一次进样, 流速为 600 nL·min^{-1}。

质谱分析采用 NSI 离子源, 喷射电压为 2.0 kV, 毛细管加热温度为 320 ℃, 扫描模式: 一级全扫描 (Full Scan, *m*/*z* 350～1 500), 数据依赖性二级质谱扫描 (data-dependent acquisistion) ddMS^2; 分辨率: 60 000 (Full Scan), 15 000 (MS/MS); AGC 目标值: 4e6 (Full Scan), 5e4 (MS/MS); 碰撞模式: 高能量碰撞解离 (HCD), 碰撞能量: NEC 35[12-14]。

PEAKS Studio 软件分析 将质谱原始数据导入 PEAKS Studio11 软件 (加拿大 Bioinformatics Solutions Inc. 公司)。样本一和样本二的鉴定采用数据库搜索 (DB search) 与从头测序 (*de novo* sequencing) 两种方法。*De novo* 测序是一种基于 MS/MS 谱图中片段离子信息直接推断肽段氨基酸序列的方法, 不依赖已有数据库, 有助于发现蛋白质数据库中未收录的潜在新肽段[15]。

母离子质量容差设置为 10 ppm, 碎片离子质量容差设置为 0.02 Da。肽段长度限定为 6～45 个氨基酸残基, 使用分类 ID 为 485572 的 UniProt 数据库进行匹配。不考虑固定或可变的翻译后修饰。肽段谱匹配的显著性筛选标准为 −10Lg*P* ≥ 20.0, *de novo* 模型置信度阈值为 ≥ 70.00%。

肽功能预测 使用 Peptide Ranker[16] (http://distill-deep.ucd.ie/PeptideRanker/) 预测 BAPs 活性, 筛选标准为得分 ≥ 0.8。通过 BIOPEP-UWM 数据库[17] (https://www. uwm. edu. pl/biochemia/index. php/pl/biopep) 对 BAPs 功能进行注释, 鉴定具有神经活性的肽段。针对神经活性肽, 分别使用 Innovagen 在线工具[18] (https://pepcalc. com/) 评估其水溶性, 采用 Expasy 工具[19] (https://web. expasy. org/protparam/) 预测其稳定性, 不稳定指数小于 40 的肽段被认为较稳定, 而不稳定指数高于 40 的肽段稳定较差。此外, 借助 ToxinPred 平台[20] (https://webs. iiitd. edu. in/raghava/toxinpred/) 预测潜在毒性, 并通过 Admet SAR[21] (http://lmmd.ecust.edu.cn/admetsar2) 系统评估肽的人体肠道吸收 (human intestinal absorption, HIA) 特性。

分子对接 选择目标基因作为蛋白质受体, 首先通过 Uniprot[22]获取其 Entry ID, 并在蛋白质数据银行 (protein data bank, PDB) 中下载蛋白质受体的三维晶体结构。随后, 使用 Pymol 软件对蛋白质受体结构进行优化, 包括去除水分子和小分子配体。多肽的三维结构通过 RPBS 在线服务器[23]生成。接下来, 使用 AutoDockTools1.5.6 对多肽和蛋白质受体进行加氢、计算电荷处理, 并进行分子对接, 最终计算蛋白质受体–多肽的结合能。结合能越低, 表示结合效果越好, 若结合能 < 0 kJ·mol^{-1}, 说明小分子配体与蛋白质受体可自发结合, 结合能 < −21 kJ·mol^{-1} 通常被认为具有较高的结合稳定性[24]。

结果

1 虻虫 BAPs 的全局分析

1.1 总离子流图分析 虻虫 BAPs 通过 C18 柱分离,

根据多肽的溶解性依次被洗脱, 并被仪器采集后进行碎裂, 得到多肽的总离子流图。在总离子流图中, 包含了多肽的一级扫描数据和二级碎裂数据。图1A展示了样本一的总离子流图, 观察到多肽信号较为均匀, 主要集中在10～80 min; 图1B展示了样本二的总离子流图, 多肽信号集中在10～120 min。

1.2 肽段鉴定统计 经过DB search, 样本一中鉴定到6 945条肽段, 样本二中鉴定到3 316条肽段, 其中1 067条共有肽段, 即共得到9 194条肽段。经*de novo*测序, 样本一得到751条肽段, 样本二得到433条肽段。两种方法共从虻虫中鉴定到10 378条肽段。经数据库搜索鉴定到的肽段, 分子质量介于430.181 2～4 686.451 7 Da, 其中大部分肽段的分子量集中于1 500 Da以下, 分子质量在2 500 Da以下的肽段占比高达97.49%。肽段长度主要分布在6～26个氨基酸之间, 其中以7～14个氨基酸的肽段最为集中, 最长的肽段由44个氨基酸构成。

2 代表性多肽段的MS/MS碎片离子解析与序列验证

为进一步验证关键肽段序列的准确性, 选取两个代表性肽段, 分别来自DB search和*de novo*测序。通过对二级质谱中所有可检测的b离子与y离子进行系统性分析, 实现肽段一级结构的完整确认, 并从离子连续性、离子强度和匹配准确度等多个维度进行了深入分析, 这一过程确保了肽段序列的高可靠性。

