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AHTPDB: a comprehensive platform for analysis and presentation of antihypertensive peptides

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ABSTRACT

AHTPDB (http://crdd.osdd.net/raghava/ahtpdb/) is a manually curated database of experimentally validated antihypertensive peptides. Information pertaining to peptides with antihypertensive activity was collected from research articles and from various peptide repositories. These peptides were derived from 35 major sources that include milk, egg, fish, pork, chicken, soybean, etc. In AHTPDB, most of the peptides belong to a family of angiotensin-I converting enzyme inhibiting peptides. The current release of AHTPDB contains 5978 peptide entries among which 1694 are unique peptides. Each entry provides detailed information about a peptide like sequence, inhibitory concentration (IC_{50}), toxicity/bitterness value, source, length, molecular mass and information related to purification of peptides. In addition, the database provides structural information of these peptides that includes predicted tertiary and secondary structures. A user-friendly web interface with various tools has been developed to retrieve and analyse the data. It is anticipated that AHTPDB will be a useful and unique resource for the researchers working in the field of antihypertensive peptides.

INTRODUCTION

High blood pressure has now become a major global health concern, affecting approximately 73 million people only in the United States (1). It is estimated that there will be around 1.56 billion people affected with hypertension throughout the world by 2025 (2) reflecting the aggressiveness of the disease.

Angiotensin converting enzyme (ACE) increases blood pressure by converting the inactive angiotensin I (decapeptide) to the active angiotensin II (octapeptide), which is a potent vasoconstrictor hormone and aldosterone-stimulating peptide that controls fluid-electrolyte balance and blood pressure. A number of chemical compounds have been designed to act as antihypertensive drugs, e.g. Captopril, Fosinopril, Lisinopril, etc., which act either by direct ACE inhibition or by blocking the angiotensin II receptors. Although these drugs are effective in controlling blood pressure, there are numerous side effects associated with them, e.g. coughing, taste disturbance, skin rashes, kidney failure, etc. (3,4). Besides chemical drugs, diet and lifestyle also play a significant role in the prevention of this disease. In the past, small bioactive peptides with antihypertensive activity have been discovered. Since many of these peptides are part of proteins present in our food, these are not toxic in general and may represent a new strategy for the prevention and treatment of hypertension. In addition, antihypertensive peptides (AHTPs) from other sources (other than food) have also been reported. Hence, there is a substantial interest in discovering peptides with antihypertensive activity from all possible sources, e.g. natural food, algae, fungi, microorganisms, insects (5–9), etc. The natural food sources include mainly milk and dairy products, egg, meat, fish, plants (cereal, wheat, rice, garlic, spinach, grapes, etc.) (10–18). These peptides have also been reported from various biological processes (e.g. enzymatic hydrolysis, fermentation) and chemical synthesis (19–21). These peptides have been known to inhibit ACE, thus popularly known as AHTPs.

Since hundreds of peptides with antihypertensive activity have already been reported and new peptides are also being discovered every day, there is requirement of a dedicated platform, which systematically catalog these peptides along with their properties, e.g. sequence, inhibitory value (IC_{50}), source, toxicity value, length of peptides, etc. There are few repositories of bioactive peptides, which provide information about few AHTPs, e.g. ACEpepDB (http://www.cftri.com/pepdb/), BIOPEP and EROP-Moscow database (22,23). Since these databases do not provide comprehensive information related to AHTPs, there is a scope of improvement in updating the knowledge about AHTPs. Therefore, we developed a dedicated and comprehensive database, 'AHTPDB', for systematic collection, storage and presentation of AHTPs. In AHTPDB, we cataloged 5978

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(1694 unique) experimentally verified AHTPs along with the information related to their sources (natural, synthetic), IC_{50} value, log value of IC_{50} (pIC_{50}), length of peptides, toxicity/bitterness value, molecular mass, isoelectric point, etc. We hope AHTPDB will be beneficial for the scientific community working on antihypertensive drug discovery.

