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Investigation of Physicochemical Properties of Isoniazid and its Target Protein -Ligand Docking

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ABSTRACT: The continuing rise in tuberculosis (TB) incidence especially in the present of human immunodeficiency virus (HIV) infection and the problem of drug resistance strains towards isoniazid have prompted the research on new drug candidates and study the physicochemical properties of isoniazid target protein, as well as the mechanism of drug resistance. In this work, we calculated parameters such as atomic charges, energy (ΔE), chemical shift anisotropy (δ), asymmetry parameter (η),chemical shift anisotropy (Δσ), dipole orientation , isotropic, anisotropic, NMR determinant, distance matrix determinant, magnetic shielding (GIAO) for isoniazid and riphampin using the HF method with 3-21 G, 6-31G, basis set. Crystal structure of isoniazid target protein (PDB-entry: 1ENY) and the active site structure is characterized by iGEMDOCK. Bioinformatics computation for isoniazid target protein was carried out with ProtParam(www.expasy.org) that computes various physico-chemical properties that can be deduced from a protein sequence. The active site structure is characterized by five amino acids, GLy-14, THR-39, PHE 41, LEU-63 andILE-95 Docked by iGEMDOCK. Energies of docking was obtained fitness=- 75.55 VDW=- 55.29 Hbond =-19.25respectively for active site of isoniazid target protein. Estimated half-life was computed to be 4.4 hours The instability index (II) was computed to be 39.8 Aliphatic index: 100.60 This classifies the protein as stable. These investigations have showed that theoretical studies can be used to successfully explain biochemical difficulties. Similar with experimental methods, they deal assumptions and interpretation, and they have their limitations, but there are many problems that are solved using theory studies.

Keyword: Isoniazid, Target protein, NMR, Tuberclusis, ProtParam, IGEMDOCK

INTRODUCTION

Tuberculosis (TB) has plagued mankind for 5,000 years, dating back to the ancient Egyptians, and was recognized by Hippocrates in 460 AD as a deadly communicable disease, called phthisis or consumption (Osler and McCrae 1907). In 1882, Robert Koch discovered that the bacillus Mycobacterium tuberculosis (Mtb) was the causative agent of TB, noting that: "If the importance of a disease for mankind is measured from the number of fatalities which are due to it, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera, and the like. Statistics have shown that one seventh of all humans die of tuberculosis (Koch, 1982). Today, TB remains a devastating disease. The World Health Organization (WHO) estimates that 2 billion people are infected worldwide, with 8.8 million people becoming newly infected, and 1.4 million succumbing each year (WHO, 2000).

Today, combined administration of antibiotics constitutes the first line chemotherapeutic response to pan-susceptible TB. In particular, the WHO recommends a two-month treatment with isoniazid, pyrazinimide, ethambutol and rifampicin, followed by an additional four to seven months of isoniazid and rifampicin (Mouri, 2003). The combination of vigilant case detection and multidrug anti-TB chemotherapy led to significant declines in the incidence of TB, at least in industrialized countries, though the disease persisted in Africa, Asia, and Latin America, and in particular in those countries with the weakest economies and poorest population bases (WHO, 2011). In spite of several initiatives and research to improve the performance of national tuberculosis control programs, pulmonary tuberculosis remains an important health problem which disproportionately affects less developed countries. During 2011, the worldwide number of cases and deaths were estimated at 8.7 and 1.4 million, respectively (WHO, 2012). Isoniazid, the most powerful mycobactericidal drug available, ensures early sputum conversion and helps in decreasing the transmission of TB. Rifampicin, by its mycobactericidal and sterilising activities is crucial for preventing relapses.

Thus, isoniazid and rifampicin are keystone drugs in the management of TB. While resistance to either isoniazid or rifampicin may be managed with other first-line drugs, resistance to both isoniazid and rifampicin (MDR-TB) demands treatment with second-line drugs. These drugs have limited sterilising capacity and are not suitable for shortcourse treatment. Thus, patients with MDR-TB require prolonged treatment with drugs that are less effective and more toxic. Therefore, it is necessary to distinguish MDR-TB from mere drug-resistant tuberculosis by performing mycobacterial culture and sensitivity testing because the therapeutic implications are different (Sharma and Mohan, 2004).