2.1 肽段KTFDKDGLIDGEK (源自DB search)的碎片离子结构解析 该肽段由数据库搜索获得, MS/MS图谱中显示出清晰且连续的b离子和y离子系列, 覆盖了整个肽段的结构区域 (图2A)。N-端的b离子中, 观测到b1 (129.103 0)、b2 (230.151 5)、b4 (492.249 9)、b5 (620.349 6)、b8 (905.470 4) 和b9 (1 018.557 4), 表明从N端起始氨基酸开始到中后段均有较好的碎裂覆盖。尤其是b8和b9具有较高的信号强度, 为N端序列的确认提供了重要支持。C-端的y离子系列更为完整, 依次检测到y1 (147.113 7)、y2 (276.158 6)、y3 (333.180 8)、y4 (448.208 9)、y5 (561.293 9)、y6 (674.376 0)、y7 (731.398 9)、y8 (846.430 1)、y9 (974.520 3)、y10 (1 089.556 6)、y11 (1 236.610 7)和y12 (1 337.658 8)。其中y4至y12形成了高度连续的碎片系列, 碎片分布密集, 信号响应强烈, 充分表征了肽段C端区域的氨基酸组成。这种完整的y系列碎片不仅增强了序列匹配的可信度, 也显示了质谱数据的质量和样品的稳定性。

2.2 肽段VLSAADKGVNK (源自*de novo*测序)的碎片离子结构解析 该肽段序列由*de novo*测序结果获得, 尽管不依赖参考数据库, 但其MS/MS谱图中也展现了高度可信的碎片离子匹配 (图2B)。N-端的b离子中, 清晰检测到b2 (213.161 4)、b3 (300.170 0)、b4 (371.242 8) 以及b6 (557.299 6), 这些关键位点覆盖了N-端至中段的氨基酸信息, 有助于从*de novo*算法角度逐步构建序列骨架。C-端的y离子系列更为完整, 几乎贯穿整个肽段结构。从y1 (147.113 6) 开始, 依次检测到y3 (360.228 0)、y4 (417.249 5)、y5 (545.345 8)、y6 (660.372 3)、y7 (731.411 7)、y8 (802.450 9) 和y9 (889.480 0), 其中y2 (131.082 2) 为双电荷离子 ($[y2]^{2+}$), 是*de novo*解析中的重要辅助线索。y系列的连续性和高信噪比, 进一步支持该序列的正确性, 并体现出*de novo*测序在无参考条件下对结构信息的有效提取能力。整体而言, 该肽段的碎片离子分布密集且信号

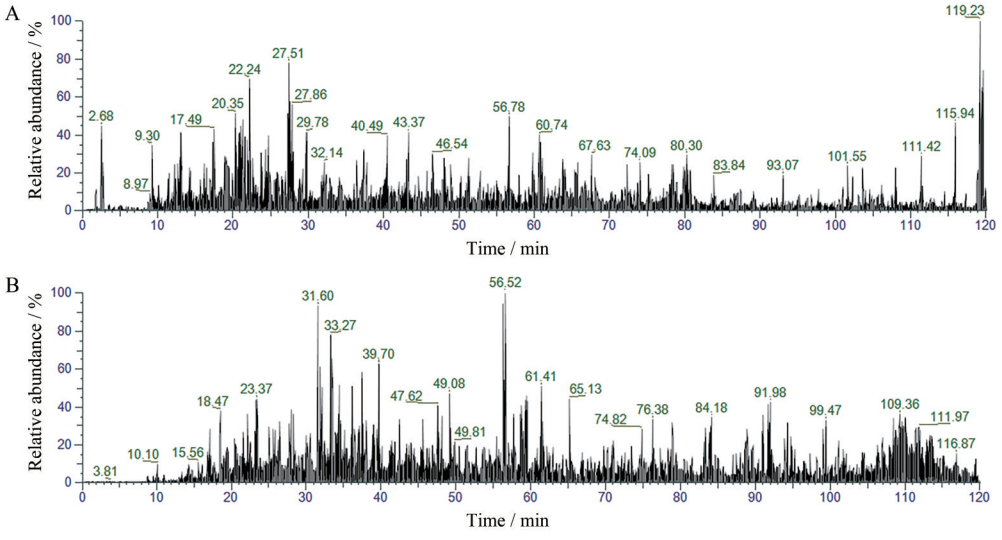


Figure 1 Total ion chromatograms of *Tabanus bivittatus* polypeptides. A: *Tabanus bivittatus* polypeptide sample 1; B: *Tabanus bivittatus* polypeptide sample 2