METHODOLOGY

Data collection

The sequences of AHTPs with other relevant information were manually collected from published literature, PubMed using keywords as 'antihypertensive peptides', 'hypotensive peptide', 'blood pressure lowering peptides', 'ACE-inhibiting peptides', etc. We collected and screened about 350 research articles extracted from the above search criteria. The AHTPs and their relevant information like inhibitory concentration (IC_{50}), source, assay method, purification techniques, *in vivo* model organism and decrease in systolic blood pressure (SBP) were extracted manually and complied systematically. There are three databases, which provide information of AHTPs, i.e. ACEpepDB (http://www.cftri.com/pepdb/), EROP-Moscow database and BIOPEP (22) database. Data related to AHTPs from these resources were also included. Only those peptides, which have been validated experimentally, were included in AHTPDB. We have made multiple entries of peptides if these peptides have different inhibitory values or have been isolated from different sources, e.g. milk, wheat or soybean, etc. In this way, we compiled total 5978 peptide entries with 3364 entries having IC_{50} values, out of which 1694 are unique peptides.

Database architecture and web interface

After data collection and compilation, the information about AHTPs was presented in the form of database, which was built on Apache HTTP server 2.2 with MySQL server 5.1.47. The front-end of database was developed using HTML, PHP and JavaScripts while MySQL was used to handle the back-end. PERL and PHP languages were used for writing all the scripts. The architecture of AHTPDB is depicted in Figure 1.

Organization of data

In AHTPDB, data were organized systematically as primary and secondary data. The primary data consists of all AHTP sequences and the other related information, which was manually curated from the literature. Each peptide has been assigned a unique entry number, providing the detailed information about each peptide. Each entry contains following main fields: (i) unique AHTPDB ID, (ii) PubMed ID (PMID) or link of reference, (iii) sequence of peptide, (iv) length, (v) inhibitory concentration (IC_{50}), (vi) log value of inhibitory concentration (pIC_{50}), (vii) source, (viii) bitterness/toxicity value, (ix) molecular weight, (xi) isoelectric point, (xii) year of publication, (xiii) purification techniques, (xiv) IC_{50} determination assay, (xv) animal model used for testing, (xvi) decrease in SBP.

One of the unique features of AHTPDB database is the structural information of peptides. The secondary data consists of structural information of all peptides. It has been shown in numerous previous studies that hydrophobic residues at C-terminal (primarily proline) are desired for effective antihypertensive activity of these peptides (24,25). Therefore, understanding the structure of these compounds will be of considerable interest.

For all the AHTPs, the structural information was obtained using PEPstr algorithm (26). PEPstr is a state-of-art algorithm used for predicting the tertiary structure of peptides. Using Mean Force Potential (MFP) energy scores, Thomas *et al.* showed that the structures predicted by PEPstr are close to NMR structures (27). Briefly, PEPstr predicts intermediate states like secondary structure by PSIPRED (28), beta-turn types information using BetaTurns (29) and assigns the ideal torsion angle of these intermediate predicted states to be used as restraints to generate an initial structure. This initial structure is further modeled using energy minimization followed by short molecular dynamics using AMBER 11 software (30) to give the final predicted structure. Although the PEPstr method was designed to handle peptides with length between 7 and 25 residues, in this study, the length restriction was slightly relaxed from 5 to 30 residues. We also extended the time of molecular dynamics simulation of all the peptide structures to 1 ns instead of 25 ps, which was originally implemented in PEPstr. The structure of 2056 (912 unique) peptides was successfully predicted using PEPstr.