Multidrug-resistant TB (MDR TB) is defined as Mycobacterium tuberculosis (*M. tuberculosis*) that is resistant at least to isoniazid and rifampicin. Extensively drug-resistant TB (XDR TB) is defined as *M. tuberculosis* resistant to isoniazid, rifampicin, any fluoroquinolone and at least one of three injectable second-line drugs (capreomycin, kanamycin and amikacin). As MDR TB and XDR TB have become more prevalent, our clinical and public health practices towards the Prevention and control of TB are challenged. In order to deal with these resistant forms of TB and avoid further MDR TB and XDR TB cases, a comprehensive approach, as with drug-susceptible TB, needs to be taken to ensure rapid detection, proper treatment and public health measures to cure the patients and prevent further transmission of the infection. Intensive case finding and contact tracing are key components of the public health action required to promptly detect infected individuals. However, the management of contacts of MDR TB and XDR TB patients is particularly challenging as the evidence base for best practises is very limited and there are few therapies available (van der *et al*, 2012).

Conventional approaches to discovering therapies for TB has met limited success, thus, demanding a more open and innovative strategy to capture the experience of experts and enthusiasm of young researchers at a global scale. To overcome this crucial bottleneck, OSDD is designed to function through a web-based platform where experts from academia, industry including individuals from varied subject areas can interact and contribute to solve complex problems associated with discovering novel therapies. The course from drug discovery to development is carefully planned, yet decentralized in nature (Anshu *et al.*, 2011). Multi-drug resistant (MDR) Tuberculosis refers to resistance in vitro to at least Isoniazide (INH) and rifampicin (RIF). Resistance to at least two drugs other than the combination of INH and

RIF is known as polyresistant tuberculosis and monoresistance is the resistance due to only one anti-tuberculosis medication. This definition is based on the fact that INH plus RIF is the most important combination and resistant to them will lead to failure of short course chemotherapy even though resistant to other drugs could also lead to failure (Evans, 2008).

Resistance to isoniazid in Mycobacterium tuberculosis can be mediated by substitution of alanine for serine 94 in the InhA protein, the drug's primary target. InhA was shown to catalyze the beta-nicotinamide adenine dinucleotide (NADH)-specific reduction of 2-trans-enoyl-acyl carrier protein, an essential step in fatty acid elongation. Kinetic analyses suggested that isoniazid resistance is due to a decreased affinity of the mutant protein for NADH. The three-dimensional structures of wild-type and mutant InhA, refined to 2.2 and 2.7 angstroms, respectively, revealed that drug resistance is directly related to a perturbation in the hydrogen-bonding network that stabilizes NADH binding (Dessen *et al.*, 1995).

Isoniazid is a key drug used in the treatment of tuberculosis. Isoniazid is a pro-drug, which, after activation by the katG-encoded catalase peroxidase, reacts nonenzymatically with NAD (+) and NADP(+) to generate several isonicotinoyl adducts of these pyridine nucleotides. One of these, the acyclic 4S isomer of isoniazid-NAD, targets the inhA-encoded enoyl-ACP reductase, an enzyme essential for mycolic acid biosynthesis in Mycobacterium tuberculosis (Argyrou *et al.*, 2006).

The resumption of tuberculosis led to an increased need to understand the molecular mechanisms of drug action and drug resistance, which should provide significant insight into the development of newer compounds. Isoniazid (INH), the most prescribed drug to treat TB, inhibits an NADH-dependent enoyl-acyl carrier protein reductase (InhA) that provides precursors of mycolic acids, which are components of the mycobacterial cell wall. InhA is the major target of the mode of action of isoniazid. INH is a pro-drug that needs activation to form the inhibitory INH-NAD adducts (Dias *et al.*, 2007).

An understanding of isoniazid (INH) drug resistance mechanism in Mycobacterium tuberculosis should provide significant insight for the development of newer anti-tubercular agents able to control INH-resistant tuberculosis (TB) (Oliveira *et al.*, 2006).

Tuberculosis, a global threat to public health, is becoming untreatable due to widespread drug resistance to frontline drugs such as the InhA-inhibitor isoniazid. Historically, by inhibiting highly vulnerable targets, natural products have been an important source of antibiotics including potent anti-tuberculosis agents (Hartkoorn *et al.*, 2012). Computational and molecular modeling tools have become a close counterpart to experiment in the understanding of molecular aspects of biological systems.

The computational approaches like homology modeling, molecular docking and quantitative structure activity relationships (QSAR) and molecular dynamics (MD) are widely employed to discover the novel hits for various therapeutic targets. In a recent report, we have highlighted the interface between computational approaches and experiment as crucial tools in the drug discovery machinery (Hezekiel *et al.*, 2015).

