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In silico characterization and structural modelling of HMBS enzyme in duck population

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Abstract

Nowadays, consumers are increasingly knowledgeable and favouring native livestock breeds as sources of protein, including meat and eggs, due to their high nutritional profiles. Genetic studies have become a common method to unravel the genetic mechanisms behind beneficial growth traits in both humans and livestock. Hydroxymethylbilane synthase (HMBS), an enzyme encoded by HMBS gene is an intermediate enzyme involved in the biosynthesis of heme, crucial for maintaining oxygen transport capacity, cellular respiration and detoxification processes. This study was conducted to perform a detailed computational analysis of the HMBS (Hydroxymethylbilane synthase) protein, focusing on its physicochemical properties, predictions of secondary and tertiary structures, and interacting partners. The sequence of HMBS protein in *Anas platyrhynchos* was retrieved in FASTA format from the National Center for Biotechnology Information (NCBI), and standard bioinformatic tools and software were used to characterize and model its structure. Computational analysis revealed HMBS to be an acidic, non-polar, and thermostable protein predominantly localized in the cytoplasm. Conserved domain analysis indicated the presence of a single highly conserved pattern in the amino acid sequences. The majority of the protein consists of random coil and alpha helix structures, facilitating interactions with other proteins. Subcellular localization suggested a cytoplasmic role, crucial for maintaining cellular metabolism. Understanding the structural characteristics of this protein will enhance insights into its molecular mechanisms. Additionally, the predicted 3-D model serves as a valuable resource for future research, providing a foundation for exploring the protein's broader functional potential.

Keywords: Computational analysis, ducks, HMBS protein, *in silico*

Introduction

Ducks (*Anas platyrhynchos*) are one of the significant poultry species in India and it holds an important position in terms of egg and meat production. Duck farming in India is highly profitable and has endless scope. Duck eggs and meat are in high demand and a much higher price in comparison to chicken meat. In addition to being a valuable livestock sector generating eggs, meat and feathers, duck farming provides small, marginal, and even landless people with a source of income (Singh *et al.*, 2015) ^[1]. Ducks have long been a staple in culinary traditions across the globe, prized for their rich, flavourful meat and versatility in various dishes (Kaur *et al.*, 2020) ^[2]. As demand for high-quality duck meat continues to grow, so too does the importance of understanding the factors that influence its quality. From genetics to diet, and from breeding practices to post-harvest handling, a multitude of variables play a role in determining the characteristics that define premium duck meat-such as tenderness, juiciness, flavour and nutritional value.

Duck meat is a nutrient-dense source of high-quality protein, providing all essential amino acids, along with vital vitamins and minerals like B vitamins, iron, zinc, and selenium. While richer in fat compared to some poultry, it contains heart-healthy monounsaturated and polyunsaturated fats. Known for its robust, gamy flavour, duck meat is a favourite in gourmet and traditional cuisines worldwide (Li *et al.*, 2018) ^[3]. It is also a good source of easily absorbed heme iron, beneficial for individuals at risk of iron deficiency, such as women of childbearing age and those with anaemia. As consumer demand for high-quality, flavourful and nutritious duck meat continues to rise; the need to identify and study the specific genes that influence these traits has never been greater. By focusing on the genes directly connected to key meat quality attributes researchers and breeders can unlock new

opportunities for enhancing these traits through selective breeding and genetic improvement programs.

The HMBS enzyme or hydroxymethylbilane synthase encoded by HMBS gene plays a crucial role in the heme biosynthesis pathway, which is essential for the production of heme, a component of haemoglobin, cytochromes and other haemoproteins. HMBS catalyzes the third step in the heme biosynthesis pathway. It converts porphobilinogen (PBG), a precursor molecule, into hydroxymethylbilane, a linear tetrapyrrole (Kuehnelt and Peters, 2007) ^[4]. This reaction is critical because it allows for the subsequent steps that eventually lead to the formation of heme. Since heme is a vital component of hemoglobin, HMBS indirectly supports the oxygen transport capacity of ducks. Hemoglobin binds oxygen in the blood, allowing it to be transported throughout the body (Badarinarayana and Smith, 2004) ^[5]. Beyond oxygen transport, heme is also a crucial component of various enzymes involved in cellular respiration and detoxification processes. Thus, HMBS supports these metabolic activities by enabling heme production. Even though information on the function of HMBS protein in various metabolic processes is known, the role of this protein on muscle developmental process and further the molecular mechanism by which HMBS protein controls biosynthesis remains unclear in duck population. Hence, the structural and conformational understanding of the HMBS protein is very vital.