The tertiary structure of 3885 (745 unique) peptides, which were either di-peptides or tri-peptides or tetra-peptides, was not predicted by PEPstr but was predicted using a different approach. We used phi and psi torsion angle value of 180° as restraint to generate an initial structure having linear conformation. This initial structure was further simulated using AMBER and the whole trajectory of molecular dynamics simulation was searched for the structure having minimum energy, which was provided as the final predicted structure. 36 peptide sequences (5 peptides with less than 5 residues and 31 peptides between 5 and 30 residues) had pyroglutamine residue (denoted by 'J' letter) at the N-terminus, which is a non-natural residue. Non-natural residues require special force fields in order to be recognized by standard molecular dynamics software like AMBER. Due to the unavailability of force field for pyroglutamine, the structure of these 36 peptides was not predicted. One of the sequences in this data set had length of 81 residues and therefore the structure of that sequence was also not predicted. Once the predicted structures of the peptides were obtained using PEPstr, the secondary structural states of these peptides was assigned using DSSP software (31). The tertiary structure of peptide in PDB file format is given as input to DSSP software, which assigns eight different types of secondary structural states (H: alpha helix, G: 3/10 helix, I: pi helix, E: extended strand, B: beta-bridge, S: bend, T: turn and C: loop). The predicted structures are also represented in simplified molecular-input line-entry system (SMILES) notation by converting the tertiary structures in SMILES format using Open Babel software (32).

As anticipated, all the peptides with length less than 5 residues occurred in loop region. Majority of the residues

