PPD v1.0—an integrated, web-accessible database of experimentally determined protein pKₐ values

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ABSTRACT

The Protein pKₐ Database (PPD) v1.0 provides a compendium of protein residue-specific ionization equilibria (pKₐ values), as collated from the primary literature, in the form of a web-accessible PostgreSQL relational database. Ionizable residues play key roles in the molecular mechanisms that underlie many biological phenomena, including protein folding and enzyme catalysis. The PPD serves as a general protein pKₐ archive and as a source of data that allows for the development and improvement of pKₐ prediction systems. The database is accessed through an HTML interface, which offers two fast, efficient search methods: an amino acid-based query and a Basic Local Alignment Search Tool search. Entries also give details of experimental techniques and links to other key databases, such as National Center for Biotechnology Information and the Protein Data Bank, providing the user with considerable background information. The database can be found at the following URL: http://www.jenner.ac.uk/PPD.

INTRODUCTION

A significant proportion of chemical reactions involving proteins are mediated through electrostatic interactions of their ionizable residues (1). Such residues greatly influence the conformation of a protein and therefore its function (2,3), as demonstrated by their folding mechanisms (4–6), enzyme catalysis and protein–protein interactions (7). With respect to enzyme catalysis, residues can act as proton donors and acceptors within the catalytic site and help stabilize transition states, with a concomitant influence on the rate of reaction (8,9).

The dissociation constant (Kₐ) is a measure of the acidity of a compound, i.e., its ability to donate a proton. Kₐ values range widely from 10⁻¹⁰ for the strongest acids, such as sulphuric, to 10⁻⁵⁰ for the weakest, such as methane. Therefore a negative logarithmic scale is usually applied (pKₐ = −log₁₀ Kₐ), whereby Kₐ values for sulphuric acid and methane would become pKₐ values of −10 and 50, respectively. Generally, more negative pKₐ values correspond to stronger acids. The pKₐ values of individual amino acid residues in proteins are determined by the ionization of their side-chain groups. For the 20 natural amino acids, pKₐ values range from 4.0 for the side-chain carboxyl of aspartate to 12.0 for the side-chain guanididium group of arginine. Main-chain groups are not ionizable, although two additional ionizable groups exist at the N- and C-termini. Residues within proteins have pKₐ values that are moderated by their micro-environments, the nature of their near neighbours, the extent of hydrogen bonding and so on and can take on a range of values different from that of a model residue.

NMR spectroscopy is the most widely used method for determining the pKₐ values of individual residues, with an accuracy of ~0.1 pH units. Although many NMR methods are available, most entries in the Protein pKₐ Database (PPD) are derived using ¹H, ¹³C and ¹⁵N experiments. Inaccuracies in NMR experiments stem from the range of pH values tested, variations in ionic strength and the reversibility of the titration (10). In light of this, new combination methods are being used based on NMR spectroscopy coupled with site-directed mutagenesis, which leads to more accurate pKₐ values (10,11).

The functional importance of ionizable residues has led to numerous attempts to predict individual residue-specific pKₐ values (12–16). pKₐ values are usually calculated from 3D structures using the Poisson–Boltzmann equation. However, variations occur between calculated and experimentally measured pKₐ values (13). Molecular dynamic simulations have also been used for such predictions, although this only gives rise to a marginal increase in accuracy (17).

As only a small handful of reviews have attempted to compile residue-specific protein pKₐ values (10,18,19), it was decided to develop a database that would serve as a standard compendium against which to compare new experimental or theoretical results. The PPD v1.0 contains >1400 amino acid pKₐ values, sourced from experimental data. Cross-references to several external databases—the Protein Data Bank (PDB) (20), the Enzyme Nomenclature and Classification database (21), the Protein–Protein Interaction database (22), the Reliable Protein Structure database (23), the Secondary Structure database (24), the OPM database (25), the SCOP database (26), the CATH database (27), the Pfam database (28), the SMART database (29), the PROSITE database (30), the DAS database (31), the Gene Ontology database (32), the GO Annot database (33), the GSD database (34), the MGD database (35), the SGD database (36), the YPD database (37), the KOG database (38), the Kyoto Encyclopedia of Genes and Genomes database (39), the SwissProt database (40), the Tamil Nadu Protein Database (41), the Swiss Institute of Bioinformatics database (42), the National Center for Biotechnology Information database (43), and the Global Protein Database (44)—give details of experimental techniques and links to other key databases, such as National Center for Biotechnology Information and the Protein Data Bank, providing the user with considerable background information. The database can be found at the following URL: http://www.jenner.ac.uk/PPD.