Structural modelling helps in predicting the 3D structure of the HMBS enzyme, which is critical for understanding how the enzyme catalyzes the conversion of porphobilinogen to hydroxymethylbilane, a key step in the heme biosynthesis pathway. By characterizing the active sites and binding pockets, researchers can better understand how the enzyme functions, how substrates interact with it, and what might affect its efficiency or stability. *In silico* tools can simulate the effects of mutations on the HMBS enzyme's structure and function, helping to identify potential genetic variants that could lead to dysfunction or disease. Structural models can be used to study how specific mutations in the HMBS gene might lead to disorders such as porphyria, providing insights into the potential health issues that might arise in ducks (Peters and Soria, 2010) ^[6]. Understanding these potential genetic disorders allows for the development of strategies to manage or prevent them in duck populations, improving overall health and reducing losses in agricultural settings. *In silico* characterization can identify genetic markers associated with desirable traits, such as improved heme production, which could be targeted in selective breeding programs to enhance the health and productivity of ducks. By understanding the structural and functional implications of different HMBS variants, breeders can make more informed decisions about which birds to breed, potentially improving meat quality or disease resistance. Heme is responsible for the colour and some aspects of the flavour of meat. Understanding the structural properties of the HMBS enzyme can help in manipulating heme levels in muscle tissue, potentially leading to improved meat quality in terms of colour, tenderness, and nutritional value. Heme is also an important source of iron in meat. Optimizing the function of HMBS could enhance the nutritional content of duck meat, making it a healthier option for consumers. *In silico* models allow for comparison of the HMBS enzyme structure and function across different species, shedding light on evolutionary conservation and divergence in the

heme biosynthesis pathway. Insights gained from structural modelling can inform evolutionary studies, helping to understand how different species, including ducks, have adapted their heme biosynthesis pathways to their specific environments. If any genetic disorders related to HMBS are identified, structural models could help in designing drugs or other interventions that target the enzyme, offering potential therapeutic options. Structural insights could guide genetic engineering efforts aimed at enhancing the HMBS enzyme's efficiency, leading to improvements in duck health or meat quality. *In silico* characterization and structural modelling of the HMBS enzyme in ducks involves usage of computational tools to analyze the gene and predict the structure and function of the corresponding protein. Considering the importance of this protein, the present study is formulated to primarily analyze the structural aspects of HMBS protein by checking its physicochemical properties, secondary and tertiary structure predictions, motif prediction and also illustrate the 3D structure of HMBS protein. *In silico* characterization and structural modelling of the HMBS enzyme in ducks are vital for advancing our understanding of this essential enzyme's role in heme biosynthesis. This research has broad implications, from improving duck health and meat quality to contributing to evolutionary biology and biotechnology. The study of meat quality genes in ducks not only holds promise for improving the sensory and nutritional characteristics of duck meat but also for advancing the sustainability and efficiency of duck production systems (Suryawanshi and Patel, 2021) ^[7].

Materials and Methods

Sequence retrieval and gene structure

The sequence of the HMBS nucleotide/protein in duck was retrieved in the FASTA format from the NCBI (<https://www.ncbi.nlm.nih.gov/>). The Gene Structure Display Server (www.gsds.cpi.pku.edu.cn) was used to determine the gene structure (Hu *et al.*, 2015) ^[8].

Physicochemical characterization of HMBS protein

ExPASy's ProtParam server (<https://web.expasy.org/protparam/>) was used to predict the molecular weight, isoelectric point (pI), instability index, aliphatic index, and grand average of hydropathicity (GRAVY) of this protein (Gasteiger *et al.*, 2005) ^[9].