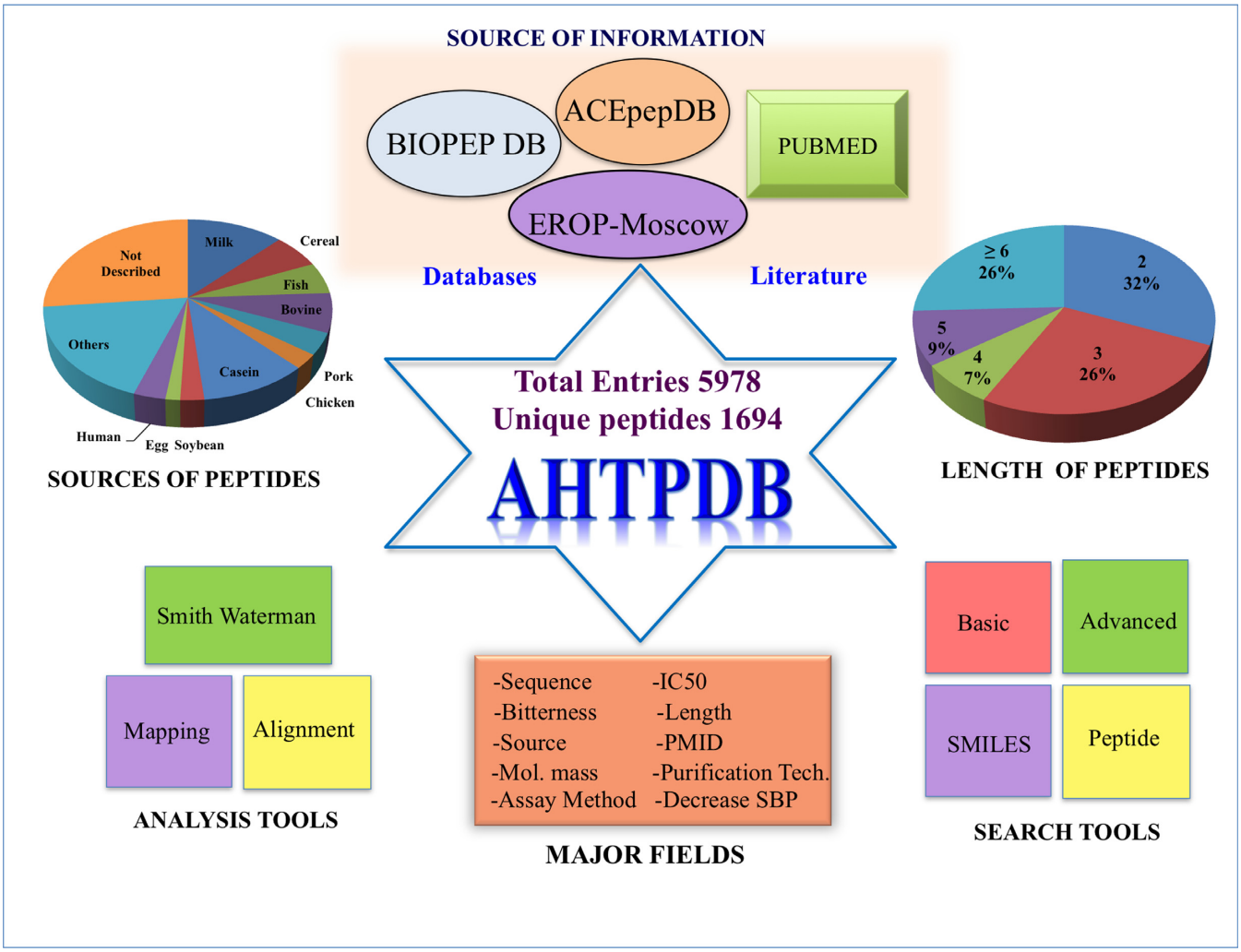

Figure 1. Architecture of AHTPDB database.

(~76%) of peptides having length between 5 and 10 occurred in loop region. Other prominent local structures occurring in these peptides were turns (~7%) and bends (~16%) with very less residues (~1%) forming ordered structures like helix/strand. In case of peptides with length between 11 and 30, the proportion of ordered secondary structural elements increased (~7%) along with proportion of turns (~12%) and bends (~25%) while the proportion of loop region decreased (~55%). Turns provide structural stability to the peptides (33) and play a helping role in molecular recognition processes between peptide and their receptors (34). Bends provide high curvature (at least 70°) (31) and are therefore important in constraining the peptides to adopt non-linear conformation. Therefore, most of the AHTPs (~92%) in this database are dominated by loop regions followed by turns and bends with very less proportion of helix/strand.

In addition to structural information, we have computed amino acid frequency and composition of all the AHTPs. Also, composition and frequency of various physicochemical properties of amino acids like aromatic residues, aliphatic residues, positive charge, negative charge, neutral, polar residues and hydrophobic residues have been computed and stored as secondary information in the database. The knowledge of amino acid composition and various physicochemical properties of AHTPs is very important to understand the nature of the AHTP.

RESULTS

Data statistics

The current release of AHTPDB database contains 5978 entries collected from around 350 research articles. Among these, 3364 entries have provided information of IC_{50} values of peptides. During data curation from the literature, it was noticed that many of the identical peptides have been isolated from different sources and exhibited different IC_{50} values. In order to provide comprehensive information, we have made multiple entries of a single peptide if the identical peptides have been reported from more than one source or have been reported to have different IC_{50} values. Thus, the total number of unique peptides in AHTPDB database is 1694.

AHTPs have been isolated from various sources, including natural food, fungi, algae, microorganism, insects or snake venom. Among natural food sources, milk is the most explored source for identification of AHTPs with 834 entries (Figure 2). The other major sources include cereals (419 entries), fish (384 entries), bovine (477 entries), pork/porcine (333 entries), chicken (177 entries), casein (723 entries), soybean (159 entries), egg (97 entries), human (215) and others (1152 entries). No source information was provided in case of 1805 entries hence marked as 'ND', i.e. Not Described. Some peptide entries occur in more than one class and are therefore overlapping. For example, an entry having source described as 'Milk casein' occurs in 'Milk' class as well as in 'Casein' class. In AHTPDB, almost all AHTPs target 'angiotensin-I converting enzyme (ACE)'. Many such peptides have also been used as food additives. Therefore, toxicity/bitterness values of these peptides are of much importance and thus we have compiled these values (156 entries) in the database. Toxicity values may help in designing significant AHTPs with high efficacy but least toxicity or bitterness to be used as food additives. In AHTPDB, most of the peptides have been evaluated by two assays developed by Cushman & Cheung (35) and Kasahara & Ashihara (36). A total of 1182 and 218 entries have been made for these two assays, respectively. Most of the AHTPs have been identified and purified by using RP-HPLC, gel filteration chromatography (GFC), size exclusion chromatography (SEC), ion exchange chromatography (IEC), ultrafiltration (UF), thin layer chromatography (TLC), etc. A number of peptides have been tested on Spontaneously Hypertensive Rats (SHR) or Dahl Salt-Sensitive Rats (DSSR) in order to check the antihypertensive effect of peptides. In this study, we collected all the values (maximum decrease in SBP) wherever provided in the literature. Also, we approximated the value of decrease in SBP where exact value was not given but represented in the form of graphs.