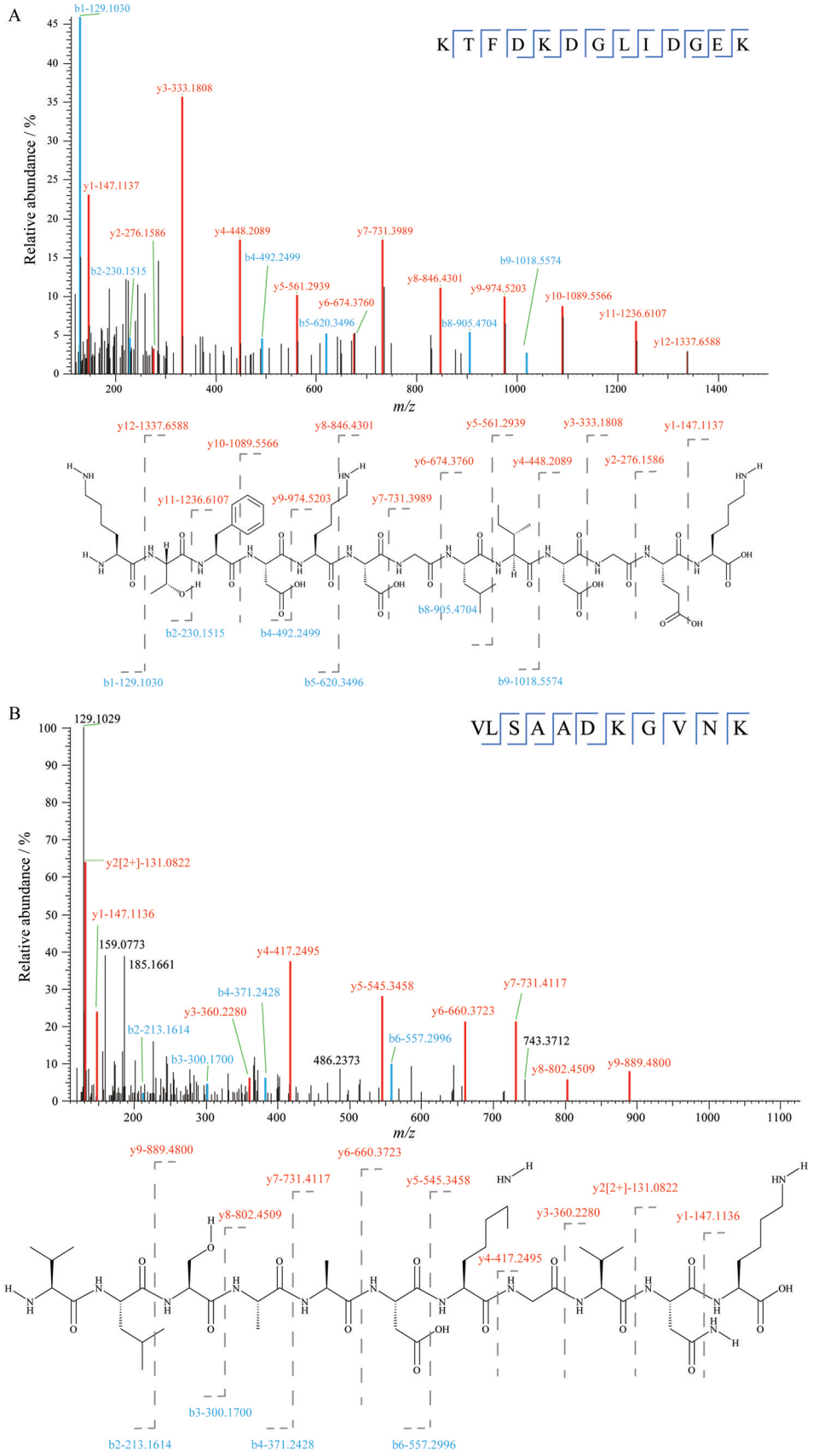


Figure 2 Peptide sequence analysis based on MS/MS spectra. A: Peptide sequence analysis from DB search results. The MS/MS spectrum shows the b-ion and y-ion fragments of the peptide sequence (KTFDKDGLLIDGEK); B: Peptide sequence analysis from *de novo* sequencing results. The MS/MS spectrum shows the b-ion and y-ion fragments of the peptide sequence (VLSAADKGVNK)

明确, 验证了 *de novo* 测序的准确性, 也印证了在数据库不可得或低覆盖情况下 *de novo* 策略的实用性与价值。

3 利用生物信息学工具预测多肽潜在的生物活性

3.1 活性肽的初步筛选与 Peptide Ranker 评分

Peptide Ranker 可根据多肽的氨基酸组成, 预测其潜在的生物活性。得分范围为 0～1, 分值越高, 表明该肽段具有生物活性的可能性越大。一般认为, 当 Peptide Ranker 评分 ≥ 0.50 时, 该肽段即具有一定的生物活性[25]; 而设置更高的评分阈值 (如 ≥ 0.80) 有助于降低假阳性率, 提高预测结果的可靠性。本研究共获得 95 条高评分肽段, 其中 74 条来源于 DB search, 21 条来源于 *de novo* 测序。这些肽段均为由 5～18 个氨基酸组成的短肽, 具有进一步开展功能分析的潜力。

