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SIMILARITIES AND DIFFERENCES BETWEEN HAPTOGLOBIN IN MAMMALS AND PIT 54 IN POULTRY – A REVIEW

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Summary

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The acute phase response (APR) is a natural systemic reaction raised to neutralize the effect of challenging agents (infections, traumas, neoplasms, immune disorders) that impair the homeostasis. Acute phase proteins (APP) are a component of APR. The purpose of the present review is to summarize the available information about haptoglobin (Hp) in mammals and its analogue PIT 54 in *Gallus gallus domesticus*. Hp is a major APP in ruminants and a representative of moderate APP in men, carnivores, horses and rabbits. Chicken PIT 54 is a soluble member of the so-called scavenger receptor cystein rich proteins family. Hp is an α_2-glycoprotein that belongs to transport (metal-binding) conjugated proteins. The primary function of Hp is to bind free haemoglobin and thus, to protect the organism from losing iron. The peroxidase activity of Hb is at the basis of assay methods for this protein. The review presents data about the development of knowledge about mammalian Hp and analysis of its biochemical properties. A special attention is paid on the information relation to biosynthesis and properties of mammalian Hp and the avian haemoglobin-binding protein called PIT 54. The investigation of the latter is essential for the timely detection of subclinical infections.

Key words: function, haptoglobin, PIT 54, properties

INTRODUCTION

Blood serum contains numerous proteins that are either actively secreted or released by various blood cells or organs. Acute phase proteins (APPs) are an element of acute phase response (APR), a natural systemic reaction defense against the challenge with agents of infectious, neoplastic, stress, traumatic or parasitic origin. As a component of innate resistance (Pannen & Robotham, 1995), APR aims to limit and neutralize the adverse effect of factors enumerated (Kushner & Mackiewicz, 1987; Stadnyk & Gauldie, 1991), as well as to prevent the further damage of affected tissues, to remove toxins and their by-products and to activate processes related to recovery of the normal function of damaged organs (Dinarello, 1984; Baumann & Gauldie, 1994). This way, APR contributes to the maintenance of homeostasis (Dinarello, 1984). In a narrower sense, the term is used to designate the changes in the plasma concentrations of a large number of proteins, synthesized mainly in the liver.

CLASSIFICATION OF ACUTE PHASE PROTEINS

Various classifications of APPs have been proposed (Chamanza *et al.*, 1999; Eckersall, 2000a; Murata *et al.,* 2004; Petersen *et al.*, 2004). It should be noted that the significance of the different APPs varies for the different animal species and this largely impedes the elaboration of an uniform classification. According to the extent of increase, APPs are divided into major, moderate and minor. Depending on the period of time when APPs concentration changes, they are rapidly reacting and slowly reacting. APPs whose levels increased after the challenge are called positive and those whose levels are reduced – negative.

According to Murata *et al.* (2004), major APPs with 10- to 100-fold increase are as follows: in chicken – not known, in dogs – C-reactive protein (CRP), in cats and horses – serum amyloid A (SAA); in pigs – major acute phase protein (MAP), in ruminants – haptoglobin (Hp); in rabbits – SAA, CRP, lipopolysaccharide-binding protein (LBP); in fish – CRP, SAA. Moderate APPs that exhibit a 2 to 10-fold increase are: in chickens: α1-acid-glycoprotein (AGP), ceruloplasmin (Cp), ovotransferrin (ovoTf), SAA; in dogs: AGP, Cp, fibrinogen (Fb), Hp, SAA; in cats: CRP, Hp, Cp; in horses: AGP, CRP, Fb, Hp; in swine: AGP, CRP, Fb, Hp; in ruminants: AGP, CRP, Fb, SAA, in rabbits: α2-macroglobulin. Minor APPs with no more than twofold increase are: in chickens – Fb; in dogs – not available; in horses – AGP, Cp, Fb; in swine and ruminants – Cp. According to an alternative classification (Gruys *et al.*, 1998), major chicken APPs are CRP, SAA, ovoTf, Fb, α1AGP, and in swine: MAP and Hp. Chamanza *et al.* (1999) and Eckersall (2000a) outline SAA to be one of the most appropriate APPs as a marker of infection in chickens. The classification of Petersen *et al.* (2004) shows that in swine and cattle, fibrinogen increase is from 50 to 100% and in rabbits – between 1 and 10 times. These authors also believe that in swine, cattle and rabbits, Hp increase is more than 10 times, whereas in dogs, cats, men and rats, this protein's increase is up to 10-fold.