Integration of web tools

A number of user-friendly tools have been integrated in AHTPDB which facilitates data extraction and analysis in a very convenient way. Following is the description of these tools:

Search facility. We have implemented four search options, e.g. basic search, advanced search, peptide search and SMILES search. Basic search option allows users to perform a search on any field of the database, e.g. sequence of peptide, PubMed ID, source of peptide, etc. A user can display any or all the provided fields. Using advanced search, user can search multiple options at a time by adding number of queries at a time. There are two options in peptide search (i) Containing search, which enable searching of user-defined peptide sequence (complete or partial) in database (ii) Exact search, searches peptides which are identical to user's peptides. SMILES search facilitates searching of SMILES notation of a given peptide against AHTPDB peptide database in SMILES format.

Explore. In order to search and extract data on specific fields, we have developed a powerful browsing facility that allows users to browse data on the four specific properties that include (i) source, (ii) length, (iii) IC_{50} and (iv) physicochemical properties. In AHTPDB, we have covered 35 different major sources from which AHTPs have been isolated. These peptides have shown different ACE-inhibiting property in different concentrations from different sources. Using this field, users can browse peptides isolated from a particular source. In the IC_{50} field, the peptides can be extracted based upon IC_{50} value given in different units, i.e. μM, μmol or μg/ml or μg/L. A range of IC_{50} values has also been provided and based on this, peptides with desired IC_{50} values can be searched and extracted. In AHTPDB, peptides based on their length can also be browsed. Length of AHTPs varies from 2 to 30 amino acids. However, majority of the peptides have length between 2 and 5 amino acids. One AHTP has length of 81 amino acids.

Smith Waterman algorithm. Since in case of small peptides, Smith-Waterman algorithm performs similarity search more effectively, this tool was integrated (37). This option allows users to search AHTPs in the database that are similar to their peptides. Users can search peptide sequences by submitting sequence in FASTA format.

Sequence alignment. This tool is meant for alignment of sequence provided by the user with peptides in AHTPDB database. User can submit query peptide sequence in FASTA format in sequence box and peptide IDs of AHTPDB database in ID box, for peptide alignment.

Mapping. It allows the users to map AHTPs on their peptide sequence by allowing user to run a super-search and sub-search. Super-search provides similar peptides of our database against a protein sequence as a query whereas in case of sub-search, a given peptide is mapped against all peptides of AHTPDB database.

Property. This tool has four modules: (i) amino acid (AA) composition, (ii) AA frequency, (iii) physicochemical property (PP) composition and (iv) PP frequency. It has been built to assist users to analyse and retrieve AHTPs having desired amino acid composition/frequency and physicochemical properties. For example, user may be interested to know about AHTPs having a specific range of amino acid composition or AHTPs having high positive charge residues. All these tasks can be performed using these tools.

Structure. In AHTPDB, predicted secondary and tertiary structures of each peptide have been stored. In this tool, three modules: (i) secondary structure (SS) composition, (ii) SS search and (iii) structure alignment have been developed to extract structural information of AHTPs. SS composition module assists users to search AHTPs based on their SS composition (composition H-helix, E-β strand, T-turn and C-coil). SS search module provides facility to search query peptide's secondary structure against AHTPDB. Structure alignment module allows users to align their peptide structure with any of the structures of AHTPs available in AHTPDB.