In order to be able to further our understanding of molecular recognition and to develop algorithms that improve scoring functions, it is necessary to have well established protein systems available for study. In this work we used protein systems, the isoniazid target protein, available in the protein data bank and a large number of known ligands that bind to them.

Protein-small molecule (referred to as "ligand" in what follows) interactions play central roles in numerous basic processes in life, such as enzyme catalysis, activation by naturally occurring ligands, and inhibition by human-designed drugs. Thus our capability of modeling such interactions at atomic resolution is crucial to enhance our understanding of biochemistry (Jens and David 2006).

Proteins play a pivotal, indeed essential role, in cellular (and in multicellular organisms, extracellular) activity. Their numerous biological functions together with the molecular basis of their biophysical properties behavior are therefore of multidisciplinary interest within the framework of what are generally called the biological (biochemistry, pharmacology, physiology, immunology, etc.) and physical (physics, chemistry, mathematics, computing, etc.) sciences.

Clearly, a theoretical description of the drug-target interactions would be of great value in explaining these apparently conflicting results. Here, we use ab initio methods on isoniazid and riphampin .In this work, we extend the iGEMDOCK approach to protein-ligand docking.

MATERIAL AND METHODS

A. Computational Methods

Gaussian computation

Stage 1: Start ChemDraw and construct molecules. Save the results as a ChemDraw file.

Stage 2: Reopen this file using Chem3D and perform an energy minimization. Then save the results as a gjf file.

Stage 3: Reopen this file using Gaussian 2009 and the calculations were performed using theGaussian 09 program suite. Gaussian is one of the most widely used quantum chemical program packages for molecular applications, and is used both in industry and in many scientific areas in academia. We have calculated the geometric parameters of the compounds in the ground state the using the Hartree-Fock (HFLee).

The calculation that you ask Gaussian to perform is distributed between many processors to get the answer faster. If you want to optimize geometry, it means that you want Gaussian to adjust the bond lengths, angles, and dihedrals to find the lowest energy conformation of molecule. The command to tell Gaussian to optimize the molecular geometry is “opt”. The Gaussian program does semi-empirical and ab initio calculations. In abinitio calculations the important integrals are done directly from first principles. First principles means that the integrals are done either using closed formulas or by doing the integrals numerically. The particular ab initio method that works best for calculating NMR properties. Finding a good geometry is called geometry optimization, so “OPT” are used as the keyword. We used Gaussian09 at the NMR shift calculation using the HF methods 3-21G and 6-31G, basis set [45]. There for “NMR” are used as the keyword. The calculation will generate an output file called NMR.out. The output file (NMR.out) contains a lot of information about the NMR shift calculation and are listed in the “GIAO Magnetic shielding tensor (ppm)”, such as σ Isotropic (ppm) and σ Anisotropic(ppm). As the usig the X, Y and z with Matlab program is solved determinant 3 3 and σ is calculated. Molecular orbital calculations can be used to get good estimates for chemical shifts. In this exercise we calculated the chemical shifts for each of the atom, then using the Excel program draw the diagrams which show chemical shifts for each of the atom. In the part of “GIAO Magnetic shielding tensor (ppm)” the using the σ Isotropic (ppm), σ Anisotropic(ppm) and Eigen values(σ11, σ22,σ33) are calculated parameters such as σ, η and Δσ. In this work, we calculated parameters such as atomic charges, energy (ΔE), chemical shift anisotropy (δ), asymmetry parameter (η), chemical shift anisotropy (Δσ), dipole orientation, isotropic, anisotropic, NMR determinant, distance matrix determinant, magnetic shielding (GIAO) for isoniazid and riphampin using the HF method with3-21 G, 6-31G, basis set.

IGEMDOCK docking (isoniazid for isoniazid target protein)

Structure-based virtual screening and post-screening analysis are emergent tasks in Computer-based drug discovery. Combining these two methods to effectively reduce the false positives from a large compound database is considered as a key step to finding the lead compounds.

In this study, we have developed a graphical-automatic drug discovery system, called iGEMDOCK, for integrating docking, screening, post-analysis, and visualization.

To our best knowledge, iGEMDOCK is the first system for this requirement able to be directly visualized by a molecular visualization tool and analyzed by post-analysis tools.

iGEMDOCK provides the post-analysis tools by using k-means and hierarchical clustering methods based on the docked poses (i.e. protein-ligand interactions) and compound properties (i.e. atomic compositions)(18).