3.2 神经活性肽功能位点的预测与分析

通过在线数据库 BIOPEP-UWM 预测 95 条高评分肽段的潜在生物活性位点。选择“Bioactive peptides”模块下的“ANALYSIS”功能, 使用“Profiles of potential biological activity”工具, 逐一输入 95 条肽段序列, 重点分析其是否包含与神经调节相关的生物活性氨基酸残基。结果显示, 共有 80 条肽段 (占比 84.2%) 含有神经活性氨基酸残基。其中, 59 条肽段来源于 DB search、21 条肽段来源于 *de novo* 测序。进一步分析肽段中活性位点, 高频出现的二肽位点包括 AP、GG、GP、FG、PF、GF、PR 等, 分别在所有神经活性肽段中出现 23、19、15、13、13、11 和 10 次 (图 3)。这些重复出现的短肽序列可能是神经调节功能的关键活性位点, 具有开发价值。此外, 源自 DB Search 的 59 条肽段常对应于脑啡肽酶 (enkephalinases, ENK) 抑制剂、二肽基肽酶 III 抑制剂、三肽基肽酶 II (tripeptidyl-peptidase II, TPP II) 抑制剂等酶类抑制剂 (表 1), 提示虻虫中 BAPs 可能通过抑制与神经信号调控相关的酶类, 从而在神经系统中发挥调节作用[26-28]。

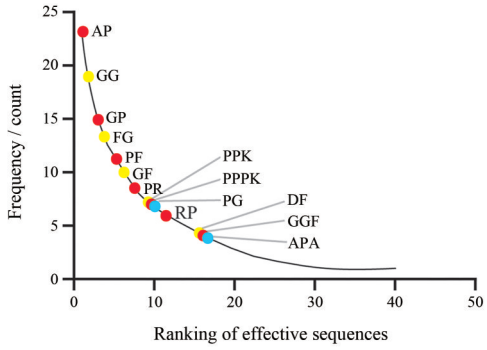


Figure 3 Frequency of neuroactive peptide functional sites identified in *Tabanus bivittatus*

Table 1 Physicochemical properties of neuroactive bioactive peptides identified in *Tabanus bivittatus*. GCP II: Glutamate carboxypeptidase II; DCP: Des-gamma-carboxy prothrombin; TPP II: Tripeptidyl-peptidase II; PPI: Peptidyl-prolyl *cis-trans* isomerase; AAP: Amino acid permease; PAM: Peptidylglycine alpha-amidating monooxygenase; PEP: Phospholipid exchang proteins; ENK: Enkephalinases; AC: Adenylate cyclase; CAAP: Caspase activity and apoptosis; Xaa-Pro AP: Xaa-Pro aminopeptidase; GCP: Glutamate carboxypeptidase

No.	Peptide sequence	Peptide rank score	Valid sequence	Bioactive peptide name	Toxicity	Solubility	Instability	ADMET	Length	Mass	Abundance rank
1	FPPRDF	0.972 134	DF	GCP II inhibitor	Non-toxic	Good	47.40	HIA−	6	777.380 9	50
			PR	DPP III inhibitor							
2	PPPPPPPPPK	0.968 593	PPK	κ-Casein-derived peptide	Non-toxic	Good	163.08	HIA−	10	1 019.580 4	2
			PPPK	DCP inhibitor							
3	APPPPPPPPP	0.960 917	AP	TPP II inhibitor	Non-toxic	Poor	182.34	HIA+	10	962.522 5	23
			AP	PPI inhibitor							
4	GFPFDR	0.958 934	GF	AAP inhibitor	Non-toxic	Good	80.53	HIA−	6	737.349 7	36
			GF, PF	DPP III inhibitor							
			GF	TPP II inhibitor							
5	GFPGGFPGGA	0.948 028	GF	AAP inhibitor	Non-toxic	Poor	63.71	HIA+	10	862.397 3	9
			GF	DPP III inhibitor							
			GF	TPP II inhibitor							
			PG	Anti-thrombotic peptide							
			PG	PAM inhibitor							
			PG	PEP inhibitor							
			GG	ENK inhibitor							
			GA	AC inhibitor							
			GGF, GGA	CAAP inhibitor							
6	FPGGGP	0.946 9	PG, GP	Anti-thrombotic peptide	Non-toxic	Poor	80.57	HIA+	6	530.248 8	24