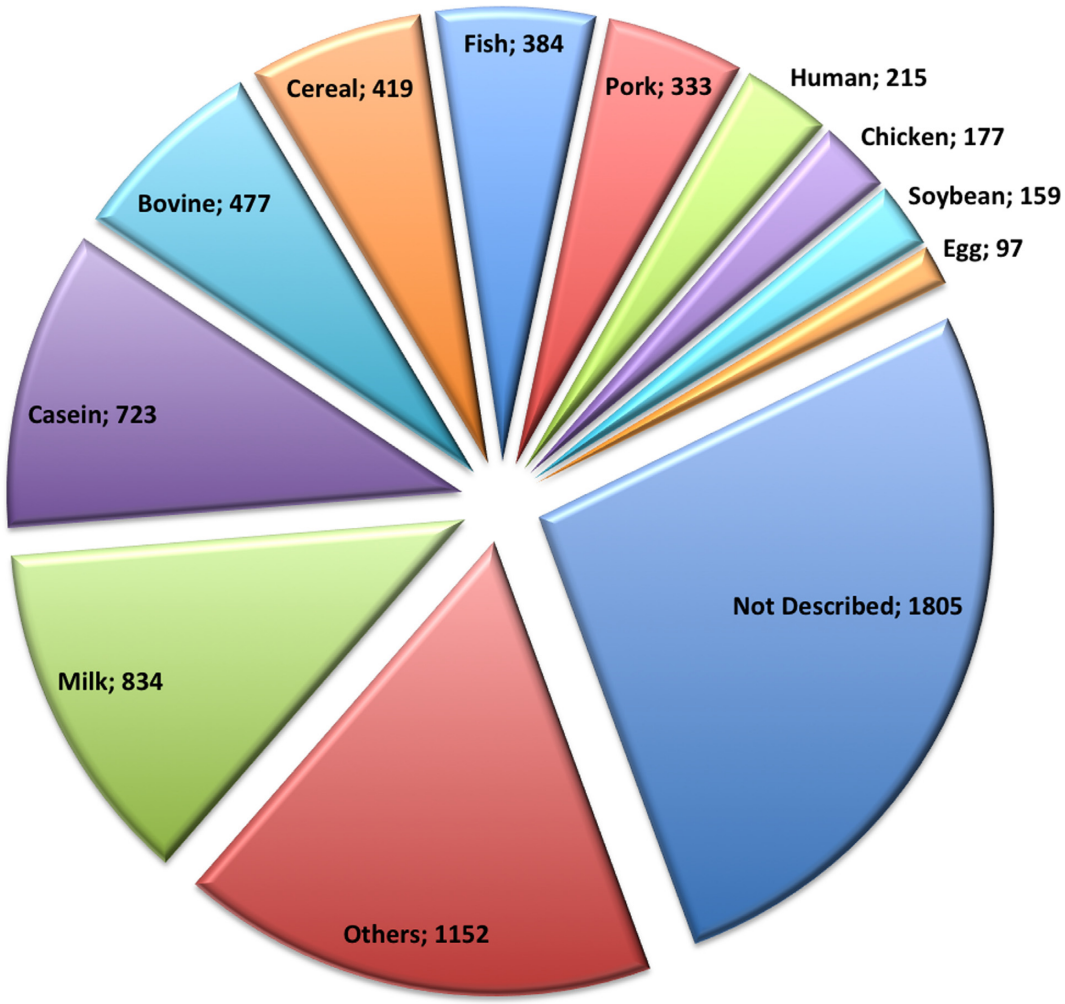

Figure 2. Distribution of antihypertensive peptides in AHTPDB database based on various sources.

DISCUSSION

Bioactive peptides are small peptides having biological activities. Such peptides are often released in the gastrointestinal track during food processing and play important role in metabolic regulation and modulation. Bioactive peptide based research is an area of tremendous opportunities as a number of peptides have been identified with different therapeutics values, e.g. antimicrobial peptides (38), anticancer peptides (39,40), antioxidative peptide (41), antifungal or tumor-homing peptides (42,43). Over the years, a number of repositories providing comprehensive information of these peptides have been developed (44–46).