We provide a structure (crystal structure of isoniazid target protein, PDB code: 1ENY) in the folder .we have done the following steps:

1. Pressing the button "Prepare Binding Site" on the "Protein Ligand Docking/Screening tag"
2. Browsing and selecting the protein file (\examples\protein\1ENY.pdb)
3. Defining the binding site type as "By bounded ligand"
4. Defining the center of binding site by selected ligand "ISONIAZID"
5. Setting the size of binding site (e.g. 8 angstrom) by the extended radius from selected ligand "THM"

Bioinformatics computation for isoniazid target protein

ProtParam (www.expasy.org) computes various physico-chemical properties that can be deduced from a protein sequence. No additional information is required about the protein under consideration. The protein can either be specified as a Swiss-Prot/TrEMBL accession number or ID, or in form of a raw sequence. White space and numbers are ignored. If you provide the accession number of a Swiss-Prot/TrEMBL entry, you will be prompted with an intermediary page that allows you to select the portion of the sequence on which you would like to perform the analysis. The choice includes a selection of mature chains or peptides and domains from the Swiss-Prot feature table (which can be chosen by clicking on the positions), as well as the possibility to enter start and end position in two boxes. By default (i.e. if you leave the two boxes empty) the complete sequence will be analyzed.

Note: It is not possible to specify post-translational modification for your protein, nor will ProtParam know whether your mature protein forms dimers or multimers. If you do know that your protein forms a dimer, you may just duplicate your sequence (i.e. append a second copy of the sequence to the first), as all computations performed by ProtParam are based on either compositional data, or on the N-terminal amino acid.

RESULTS AND DISCUSSIONS

Fig. 1 shows the 3D dimensional structure of isoniazid target protein. This model indicated to attachment of isoniazid to target protein. Docking methods are use to identify possible drugs against a given protein target. This experiment is designed to mimic the situation in drug discovery where a cry with a ligand bound in the binding site is frequently known. After giving a set of poses, iGEMDOCK will recalculate the energy of each pose and list on the summarized Table 2. The interaction data includes summarized energy and individual energy terms. Fitness is the total energy of a predicted pose in the binding site.

Fig. 1. 3D dimensional structure of isoniazid target protein.

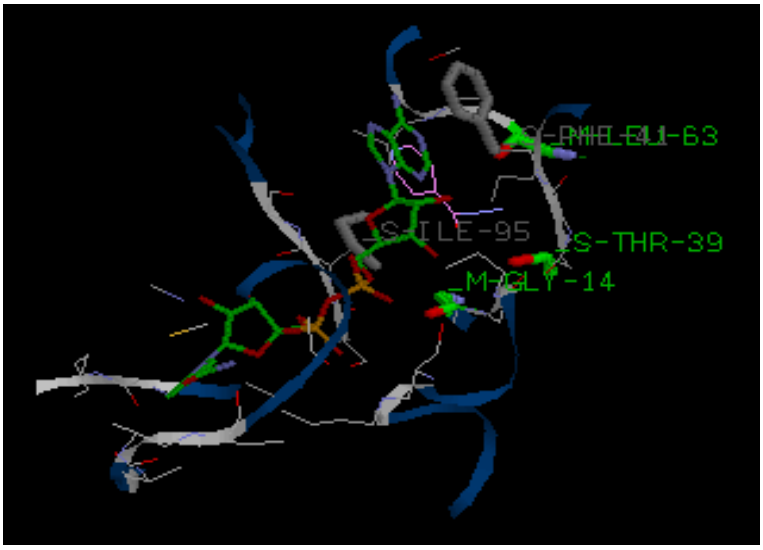

Fig. 2. Crystal structure of isoniazid target protein (PDB-entry: 1ENY). The active site structure is characterized by five amino acids, GLy-14, THR-39, PHE 41, LEU-63 andILE-95 Docked by iGEMDOCK.

Table 1: Summarized energy vdW + Hbond of protein -ligand (isoniazid) interaction.

Ligand	Fitness	VDW	HBond
cav1ENY_NAD-ISONIAZID-0.pdb	-75.55	-56.29	-19.25

Compound	fitness	H-M GLY 14	H-S THR 39	H-M LEU 63	V-S PHE 41	V-S ILE 95
		☑	☑	☑	☑	☑
cav1ENY_NAD-ISONIAZID-0...	-75.5	-3.5	-6.7	-7	-10.5	-9

The empirical scoring function of iGEMDOCK is estimates as: Fitness = vdW + Hbond + Elec. Here, the vdW term is van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively. Here, we represent of interaction profile of 1ENY and the ligand 1ENY-ISONIAZID. Such interactions will be measured by GEMDOCK scoring function and outputted onto the Table.