Continued

No.	Peptide sequence	Peptide rank score	Valid sequence	Bioactive peptide name	Toxicity	Solubility	Instability	ADMET	Length	Mass	Abundance rank
			GG, GP	ENK inhibitor							
			PG	PAM inhibitor							
			PG, GP	PEP inhibitor							
7	WPPLNP	0.946 177	PL	Xaa-Pro AP inhibitor	Non-toxic	Poor	35.63	HIA+	6	722.375 1	10
8	GGFGGGF	0.942 854	GG, FG	ENK inhibitor	Non-toxic	Poor	61.46	HIA+	7	597.254 7	20
			GF	AAP inhibitor							
			GF	DPP III inhibitor							
			GF, GGF	TPP II inhibitor							
			GGF	CAAP inhibitor							
9	FGGGFG	0.939 998	FG, GG	ENK inhibitor	Non-toxic	Poor	49.47	HIA+	6	540.233 2	30
			GF	AAP inhibitor							
			GF	DPP III inhibitor							
			GF, GGF	TPP II inhibitor							
			GGF	CAAP inhibitor							
10	APPPPPPKPA	0.936 869	PPK	*κ*-Casein-derived peptide	Non-toxic	Good	136.28	HIA−	10	967.549 1	3
			PPPK	DCP inhibitor							
			AP	TPP II inhibitor							
			AP	PPI inhibitor							
11	PPPPPPKPA	0.933 505	PPK	*κ*-Casein-derived peptide	Non-toxic	Good	136.28	HIA+	9	896.512	50
			PPPK	DCP inhibitor							
12	APPPPPPKPAAP	0.922 726	AP, AA	TPP II inhibitor	Non-toxic	Good	128.91	HIA−	12	1 135.638 9	38
			AP	PPI inhibitor							
			PPK	*κ*-Casein-derived peptide							
			PPPK	DCP inhibitor							
			AA	CAAP inhibitor							
13	APPPPPPKPAAPA	0.918 189	AP, APA, AA, AAPA	TPP II inhibitor	Non-toxic	Good	131.28	HIA−	13	1 206.676	50
			AP	PPI inhibitor							
			PPK	*κ*-Casein-derived peptide							
			PPPK	DCP inhibitor							
			AA	CAAP inhibitor							
14	APGPINF	0.917 894	PGP, PG, GP	PEP inhibitor	Non-toxic	Good	136.77	HIA+	7	714.370 1	22
			GP	ENK inhibitor							
			PG	PAM inhibitor							
			PGP, PG, GP	Anti-thrombotic peptide							
			AP	TPP II inhibitor							
			AP	PPI inhibitor							
15	AAPPPPPPKPA	0.910 258	AA, AP	TPP II inhibitor	Non-toxic	Poor	14.61	HIA−	11	1 038.586 2	4
			AA	CAAP inhibitor							
			AP	PPI inhibitor							
			PPK	*κ*-Casein-derived peptide							
			PPPK	DCP inhibitor							
16	GPPGPMGPA	0.908 978	PGP, PG, GP	PEP inhibitor	Non-toxic	Good	124.8	HIA+	9	779.363 6	29
			PGP, PG, GP	Anti-thrombotic peptide							
			GP, GPA	ENK inhibitor							
			PG	PAM inhibitor							
			PPGP	DCP inhibitor							

Continued

No.	Peptide sequence	Peptide rank score	Valid sequence	Bioactive peptide name	Toxicity	Solubility	Instability	ADMET	Length	Mass	Abundance rank
17	RPPPPR	0.904 961	RP	Anti-injury peptide	Non-toxic	Poor	43.31	HIA−	6	718.423 8	46
			RP	ENK inhibitor							
			RP	Neurolysin inhibitor							
			PR	DPP III inhibitor							
18	KDPSFDPNFG	0.904 686	FG	ENK inhibitor	Non-toxic	Good	124.17	HIA−	10	1 122.498 2	47
19	GFGLFD	0.902 149	FG	ENK inhibitor	Non-toxic	Good	25.57	HIA+	6	654.301 3	28
			GF	AAP inhibitor							
			GF	DPP III inhibitor							
			GF	TPP II inhibitor							
20	PPPPPKPA	0.896 498	PPK	κ-Casein-derived peptide	Non-toxic	Poor	28.9	HIA+	8	799.459 2	27
			PPPK	DCP inhibitor							
21	VGKFPF	0.896 363	PF	DPP III inhibitor	Non-toxic	Good	119.7	HIA+	6	693.384 9	13
22	SIFGNGF	0.890 525	FG	ENK inhibitor	Non-toxic	Poor	44.23	HIA+	7	740.349 3	31
			GF	DPP III inhibitor							
			GF	TPP II inhibitor							
			GF	AAP inhibitor							
23	APAPPP	0.885 383	AP	PPI inhibitor	Non-toxic	Poor	−25.03	HIA+	6	548.295 8	19
			AP, APA	TPP II inhibitor							
24	GIPDYDHPFG	0.884 643	DY	Peptide modulation of ion flux	Non-toxic	Poor	168.83	HIA+	10	1 116.487 5	50
			HP, PF	DPP III inhibitor							
			FG	ENK inhibitor							
25	GVPAPF	0.881 908	PF	DPP III inhibitor	Non-toxic	Good	30.15	HIA+	6	586.311 5	16
			AP	TPP II inhibitor							
			AP	PPI inhibitor							
26	GPPSHDFHEILM	0.881 216	GP	PEP inhibitor	Non-toxic	Poor	136.73	HIA+	12	1 378.633 9	8
			GP	Anti-thrombotic peptide							
			GP	ENK inhibitor							
			DF	GCP II inhibitor							
			HE	GCP inhibitor							
			IL	Neuropeptide							
27	DDMGFG	0.879 153	DD	GCP II inhibitor	Non-toxic	Poor	67.08	HIA+	6	640.216 2	14
			GF	DPP III inhibitor							
			GF	TPP II inhibitor							
			GF	AAP inhibitor							
			FG	ENK inhibitor							
28	RDPILFP	0.873 158	IL	Neuropeptide	Non-toxic	Good	8.33	HIA+	7	856.480 7	50
29	GPSGKLW	0.867 823	GP	PEP inhibitor	Non-toxic	Good	63.6	HIA+	7	743.396 6	43
			GP	Anti-thrombotic peptide							
			GP	ENK inhibitor							
			LW	DPP III inhibitor							
30	FSGSFG	0.867 148	FG	ENK inhibitor	Non-toxic	Poor	45.66	HIA−	6	600.254 3	18
31	PSPAGPIGGIP	0.863 189	GP	PEP inhibitor	Non-toxic	Poor	8.33	HIA+	11	961.523 3	42
			GP	Anti-thrombotic peptide							
			GP	ENK inhibitor							
32	VPFDPRFPN	0.856 118	PF, PR, RF	DPP III inhibitor	Non-toxic	Poor	84.94	HIA+	9	1 087.545	37
33	VWTRPW	0.845 733	RP	Anti-injury peptide	Non-toxic	Good	78.42	HIA+	6	843.439 1	34
			RP	Neurolysin inhibitor							
			RP	ENK inhibitor							