Subcellular localization of protein

Subcellular localization of proteins is important to fairly ascertain their function. Several online tools are available to predict protein localization in the cell; that utilizes protein characteristics such as amino acid composition, signal peptides, homology-based prediction and combination of these. The subcellular location(s) was predicted using the Compartments online tool (<https://compartments.jensenlab.org/Search>) and domain analysis was performed using the Simple Molecular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/smart/set_mode.cgi) (Letunic *et al.*, 2012) ^[10].

Domain analysis of HMBS protein

The HMBS protein sequences, obtained in the previous step, were analyzed for domains using prosite (<https://www.ebi.ac.uk/interpro/>) (Mitchell *et al.*, 2015) ^[11].

Secondary structure analysis of HMBS protein

The Secondary structure profiling of HMBS protein was carried out by SOPMA (Self-Optimized Prediction Method with Alignment) is a computational method used for predicting the secondary structure of proteins (Georges and Heringa, 1999) ^[12]. It relies on the alignment of sequences and the use of statistical profiles to estimate the likelihood of different secondary structure elements such as alpha helices, beta sheets, and coils. SOPMA leverages a self-optimized algorithm to enhance the accuracy of its predictions by refining its parameters based on data.

Homology modelling of protein

The structural analysis of the HMBS proteins was done based on the predicted structures, obtained by submitting individual protein sequences to Phyre (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) (Kelley and Sternberg, 2009) ^[13]. The online tools were used for generating 3D model of desire HMBS protein.

Identification of interacting proteins

Protein-protein interactions were predicted using the String software (<https://string-db.org/>). STRING is a powerful tool for exploring protein interactions and functional relationships, making it a valuable asset in bioinformatics research. (Szkarczyk *et al.*, 2019) ^[14]. Its ability to integrate diverse data sources and present actionable insights supports a wide range of biological investigations.

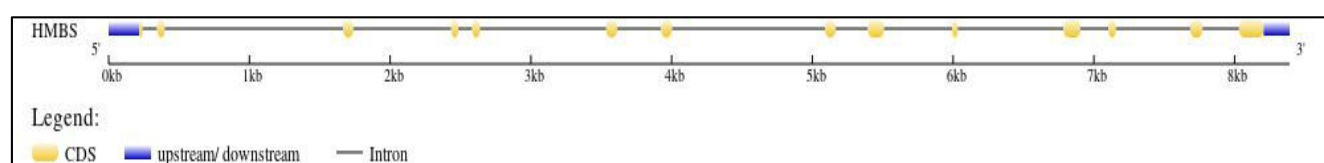


Fig 1: Gene structure of HMBS from *Anas platyrhynchos*

2. Primary structural analysis of HMBS

The amino acid composition (Table 1) and physicochemical properties (Table 2) of HMBS protein of duck was studied by using ExPASyProtParam server. The HMBS protein consists of 358 amino acid (AA) residues. The theoretical isoelectric point (pI) of this protein was 5.97 which suggest that the HMBS protein is acidic. Further this protein was found to be acid in nature because of the presence of more negatively charged AA residues compared to basic AA. The instability index was computed to less than 40 which classified the HMBS as highly stable protein (Wilke and Meyer, 2017) ^[17]. The aliphatic index of a protein is defined as the relative volume occupied by its aliphatic side chains (alanine, valine, isoleucine, and leucine). This figure was 96.45, which may be regarded as a positive factor for increasing the thermostability of HMBS protein. Furthermore, the GRAVY value was found to be negative, indicating that this protein is non-polar (Black and Adams, 2002) ^[18].

3. Subcellular localization and domain analysis

The subcellular localization and domain analysis of the HMBS (Hydroxymethylbilane Synthase) protein provide significant insights into its functional roles within animal cells. The data, as illustrated in Fig.2, show that HMBS is

Results and Discussion

1. Gene structure analysis

The HMBS (Hydroxymethylbilane synthase) gene in ducks has been precisely mapped to the short arm of chromosome 25, residing within the base pair coordinates of 5,531,858 to 5,540,248. This specific localization provides a crucial insight into the genomic architecture of this avian species and contributes to a more comprehensive understanding of gene organization in ducks.