The problem of hypertension is growing in both developed and developing countries. In order to control this disease, a number of peptides from natural sources have been identified. Since these peptides have antihypertensive effect without any adverse side effects, there is a huge surge in exploring such peptides. Although a number of AHTPs from natural sources have been explored but most of them have been reported in independent studies and information related to these peptides is scattered in the literature. To date, there is no dedicated platform, which provides comprehensive information related to AHTPs. However, few peptide databases contain AHTPs but the information is not comprehensive.

BIOPEP database provides the information regarding major bioactive peptides including AHTPs. However, out of 2609 entries, only 556 entries are of AHTPs, which are mainly ACE-inhibiting peptides (22). ACEpepDB (http://www.cftri.com/pepdb/) is another database, which consists of 865 ACE-inhibiting peptides acting as AHTPs. In EROP-Moscow database, only 313 peptides as 'ACE inhibitor' have been provided in 'enzyme inhibitor' category with details about their source, sequence, molecular mass, IC_{50} and reference (23).

Information like bitterness/toxicity values of peptides is very important as it is directly related with chances of peptides to be used as food additive. It has been reported in literature that small dipeptides are very much effective in blocking ACE activity but their taste is highly bitter so they cannot be used as antihypertensive food additive, although being able to act as ACE inhibitor (47). Similarly, therapeutic value of peptide in terms of its efficacy in mice or rat model is very important. Earlier developed databases did not provide any such information, e.g. decrease in SBP

of animal after having peptide treatment. Thus, it was imperative to develop a new platform, which integrates such crucial information with largest possible peptide coverage. Hence, we developed 'AHTPDB' which covers all essential aspects of peptide biology, specially ACE inhibitor peptide, e.g. peptide sequence, IC_{50}, pIC_{50}, toxicity/bitterness value, length of peptides, sources of peptides, PubMed ID, other links (e.g. DOI number), molecular mass, isoelectric point, year, purification methods, assay methods, mice/rat study, decrease in SBP and references. Apart from natural food, AHTPDB also covers AHTPs originated from other sources, including fungi, algae, microorganism, insects or snake venom, therefore all such peptides may not be dietary peptides.

We believe that AHTPDB will be a useful resource for biologists working in area of antihypertensive drug discovery. User can search the potential AHTPs developed so far from this database and further modify them in order to achieve desired properties. User can use AHTPDB for developing *in silico* algorithms for the prediction and analysis of AHTPs. Although a number of prediction models for designing AHTPs have been developed, still there is scope of improvement in these developed models. For this purpose, AHTPDB provides new, large and latest data of AHTPs, which can be used for understanding the properties of these peptides and to further, derive rules for the prediction of these peptides. We anticipate that development of AHTPDB will expedite antihypertensive peptide based drug discovery.

UPDATE OF AHTDB

It is possible for the user to submit any new AHTP, which is experimentally annotated, by filling the HTML form on web interface. Our team will confirm the validity of new peptide entry before including into the AHTPDB database in order to maintain the high standard quality of this database. On regular basis, we will also update this database in time-dependent manner.

LIMITATIONS AND FUTURE DEVELOPMENTS

Maximum number of AHTPs in AHTPDB has been provided with all possible information available in the literature but still there are chances of further improvement. We reported the maximum decrease in SBP, whereas it should be subdivided into time-dependent and dose-dependent effect of any AHT peptide, if provided in the literature. Also, since the bitterness/toxicity values of peptides are given in a very few studies, thus the number of peptides with this information in AHTPDB database is also very much limited. We have attempted to provide the predicted tertiary structure of most of the peptides in AHTPDB. However, the tertiary structure of few peptides could not be predicted due to the presence of non-natural residue (pyroglutamine) and unavailability of special force fields. We will try to predict the structures of such peptides if the relevant information will be available in future studies.

AVAILABILITY

AHTPDB is available at crdd.osdd.net/raghava/ahtpdb/.

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