Continued

No.	Peptide sequence	Peptide rank score	Valid sequence	Bioactive peptide name	Toxicity	Solubility	Instability	ADMET	Length	Mass	Abundance rank
34	GPPVFAKL	0.842 499	GP	PEP inhibitor	Non-toxic	Poor	10.58	HIA+	8	827.490 5	50
			GP	Anti-thrombotic peptide							
			GP, AK	ENK inhibitor							
			VF	TPP II inhibitor							
			FA	AC inhibitor							
			FA	DPP III inhibitor							
35	FIDNIFR	0.842 058	FR	DPP III inhibitor	Non-toxic	Poor	46.29	HIA+	7	843.439 1	39
36	GPPSHDFHEILMK	0.841 921	GP	PEP inhibitor	Non-toxic	Good	71.34	HIA+	13	1 506.728 9	5
			GP	Anti-thrombotic peptide							
			GP	ENK inhibitor							
			DF	GCP II inhibitor							
			HE	GCP inhibitor							
			IL	Neuropeptide							
37	APGPINFTQL	0.836 244	AP	TPP II inhibitor	Non-toxic	Good	62.69	HIA+	10	1 056.560 4	26
			AP	PPI inhibitor							
			PGP, PG, GP	Anti-thrombotic peptide							
			GP	ENK inhibitor							
			PG	PAM inhibitor							
			PGP, PG, GP	PEP inhibitor							
38	PRPANF	0.835 643	PR	DPP III inhibitor	Non-toxic	Poor	5.69	HIA+	6	700.365 7	50
			RP	Anti-injury peptide							
			RP	Neurolysin inhibitor							
			RP	ENK inhibitor							
39	GFGLFE	0.834 108	GF	DPP III inhibitor	Non-toxic	Good	34.92	HIA+	6	668.317	41
			GF	TPP II inhibitor							
			GF	AAP inhibitor							
			FG	ENK inhibitor							
			FE	GCP inhibitor							
40	QIFRPDNFVFG	0.829 491	FR	DPP III inhibitor	Non-toxic	Poor	8.33	HIA+	11	1 338.672	40
			RP	Anti-injury peptide							
			RP	Neurolysin inhibitor							
			VF	TPP II inhibitor							
			VF	Calpain-1 inhibitor							
			RP, FG	ENK inhibitor							
41	DGPSGKLW	0.828 314	GP	PEP inhibitor	Non-toxic	Poor	6.08	HIA+	8	858.423 5	44
			GP	Anti-thrombotic peptide							
			GP	ENK inhibitor							
			LW	DPP III inhibitor							
42	APPAPAPP	0.825 575	AP, APA	TPP II inhibitor	Non-toxic	Good	41.2	HIA+	8	716.385 7	50
			AP	PPI inhibitor							
			PPAP	DCP inhibitor							
43	GLPPAP	0.825 471	AP	TPP II inhibitor	Non-toxic	Poor	177.28	HIA−	6	550.311 5	6
			AP	PPI inhibitor							
			PPAP	DCP inhibitor							
44	GGGGPP	0.822 72	GP	PEP inhibitor	Non-toxic	Poor	136.73	HIA−	6	440.201 9	45
			GP	Anti-thrombotic peptide							
			GG, GP	ENK inhibitor							