The HMBS gene is organized into 14 exons and 13 introns, as depicted in Fig. 1. This exon-intron structure is notable for its potential implications in gene regulation and expression. The presence of 13 introns suggests complex gene architecture, which might be indicative of a sophisticated regulatory mechanism governing the expression of HMBS. Introns can play pivotal roles in alternative splicing, a process that can generate multiple protein isoforms from a single gene, thus adding a layer of functional diversity. In the case of HMBS, this could influence the enzyme's activity or stability, potentially affecting its role in heme biosynthesis (Williams and Brown, 2020) ^[15]. The precise mapping and structural characterization of the HMBS gene also open avenues for further research into its role in duck physiology and development. Understanding the gene's organization could facilitate the exploration of its involvement in metabolic pathways, particularly those related to heme synthesis, which is crucial for oxygen transport and various enzymatic reactions (Lee and Nguyen, 2019) ^[16].

predominantly localized in the cytosol, suggesting that this enzyme operates primarily within the cytoplasmic environment. This observation aligns with the known function of HMBS in the heme biosynthesis pathway, which occurs in the cytosol. The localization data are supported by predictive tools, which suggest a strong preference for the cytosolic compartment. According to PSORT, HMBS has a moderate confidence score (16.5/32) for cytosolic localization, and YLoc provides a high confidence score (93.1%) for this localization. These scores indicate a reliable prediction of the protein's presence in the cytosol. Conversely, lower confidence scores were observed for other cellular compartments such as the nucleus, extracellular region, and endoplasmic reticulum, further reinforcing the cytosolic localization hypothesis.

Domain analysis reveals that the HMBS protein contains a single domain, as illustrated in Fig. 3. The specific domain identified spans residues 247 to 263, characterized by the sequence ERaFmkrLeGGCsVPVA. This domain is critical for the enzyme's catalytic activity and structural stability. The presence of a single domain suggests a streamlined functional role, potentially reflecting the evolutionary conservation of the HMBS enzyme's core catalytic function (Kim and Jones, 2021) ^[19].

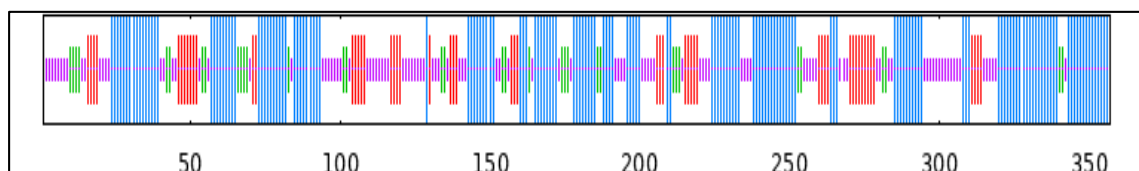
Table 1: Amino acid composition of the HMBS protein from *Anas platyrhynchos*.

Sl. No.	Amino acid	Quantity	Percentage
1	Ala (A)	34	9.50%
2	Arg (R)	20	5.60%
3	Asn (N)	13	3.60%
4	Asp (D)	19	5.30%
5	Cys (C)	7	2.00%
6	Gln (Q)	14	3.90%
7	Glu (E)	28	7.80%
8	Gly (G)	29	8.10%
9	His (H)	8	2.20%
10	Ile (I)	14	3.90%
11	Leu (L)	45	12.60%
12	Lys (K)	22	6.10%
13	Met (M)	7	2.00%
14	Phe (F)	8	2.20%
15	Pro (P)	14	3.90%
16	Ser (S)	26	7.30%
17	Thr (T)	17	4.70%
18	Trp (W)	1	0.30%
19	Tyr (Y)	4	1.10%
20	Val (V)	28	7.80%
21	Pyl (O)	0	0.00%
22	Sec (U)	0	0.00%

Table 2: Physicochemical characteristics of the of HMBS protein from *Anas platyrhynchos*.