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(21) and the National Center for Biotechnology Information (NCBI) Entrez-Protein—have also been incorporated into the database.

DATABASE DEVELOPMENT

PPD v1.0 has been implemented using a PostgreSQL relational database, which provides an appropriate infrastructure for all foreseeable future developments of the archive. The data were initially compiled in a Microsoft ACCESS database after exhaustive searching of the primary literature, which included using keyword searches of the NCBI PubMed database (http://www.ncbi.nlm.nih.gov/pubmed). The PostgreSQL database is structured into seven normalized tables, populated from a flat-file export of the ACCESS database using PERL scripts integrated with SQL. As data are continually accumulating, archiving data is an on-going process: automatic, periodic updates will be made to the PostgreSQL database.

The PPD user interface is provided by a series of HTML pages. There are two searchable forms available within the PPD site. One offers either a broad or focussed PPD search. The other searches PPD using Basic Local Alignment Search Tool (BLAST). These forms target either a PERL/SQL script or a CGI script which in turn queries the database. The bespoke search engine facilitates fast, efficient and flexible data retrieval (Searching the Database). PPD is freely available on the world wide web (http://www.jenner.ac.uk/PPD).

DATABASE CONTENT

The data within PPD was sourced from the primary literature to give >1400 entries, containing pH values for >160 proteins (Table 1). The database contains pH values for amino acid side-chains, as well as the N- and C-termini. Data are archived for all amino acid residues, with the exception of methionine. However most entries focus on glutamate, lysine, histidine and aspartate, which together account for >75% of the data. As these four are all key ionizable residues, the apparent bias is not driven by our selection, but by the available experimental data. Very little data are currently available for arginine: its pK_a values from certain selected techniques: NMR spectroscopy, Raman Difference spectroscopy and UV spectroscopy.

Protein pK_a values are dependent on both intrinsic and extrinsic factors. Intrinsic factors include invariant properties of the protein investigated, such as sequence and structure. Extrinsic factors include the experimental conditions used, such as the temperature, the range of pH tested, protein concentrations as well as the experimental method. Thus we attempt to record all relevant experimental conditions when available. As logistic considerations preclude us from undertaking independent verification of the data, we are obliged to trust the values reported in the literature. It should be noted that the phenomenon of cooperative deprotonation can create circumstances under which pK_a values can not be used as a parameter that describes the ionization behaviour of the corresponding group (22–24).

SEARCHING THE DATABASE

Two methods to search PPD are available: an amino acid query-based interface (Figure 1) and a BLAST (25) interface. The implementation of a bespoke search system allows the user to perform extensive or focussed searches from a single user interface. The simplest search, using the amino acid query interface, would specify one amino acid residue only.

Table 1. Database summary

| Entry field         | Description                          |
|---------------------|--------------------------------------|
| Database entries    | 1401                                 |
| Proteins            | 163                                  |
| Total               | 146                                  |
| Sequences           | 115                                  |
| Enzymes             | 49                                   |
| Experiments         |                                      |
| Technique           | 13C* 1H* 15N* 2D* RS                 |
| Entries             | 235 780 46 112 56                    |
| Journals            | 189                                  |

RS = Raman Difference Spectroscopy and * = NMR spectroscopy.
A complex search would accommodate up to four amino acids and pKₐ ranges, along with experimental method, protein name and species. The search engine allows the choice of how results are presented. The default option returns amino acids and their associated properties (Figure 1B); while the second option returns proteins which contain the specified amino acids (Figure 1C).