Continued

No.	Peptide sequence	Peptide rank score	Valid sequence	Bioactive peptide name	Toxicity	Solubility	Instability	ADMET	Length	Mass	Abundance rank
45	VGKFPFLA	0.820 061	PF, FL, LA	DPP III inhibitor	Non-toxic	Poor	102.13	HIA+	8	877.506 2	32
46	PASGGPPR	0.817 869	GP	PEP inhibitor	Non-toxic	Poor	35.68	HIA−	8	737.382	49
			GP	Anti-thrombotic peptide							
			GG, GP	ENK inhibitor							
			PR	DPP III inhibitor							
47	GIPPAPR	0.816 334	AP	TPP II inhibitor	Non-toxic	Good	62.9	HIA+	7	706.412 6	48
			AP	PPI inhibitor							
			PPAP	DCP inhibitor							
			PR	DPP III inhibitor							
48	SERFGPNGW	0.814 976	RF	DPP III inhibitor	Non-toxic	Good	64.1	HIA+	9	1 048.472 7	50
			GP	PEP inhibitor							
			GP	Anti-thrombotic peptide							
			GW	TPP II inhibitor							
			ER, FG, GP	ENK inhibitor							
49	LDPINRPTFWP	0.814 192	RP	Anti-injury peptide	Non-toxic	Good	27.3	HIA+	11	1 425.740 5	25
			RP	Neurolysin inhibitor							
			TF	DPP III inhibitor							
			RP, FW	ENK inhibitor							
50	VDIPPAPFR	0.811 676	PPAP	DCP inhibitor	Non-toxic	Poor	37.82	HIA+	9	1 010.554 9	1
			AP	TPP II inhibitor							
			AP	PPI inhibitor							
			PF, FR	DPP III inhibitor							
51	GIPPAP	0.811 66	AP	TPP II inhibitor	Non-toxic	Good	74.59	HIA+	6	550.311 5	7
			AP	PPI inhibitor							
			PPAP	DCP inhibitor							
52	GNDNPFDM	0.807 618	PF	DPP III inhibitor	Non-toxic	Poor	85.68	HIA+	8	908.333 4	11
53	FLDSNFKM	0.805 552	FL	DPP III inhibitor	Non-toxic	Good	34.04	HIA+	8	1 000.468 8	33
54	APPAPAPPA	0.804 761	AP, APA	TPP II inhibitor	Non-toxic	Good	36	HIA−	9	787.422 8	21
			AP	PPI inhibitor							
			PPAP	DCP inhibitor							
55	FDPRFPNQ	0.803 851	PR, RF	DPP III inhibitor	Non-toxic	Poor	180.09	HIA−	8	1 019.482 5	12
56	SQLGDLGSGAG KGGGGGG	0.802 819	GA	AC inhibitor	Non-toxic	Good	29.4	HIA−	18	1 430.674 9	35
			GD, GG	ENK inhibitor							
57	ALDPINRPTFWP	0.802 711	RP	Anti-injury peptide	Non-toxic	Good	40.27	HIA+	12	1 425.740 5	15
			RP	Neurolysin inhibitor							
			TF	DPP III inhibitor							
			RP, FW	ENK inhibitor							
58	APGIIPR	0.801 647	AP	TPP II inhibitor	Non-toxic	Poor	35.5	HIA+	7	722.443 9	17
			AP	PPI inhibitor							
			PG	PEP inhibitor							
			PG	PAM inhibitor							
			PG	Anti-thrombotic peptide							
			PR	DPP III inhibitor							
59	DNPFDMKVFK	0.800 246	PF	DPP III inhibitor	Non-toxic	Good	14.2	HIA+	10	1 239.595 8	50
			VF	Calpain-1 inhibitor							
			VF	TPP II inhibitor							

3.3 神经活性肽的成药性评估与整合筛选 为进一步评估神经活性肽的成药潜力, 使用多种生物学工具对59条神经活性肽的理化性质、生物安全性、毒性以及肠道吸收特性进行系统性分析, 并汇总其序列、长度、分子量、潜在活性类型及活性位点等基本信息 (表1)。

首先, 使用Innovagen预测水溶性, 其中29条肽段水溶性较好, 其余30条肽段水溶性较差。随后, 通过Expasy评估肽段稳定性, 38条肽段为不稳定型, 提示其体内易降解; 另外21条肽段被预测为稳定型, 体内作用时间较长[29]。进一步通过ToxinPred预测毒性, 59条肽段均被预测为无毒, 具有良好的生物安全性, 适合作为潜在的治疗性分子。最后, 采用Admet SAR预测肽段肠道吸收能力, 43条肽段为HIA阳性, 即具有较好的肠道吸收潜力; 其余16条为HIA阴性, 提示其在肠道中吸收性较差。

基于上述评估, 进一步整合分析肽段的Peptide Ranker评分、稳定性、水溶性、安全性及吸收性, 筛选出7条兼具高活性预测得分 (≥0.80)、溶解性好、稳定和肠道吸收性较强的优势肽段 (GFGLFD、GVPAPF、RDPILFP、GFGLFE、LDPINRPTFWP、FLDSNFKM、DNPFDMKVFK)。这些肽段具有良好的成药潜力, 值得在后续实验中重点验证其神经调节功能及机制。

4 分子对接结果

选择Peptide Ranker评分最高的多肽FPPRDF, 使用BIOPEP-UWM对其作用靶点进行预测, 结果显示该多肽可能与DPP III发生作用。DPP III在中枢神经系统调控疼痛、应激及神经递质平衡中发挥重要作用, 且与抑制肿瘤密切相关, 已被认为是潜在的治疗干预靶点[30]。在PDB数据库中下载DPP III三维晶体结构文件 (PDB ID: 3FVV)。采用RPBS构建FPPRDF的三维结构, 优化模型后, 通过Auto Dock Tools 1.5.6完成分子对接模拟。