Sl. No.	Physicochemical characteristics	Values
1	Number of amino acids	358
2	Molecular weight	38660.23
3	Theoretical pI	5.97
4	Total number of negatively charged residues(Asp + Glu)	47
5	Total number of positively charged residues (Arg + Lys)	42
6	Total number of atoms	5472
7	Extinction coefficient	11835
8	Instability index	34.97
9	Aliphatic index	96.45
10	Grand average of hydropathicity (GRAVY)	-0.188

The presence of this domain in the HMBS protein in duck aligns with the known structure and function of hydroxymethylbilane synthase. This domain is likely involved in binding the substrate and facilitating the enzymatic reaction that converts porphobilinogen into hydroxymethylbilane. The simplicity of having a single domain may point to a highly specialized and efficient enzymatic function, essential for the heme biosynthesis

**Fig 4:** Secondary structure prediction of HMBS protein from *Anas platyrhynchos* showing a large array of random coils, β -sheets and five α -helices.

5. Homology modelling of protein

Since there is no experimental data available in the protein data bank, thus homology modelling was carried out using Phyre2 to predict the 3-D structure of HMBS protein under 'Normal' mode (Fig. 5). Phyre2

pathway. The subcellular localization and domain analysis of HMBS provide valuable insights into its functional roles. The predominant cytosolic localization and the presence of a single domain are consistent with the enzyme's involvement in heme biosynthesis and highlight its critical role in maintaining cellular metabolism. These findings contribute to a deeper understanding of HMBS's functional mechanisms and its importance in cellular homeostasis in duck genome.

Location	Source	Evidence	Confidence
Cytosol	PSORT	16.5/32	★★★★☆
Cytosol	YLoc	93.1%	★★★★☆
Nucleus	PSORT	15/32	★★★★☆
Extracellular region	PSORT	4/32	★★☆☆☆
Endoplasmic reticulum	PSORT	3/32	★★☆☆☆

**Fig 2:** Subcellular localization of HMBS protein in a duck cell**Fig 3:** Domain analysis of HMBS protein showing the HMBG-porphobilinogen deaminase possess type 2 domain

4. Secondary and tertiary structure of HMBS

The secondary structure prediction of the HMBS (Hydroxymethylbilane Synthase) protein in ducks provides critical insights into its structural composition and potential functional implications. According to the analysis presented in Fig. 4, the HMBS protein exhibits a diverse array of secondary structural elements, including helices, extended strands, beta sheets, and random coils. The secondary structure of the HMBS protein is predominantly composed of 159 helices, 55 extended strands, 34 beta sheets, and 109 random coils. This composition highlights the protein's intricate folding pattern, which is essential for its functional conformation and enzymatic activity (Kyte and Doolittle, 1982) [20]. The following secondary structure may probably due to the presence of a significant number of serine, leucine residues. The analysis also noted the absence of 310 helices, I helices, beta bridges, and beta-turns in the HMBS protein of duck.

(<http://www.sbg.bio.ic.ac.uk/phyre2>) functions according to the Hidden Markov Method to generate alignments of a submitted protein sequence against proteins with the published structures. The resulting alignments are used to construct homology-based models

