ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2018; 4(9): 280-282
www.allresearchjournal.com
Received: 21-07-2018
Accepted: 26-08-2018

Chandra Mohan
Research Scholar, Department of Zoology, Patna University, Patna, Bihar, India

Corresponding Author:
Chandra Mohan
Research Scholar, Department of Zoology, Patna University, Patna, Bihar, India

Studies of some trematode parasites based on amino acid fragments

Chandra Mohan

Abstract
The low molecule weight hemoglobin can be contrasted with high molecular weight hemoglobin of invertebrates. The random distribution and wide diversity in the nature of invertebrate hemoglobin implies an independent line of evolution of protein. In vertebrate, hemoglobin and myoglobin are the respiratory pigment of the blood and muscles respectively. The main objective in this paper is to pursue molecular relationship with some Trematode Parasites based on amino acid fragments.

Keywords: Trematode Parasites and Amino Acid Fragments

Introduction
Most invertebrate hemoglobin is extracellular and possesses coparatively high molecular weight and low isoelectric points compared with intracellular hemoglobins of vertebrates. However, hemoglobin of relatively low molecular weight is found in Chironomus sp., some annelids, and some nemerteans and in the lamellibrach molluscs, *Arca sp*. In both *Arca sp*., and polycheate *Notomastus sp*., the hemoglobin with the molecular weight of about 30 kDa is contained within the corpuscles. This low molecular weight hemoglobin can be contrasted with high molecular weight hemoglobins of invertebrates such as *Daphnia sp*., having 360 kDa MW and *Planorbis carneres* 300 kDa (Wayman, 1948) [1].

The random distribution and wide diversity in the nature of invertebrate hemoglobin implies an independent line of evolution of this protein. In vertebrate, hemoglobin and myoglobin are the respiratory pigment of the blood and muscles respectively. In most vertebrate the hemoglobin is tetrameric, each molecule consisting of four globin chains, each chain associated with a heme group (Brounitzer, 1958; Muller, 1961 a, b) [2, 3, 4]. The mass of vertebrate hemoglobin ranges from 61kDa to 72 kDa but cosiderable differences in the primary structures of their globin chains in higher vertebrates, the isoelectric pH is restricted to a range of 6.9 – 8.0 (Gratzer and Allison, 1960) [5]. An important parameter of hemoglobin function is its oxygen affinity. The oxygen tension at half saturation; the P_{50} is generally used as an index of oxygen affinity. Affinity of most of the parasites is so high that they are completely saturated with oxygen at only 1 or 2 partial pressure.

A plot of 1% saturation of hemoglobin against the corresponding partial pressure of O_2 is known as an oxygen equilibrium curve. The O_2 tension of the half saturation, the P_{50} O_2 is generally used as a convenient index of the oxygen affinity. Although it is supposed that monoheme combines with oxygen in accordance with the "law of mass action" many multiheme pigment do not. The deviation is termed as heme-heme interaction i.e.; one heme affects other (cf. the Bohr effect). The equilibrium curve of hemoglobin is very closely approximated by the Hill equation--

Y/100	=	K pn /1+ K pn
Log y/ 100-y	=	log K + n log p
Where Y	=	percentage O2 saturation
K	=	overall equilibrium constant
P	=	partial pressure of oxygen (Generally expressed in mm)
n	=	Hill constant

The constant n is the characteristics of hemoglobin. When n = 1, there is no interaction.

Material and Methods

The trematode were immedialtely brought to the laboratory, washed in normal saline at room tempreture to shed their eggs as well as regurgitate the gut contents and then in tyrode saline prepared in distilled water, a few amount of glucose is also added to keep the parasite active. Finally parasite rinsed in distilled water, and boltted dry on filter paper and stored at -20^{0}C.

Fractional precipitation with ammonium sulfate {$(NH_4)_2 SO_4$}

About 100 g of fresh frozen trematodes were homogenized in 3-5 volumes of ice-cold 0.05M phosphate buffer, pH 7.4, containing 1mM phenylmethylsulfonyl fluoride (PMSF) an inhibitor of cystein proteases. The PMSF was made soluble by dissolving adequate amount of isopropanol before adding to the homogenate and 5mM ethylenediamine tetraacetic acid (EDTA) in an ice-cold mortar and pestle. The homogenate was centrifuged at 15000 g for 20 min 4^{0}C to remove the tissue debris. The clear supernatant was made 40% saturated with ammonium sulfate and centrifuged again. The supernatant was then brought to 70% saturation with ammonium sulfate and centrifuged; the resulting supernatant was finally saturated to 95% with ammonium sulfate to precipitate the hemoglobin containing fraction. The entire process of fractionation is called 'salting in'.