The alternative search interface is based on BLAST (25). A local database of protein sequences found in PPD was compiled from SwissProt (26) and an additional postgreSQL table was created to hold this data. The local database is searched using the NCBI BLASTP and BLASTX programs (25), allowing input of either protein or nucleotide sequences. The HTML front-end connects to a web server-based PL/CGI script which interacts with the BLASTP or BLASTX programs. The output contains links to PPD entries, which are created using SwissProt (26) accession codes.

**FUTURE WORK**

There is an obvious need to extend the number of entries through continuous addition of data from new, and...
DISCUSSION AND CONCLUSIONS

The PPD is a unique compilation of protein pK\textsubscript{a} values sourced from experimental data only. PPD is novel: no database of its kind currently exists. Compared with other post-genomic databases, the size of PPD is limited, but this reflects its highly focused nature: the burgeoning of such focussed databases is a continuing trend in modern bioinformatics (27,28). The relatively modest size of the database will increase as new data is published.

Access to PPD data is given through an interface available via the world wide web and includes both a BLAST search and an amino acid query search system. The BLAST search, which is linked to pK\textsubscript{a} entries and external databases, allows PPD to be a cohesive and integrated source of protein information. PPD facilitates data-driven in silico prediction methods addressing the relationship between ionizable groups and protein function, be that protein–protein interaction, protein folding or enzyme catalysis.

A brief summary of pK\textsubscript{a} data for each amino acid is shown in Table 3, which also includes both the mean and SD of the corresponding measured pK\textsubscript{a} values. From the PPD data, we have shown the distribution of pK\textsubscript{a} values for the six most frequent residues: glutamic acid, lysine, tyrosine, aspartic acid, histidine and cysteine (Figure 2). Certain residues (aspartate, glutamate, lysine and histidine) have pK\textsubscript{a} values which show relatively narrow distributions, while other residues (cysteine and tyrosine) show a wider dispersion of values; however, this may only be a reflection of the amount of data available for these residues. While it is clear that mean values approximate closely model values, the corresponding SDs are high, reflecting the wide distribution of ionization states in

| Amino acids | Residue | Asp | Cys | Glu | His | Lys | Tyr | N-terminus | C-terminus | Number of entries | Mean pK\textsubscript{a} | SD |
|-------------|---------|-----|-----|-----|-----|-----|-----|------------|------------|-------------------|----------------|----|
| Aspartic    |         |     |     |     |     |     |     |            |            |                   | 282            | 3.6 | 1.43 |
| Lysine      |         |     |     |     |     |     |     |            |            |                   | 25             | 6.87| 2.61 |
| Histidine   |         |     |     |     |     |     |     |            |            |                   | 297            | 4.29| 1.05 |
| Glutamic    |         |     |     |     |     |     |     |            |            |                   | 404            | 6.33| 1.35 |
| Tyrosine    |         |     |     |     |     |     |     |            |            |                   | 207            | 10.45| 1.39 |
| Cysteine    |         |     |     |     |     |     |     |            |            |                   | 207            | 9.61 | 2.16 |
| N-terminus  |         |     |     |     |     |     |     |            |            |                   | 65             | 8.71 | 1.49 |
| C-terminus  |         |     |     |     |     |     |     |            |            |                   | 26             | 3.19 | 0.76 |

Table 3. pK\textsubscript{a} data associated with each amino acid

Figure 2. Distribution pattern of pK\textsubscript{a} values. Each column represents a count of pK\textsubscript{a} values for the specified amino acid and pK\textsubscript{a}.
actual proteins. Aspartate, for example, has a mean pK_a of 3.6 versus a model value of 4.0, yet the SD is 1.4. As the data for each residue increases, trends in residue-specific pK_a data will become more evident and more certain.

In recent years, there has been an impetus to accumulate data on all scales from the atomic to the genomic; this has led to a rapid increase in the number of databases. Databases are increasingly forming the backbone of science in general and post-genomic biology in particular. PPD v1.0 was developed to provide an easily accessible compilation of protein pK_a values. Despite the small size of PPD, the data it contains has utility throughout many different disciplines and, we may hope, the database will grow, through time, into a comprehensive protein pK_a resource.

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