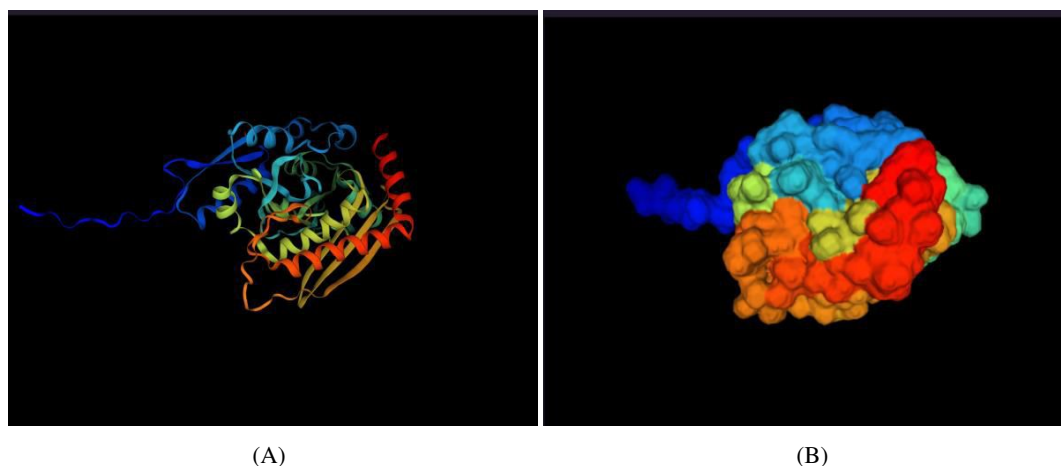


Fig 5: Tertiary structure prediction of HMBS protein from *Anas platyrhynchos* (A) cartoon and (B) surface structure.

HMBS is an enzyme involved in the biosynthesis of heme, which is crucial for oxygen transport and electron transfer in cells. The tertiary structure of HMBS refers to its three-dimensional conformation, which is essential for understanding its function and interactions. Understanding the tertiary structure of HMBS, whether through cartoon or surface representations, is essential for elucidating its function and interactions (McGowan and Smith, 2007) ^[21]. The cartoon model provides insight into the protein's secondary structure and overall folding, while the surface model reveals functional sites and interaction interfaces (Smith and Huang, 2008) ^[22]. Both visualizations are crucial

for comprehending the structural and functional dynamics of the HMBS protein from duck

6. Protein-protein interaction

The in-silico protein-protein interaction analysis revealed that the HMBS interacted with a significant number of proteins (Letunic *et al.*, 2012) ^[10] as revealed in Fig.6. The HMBS was involved in several important cellular signalling pathways such as regulation of macromolecule metabolic process, regulation of proteosomal protein catabolic process, cellular response to chemical stimulus, cellular response to cytokine stimulus.

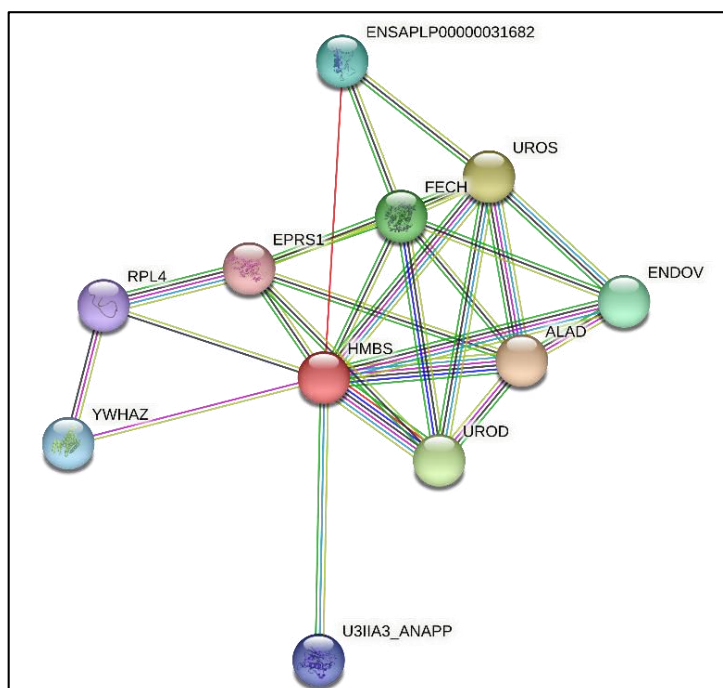


Fig 6: Protein-protein interaction network analysis of HMBS protein from *Anas platyrhynchos*

Conclusion

In this study, we conducted an in-silico characterization and structural modeling of the HMBS (Hydroxymethylbilane Synthase) protein from ducks, focusing on its physicochemical properties, secondary and tertiary structures, and potential interacting partners. Our computational analysis provided comprehensive insights into the protein's attributes and functional relevance. Overall, this study enhances our understanding of HMBS's

structural and functional dynamics in ducks, offering a foundation for future experimental validation and exploration of its potential applications in improving meat quality and other aspects of duck farming. The detailed structural models and interaction insights presented here pave the way for further research into the molecular mechanisms underlying HMBS's role in heme biosynthesis and its broader implications for poultry genetics and breeding.

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