The precipitate was dialyzed twice against 2000 volumes of 0.05 phosphate buffer, pH 7.4 for 18 hours at 4^{0}C and concentrated against sucrose crystal. The process of removal of salt through dialysis is called 'salting out'.

Proteins with surface patches that are easily dehydrated precipitate at lower ammonium sulfate concentrations than those with a tightly bound solvation shell. Once the concentration at which the target protein precipitates is known, fractional precipitation with ammonium sulfate can be used as an early step in a purification protocol. A pilot experiment should be performed using a small volume (10-20 ml) of the protein solution to ascertain the range of ammonium sulfate concentrations over which the target protein precipitates.

When an increasing amount of $(NH_4)_2 SO_4$ added to protein solution, the salt dissolve and resulting ions interacts with freely available water molecules, as more and more $(NH_4)_2 SO_4$ is added, these free available water molecule become scarce, so water molecules are preferentially pulled off from the hydrophobic patches on the protein surface. When the protein is dissolved in water the hydrophobic patches are forced into contact with surrounding water molecules which again dissolved salt into ions. The water molecule associated with the charged and polar groups on the surface of the proteins are bound by electrostatic interaction and are far less easily give up. As the $(NH_4)_2 SO_4$ concentration increases, the hydrophobic surface on the protein are progressively exposed and protein comes together to form insoluble protein aggregate via hydrophobic interactions; i.e. protein– protein interaction. Obviously those proteins with the largest number of hydrophobic patches aggregates first, where as those with no hydrophobic surface probably will not precipitate even at high conc· of $(NH_4)_2 SO_4$.

In crude mixture, co-aggregation is obviously extensive; like molecules do not necessarily all stick together. The solubility of the protein depends upon the ionic strength of a solution.

Results

The molecular mass of all trematode hemoglobin fractions are found to be about 17 kDa except that *G. crumenifer* hemoglobin fraction I was of 35 kDa. The conversion of *G. crumenifer* oxyhemoglobins I to methemoglobins by the addition of K_3 Fe $(CN)_6$ resulted in the elution of these hemoglobins to an elution volume corresponding to the molecular weights of 17 kDa.

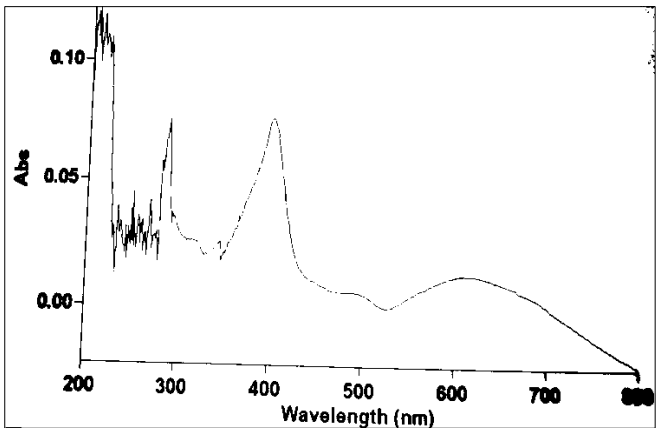

Fig 4: Spectral profile of the crude hemoglobin preparation of *Fasciola gigantica* after 95% ammonium sulfate precipitation

Table 1: Purification of *Fasciola gigantica* hemoglobin

Fraction	A412/ A280	Fold Purification
Homogenate	0.210	-
70% Ammonium sulfate saturation	0.308	1.466
95% Ammonium sulfate saturation	0.690	3.270
*Fraction I obtained by sephadex G-100 gel filtration	0.251	1.189
Fraction II obtained by ephadex G-100 gel filtration	1.069	5.065
*Fraction III obtained by Sephadex G-100 gel filtration	0.4	1.87

*Protein impurities not related to hemoglobin

Table 2: Purification of *Gastrothylax crumenifer* hemoglobin

Fraction	A412/ A280	Fold purification
Homogenate	0.758	-
70% Ammonium sulfate saturation	1.272	1.678
95% Ammonium sulfate saturation	1.531	2.019
*Fraction I obtained by sephadex G-100 gel filtration	0.428	0.564
Fraction II obtained by sephadex G-100 gel filtration	2.114	2.780
*Fraction III obtained by sephadex G100 gel filtration	2.910	3.832