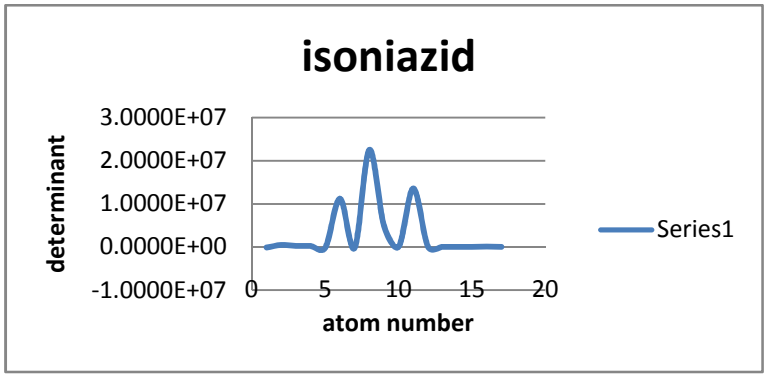

Fig. 3. The shielding tensor determinant of isoniazid calculated with HF/3-21G.

The chemical shielding refers to the phenomenon which associated with the secondary magnetic field created by the induced motions of the electrons that surrounding the nuclei when in the presence of an applied magnetic field. The energy of a magnetic moment μ, in a magnetic field, B, is as follow: E = -μ. (1 - σ) B where the shielding μ, is the differential resonance shift due to the induced motion of the electrons. The chemical shielding is characterized by a real three-by-three Cartesian matrix, which can be decomposed into a single scalar term, three antisymmetric pseudo vector components, and five components corresponding to a symmetric tensor .Only the single scalar and the five symmetric tensor elements can be observed in the normal NMR spectra of the solids. For brevity, these six values are usually referred to as the shielding tensor:

$$\begin{bmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{zx} & \sigma_{zy} & \sigma_{zz} \end{bmatrix}$$

That can be obtained by averaging the off-diagonal values of the complete tensor. The chemical shielding tensor is commonly referred to the chemical shift anisotropy (CSA) tensor according to the possession of second rank properties. The measurement or calculation of the diagonal components (σxx, σyy, σzz) or (σ11, σ22 ,σ33) in the principle axis system (PAS) allows the complete description of the CSA tensor [sandia]. The CSA tensor can also be described by three additional parameters:

a) The isotropic value (or trace portion of the CSA tensor) σ_{iso}, of the shielding tensor which is defined as $\sigma_{iso} = 1/3(\sigma 11 + \sigma 22 + \sigma 33)$

b) The anisotropy (Δσ) of the tensor, due to the following expression: $\Delta\sigma = \sigma 33 - 1/2\ (\sigma 11 + \sigma 22)$ (4) and

c) The shielding tensor asymmetry parameter (η) which is given by:

$$\eta = \frac{|\sigma_{22} - \sigma_{11}|}{|\sigma_{33} - \sigma_{iso}|}$$

The extinction coefficient indicates how much light a protein absorbs at a certain wavelength. It is useful to have an estimation of this coefficient for following a protein which a spectrophotometer when purifying it.

It has been shown that it is possible to estimate the molar extinction coefficient of a protein from knowledge of its amino acid composition.

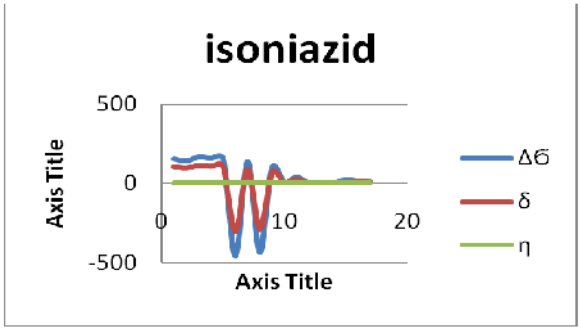

Fig. 4. The shielding tensor elements of ionized calculated with HF/3-21G.

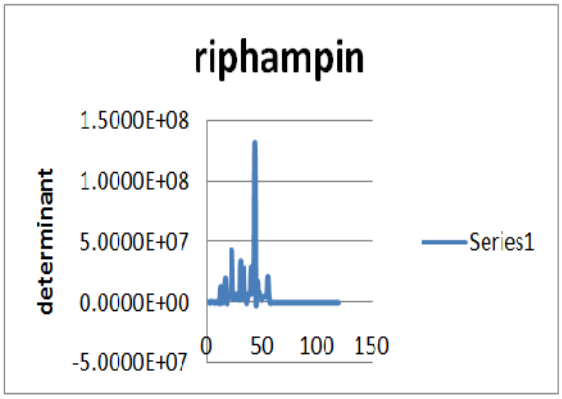

Fig. 5. The shielding tensor -determinant of riphampin calculated with HF/3-21G.