分子对接结果表明, 肽段FPPRDF可稳定嵌合于DPP III的活性腔中, 并与关键氨基酸残基形成稳定的相互作用。FPPRDF与GLU-690形成两个氢键, 键长分别为2.8和1.7 Å, 同时与GLU-688形成一个2.5 Å的氢键 (图4)。对接构象显示, 该肽段被稳定包埋于蛋白内部的结合口袋中, 形成典型的氢键网络, 呈现良好的空间互补性。其结合能为−22.554 kJ·mol^{-1}, 显著低于−21 kJ·mol^{-1}的稳定结合阈值, 提示该结合过程具有热力学自发性和较强的稳定性。这一结果为FPPRDF可能通过调控DPP III发挥生物活性提供了初步理论支持, 并为其后续功能验证和作用机制研究奠定了基础。

讨论

近年来, 虫类被认为是BAPs的重要来源, 如蜜蜂、蚂蚁、苍蝇等虫类体内的BAPs在免疫调节、神经保护和抗菌等方面展现出广泛潜力[31-33]。然而, 目前尚无系统研究聚焦于虻虫BAPs的神经调节功能。本研究首次系统鉴定出虻虫体内具有神经活性的多肽, 并通过多种生物信息学工具对其功能潜力和药代特性进行评估[34], 丰富了虫类BAPs资源库, 为后续功能验证与药物开发提供了候选分子。

在实验过程中, 不同样本处理方式对肽段获取量具有显著影响[35]。样本一直接收集上清液避免了后续溶解和洗涤步骤, 减少了操作损失。而样本二在沉淀复溶时, 疏水性肽段因溶解度低或再聚集无法完全进入水相, 且重复离心可能导致部分肽段被弃去, 造成系统性损耗[36]。通过功能预测, 共筛选出95条BAPs, 其中59条具有神经调节潜力, 提示虻虫中含有丰富的天然神经活性肽资源。进一步评估59条神经活性肽的药用潜力, 发现其中约49.2%的肽段具有良好水溶性, 但仅有约35.6%的肽段在稳定性预测中表现良好, 可能限制其在体内的持续活性。值得注意的是, 所有候选肽均被预测为无毒, 43条肽段具备良好的肠道吸收能力, 具有作为口服药物开发的潜力。此外, FPPRDF作为虻虫代表性BAP, 与DPP III进行分子对接, 结合能为−22.554 kJ·mol^{-1}, 提示FPPRDF可能通过靶向DPP III参与神经信号调控。尽管本研究结合多种手段系统评估了BAPs的潜力, 仍存在一定局限性。首

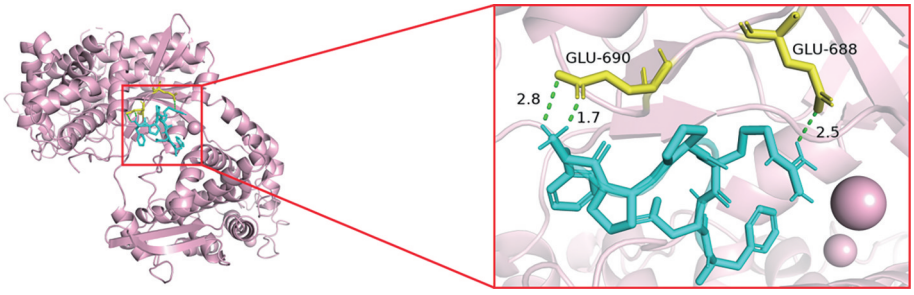


Figure 4 Schematic illustration of the molecular docking between peptide FPPRDF and the DPP III protein, showing the binding site

先, 预测过程中未考虑翻译后修饰对肽段结构和功能的潜在影响, 而PTMs在活性肽的活性调控中具有重要生物学意义, 可能改变其稳定性、靶点亲和性及生物分布等性质[37]。其次, 相关功能预测依赖于算法模型, 可能存在假阳性或假阴性, 其预测结果需要体外实验加以验证。

综上所述, 本研究系统揭示了虻虫中具有潜力的BAPs资源, 为新型神经活性肽的发现提供了重要线索。通过多种活性预测模型的联合筛选, 本研究识别出多个具有潜在神经活性的优势肽段, 其中FPPRDF等候选肽段评分较高, 具有较强的研究价值。未来研究将重点优化肽段提取和分离工艺, 针对高潜力肽段如FPPRDF开展体外功能验证。具体包括在神经细胞模型中评估FPPRDF对相关信号通路的调控作用, 并建立急性神经损伤或神经退行性疾病动物模型, 验证其体内神经保护或神经修复效应。结合质谱分析和转录组学技术, 进一步挖掘FPPRDF的潜在靶点, 深入解析其分子调控机制, 为新型神经活性肽药物的开发奠定坚实基础。

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利益冲突: 全部作者声明本文无任何利益冲突。

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