*Protein impurity not related to hemoglobin

Table 3: Readings of elution profile at 280nm & 412nm of hemoglobin of *Fasciola gigantica* after Sephadex G-100 column purification

S. No. of tubes	A 412	A280	S.No. of tubes	A412	A280
1	0.292	0.629	27	0.284	0.572
2	0.301	0.9	28	0.4	0.658
3	0.354	1.407	29	0.535	0.731
4	0.296	1.216	30	0.654	0.816
5	0.204	0.853	31	0.77	0.913
6	0.123	0.547	32	0.858	0.995
7	0.077	0.39	33	0.922	1.032

8	0.063	0.348	34	1.112	1.04
9	0.053	0.331	35	0.901	0.982
10	0.045	0.317	36	0.866	0.946
11	0.038	0.319	37	0.726	0.832
12	0.038	0.344	38	0.681	0.78
13	0.04	0.383	39	0.522	0.675
14	0.035	0.44	40	0.427	0.571
15	0.043	0.493	41	0.305	0.461
16	0.069	0.539	42	0.237	0.394
17	0.045	0.591	43	0.171	0.352
18	0.053	0.615	44	0.161	0.318
19	0.053	0.629	45	0.082	0.293
20	0.057	0.607	46	0.071	0.279
21	0.066	0.574	47	0.13	0.57
22	0.074	0.572	48	0.209	0.699
23	0.071	0.501	49	0.32	0.8
24	0.088	0.498	50	0.265	0.799
25	0.162	0.499	51	0.21	0.683
26	0.214	0.521	52	0.135	0.423

Discussion

The hemoglobins in trematodes are present in their body tissues. They were purified from the whole animal cell lysate, by precipitating proteins through ammonium sulfate fractionation in three broad cuts, achieving up to 95% saturation. The partially purified trematode hemoglobins were further purified by gel filtration chromatography over Sephadex G-100. The degree of purification of hemoglobins in these fractions was judged by A_{412}/A_{280} ratios. The rechromatography of the hemoglobin peak fractions did not further purify the hemoglobins. Different levels of purification obtained in different trematode hemoglobins as shown in the hemoglobin purification Table 2 to 3 could be due to different protein content in the trematodes of different genera under study.

Haque and Siddiqi (1982) [6] have reported different protein content from seven different trematodes of various habitats including four trematodes under present study. Von Brand (1973) [7] pointed out that the total proteins, determined from the total nitrogen content, vary from parasite to parasite and even in the same species of different age or in the different parts of the same species. It is the difference in the protein composition and content of different trematodes under study that leads to different levels of purification of trematode hemoglobin. The homogeneity of the trematode hemoglobin preparations were assessed by comparing the simple PAGE patterns of hemoglobins stained by Benzidine Reagent as well as Coomassie Brillient Blue.

Conclusion

The appearance of single bands of trematodes and their host hemoglobins by SDS-PAGE is also an indication of homogenous hemoglobin preparations. Often difficulties were experienced in obtaining clear electrophoretic pattern in SDS-PAGE system, of hemoglobins which were purified without the addition of proteolytic enzyme inhibitors such as phenylmethylsulfonyl fluoride (PMSF). However, reasonably improved results were obtained when 1 mM PMSF and 5 Mm ethylenediamine tetraacetic acid (EDTA) were added before homogenizing the parasites.

References

1. Wayman J. In: heme proteins- Advances in Protein chemistry, 4, Academic Press New York 1948.
2. Braunitzer G. The primary structure of the protein components of several hemoglobins. Zeitschrift Fuer Physiologiche Chemie 1958;312:72-84
3. Muller CJ. Inaugural Dissertion. A comparative study on the structure of mammalian and avian hemoglobins. Groningen 1961a.
4. Muller CJ. Molecular Evolution, van Gorcum. Assen 1961b, P46.
5. Gratzer WB, Allison AC. Multiple hemoglobins. Biological Reviews (Cambridge) 1960;35:459-506.
6. Haque M, Siddiqi AH. The biochemical composition of digenetic trematodes. The Indian Journal of Parasitology 1982;6:37-41.
7. Von Brand T. Biochemistry of parasites, 2nd edition, Academic Press, New York 1973.