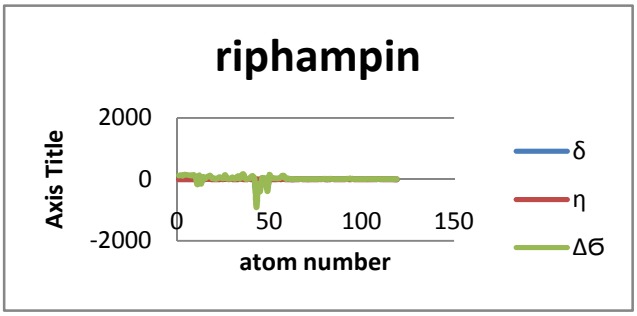

Fig. 6. The shielding tensor elements of riphampin calculated with HF/3-21G.

From the molar extinction coefficient of tyrosine, tryptophan and cystine (cysteine does not absorb appreciably at wavelengths >260 nm, while cystine does) at a given wavelength, the extinction coefficient of the native protein in water can be computed using the following equation:
E(Prot) = Numb(Tyr)*Ext(Tyr) + Numb(Trp)*Ext(Trp) + Numb(Cystine)*Ext(Cystine)
Estimated half-life:
The N-terminal of the sequence considered is A (Ala).The estimated half-life is: 4.4 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (*Escherichia coli*, in vivo).
The half-life is a prediction of the time it takes for half of the amount of protein in a cell to disappear after its synthesis in the cell. ProtParam relies on the "N-end rule", which relates the half-life of a protein to the identity of its N-terminal residue; the prediction is given for 3 model organisms (human, yeast and *E. coli*). The N-end rule (Yan and Chen 2004) originated from the observations that the identity of the N-terminal residue of a protein plays an important role in determining its stability in vivo, (Tobias *et al.,* 1991). The rule was established from experiments that explored the metabolic fate of artificial beta-galactosidase proteins with different N-terminal amino acids engineered by site-directed mutagenesis. The beta-gal proteins thus designed have strikingly different half-lives in vivo, from more than 100 hours to less than 2 minutes, depending on the nature of the amino acid at the amino terminus and on the experimental model (yeast in vivo; mammalian reticulocytes in vitro, *Escherichia coli* in vivo). In addition, it has been shown that in eukaryotes, the association of a destabilizing N-terminal residue and of an internal lysine targets the protein to ubiquitin-mediated proteolytic degradation Note that the program gives an estimation of the protein half-life and is not applicable for N-terminally modified proteins.

Instability index: The instability index provides an estimate of the stability of your protein in a test tube. Statistical analysis of 12 unstable and 32 stable proteins has revealed (Guruprasad *et al.,* 1990). that there are certain dipeptides, the occurence of which is significantly different in the unstable proteins compared with those in the stable ones. The authors of this method have assigned a weight value of instability to each of the 400 different dipeptides (DIWV). Using these weight values it is possible to compute an instability index (II) which is defined as: I = L-1, II = (10/L)* Sum DIWV(x[i]x[i+1 I = 1 where: L is the length of sequence DIWV(x[i]x[i+1]) is the instability weight value for the dipeptide starting in position i.
The instability index (II) is computed to be 39.8. This classifies the protein as stable.

Aliphatic index: 100.60
The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). It may be regarded as a positive factor for the increase of thermostability of globular proteins. The aliphatic index of a protein is calculated according to the following formula (Ikai,1980):
Aliphatic index = X(Ala) + a * X(Val) + b * (X(Ile) + X(Leu))
Where X (Ala), X (Val), X (Ile), and X (Leu) are mole percent (100 X mole fraction)
of alanine, valine, isoleucine, and leucine.
The coefficients and b are the relative volume of valine side chain (a = 2.9). And of Leu/Ile side chains (b = 3.9) to the side chain of alanine.

CONCLUSION

These investigations have showed that theoretical studies can be used to successfully explain biochemical difficulties. Similar with experimental methods, they deal assumptions and interpretation, and they have their limitations, but there are many problems that are solved using theory studies. Consequently, theoretical calculations are a competitive alternative to experiments and to successfully assumptions and also interpretation for biochemical investigations.

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