The comparison of these data evidence that Hp is a major APPs in ruminants and possibly in swine and rabbits. In men and carnivores, it belongs to the group of moderate APPs (Eckersall, 2000a; 2000b, Gryus *et al.,* 1998, Murata *et al.*, 2004). According to the first and second classifications cited, Hp is absent in chickens. The purpose of this review is therefore to perform a comparative analysis of the available information for the structure and significance of haptoglobin in mammals and its analogue in chickens.

HAPTOGLOBIN IN MAMMALS: FUNCTION, STRUCTURE, PROPERTIES, ENCODING GENES

Haptoglobin, together with Cp, haemopexin (Hmp) and transferrin belongs to transport (metal-binding) blood proteins that increase in the course of APR (Pannen & Robotham, 1995).

Human Hp is a continuously secreted plasma protein with moderate increase in APR. In healthy ruminants, it is normally absent from the plasma whereas during APR its levels increase many times.

Structurally, Hp belongs to blood plasma α2-glycoproteins. Its name comes from its function – to bind to the globin of the free blood haemoglobin (Hb) forming a stoichiometric complex ("hapto" means "to bind"). It is discovered in 1938, but the report for its discovery is published in

1959 by Smithies, who described some genetically determined variants.

According to Wicher & Fries (2006), Hb is a protein existing in the erythrocytes of many animal species. Its main function is to bind and transport oxygen and part of carbon dioxide. In some pathological conditions, i.e. infections and chemical intoxications, the erythrocytic membrane could be damaged and Hb is released into the plasma. Free Hb is the richest source of iron in the organism (Fairbanks & Beutler, 2000). Iron enhances the oxidation of lipids and proteins of adjacent tissues, the peroxidation of polyunsaturated fatty acids (Sadrazdeh *et al.*, 1984), low-density lipoprotein oxidation and is responsible for the damage of capillary endothelial cells (Vercellotti *et al.*, 1994).

Hp has several specific functions. The most important, as already mentioned, is its very high affinity (Kd ~ 1×10^{-15} mol/L) to bind free Hb in equimolar amounts (Mc Cormick & Atassi, 1990). The complex between Hp and Hb is large, not able to pass through the renal glomeruli and could be removed only by the reticuloendothelial system. The binding of Hp to Hb prevents the systemic loss of iron, occurring after intravascular haemolysis. Also, iron is one of the essential elements necessary for bacterial growth. The combination of blood effusion and tissue infection is unfavourable as iron from haemoglobin creates preconditions for microbial growth (Eaton *et al.*, 1982). This is the essential role of Hp: to make iron unavailable for microorganisms after binding with haemoglobin (Barclay, 1985). Later, it was discovered that Hp has antioxidant and anti-inflammatory properties. The antioxidant power of Hp is attributed to the fact, that binding to Hb and its removal from blood largely prevents iron-stimulated formation of oxygen radicals (Gutteridge, 1987).

Hp is present in blood plasma of all mammals. The binding of Hb to Hp prevents the passage of Hb through the glomeruli. It was found out that the absence of Hp in mice resulted in kidney damage. It is still questionable whether lower vertebrates use Hp as a means of protection against the damaging effect of Hb or not (Lim *et al.*, 2000).

Hb exhibits peroxidase activity, on which is based the method for detection of Hb-binding proteins. Using this indirect method, it was found out that blood plasma of animals from different classes, as fish and reptiles, contained Hp. Hb-binding proteins have been isolated from chicken plasma too, and their amino acid sequence has not been determined until 1984 (Delers *et al.*, 1983).

The primary translation product of Hp from mammalian mRNA is a polypeptide, that is cotranslationally isomerized and thereafter, its end fragments are proteolytically detached when still in the endoplasmatic reticulum (Hanley *et al.*, 1983). The removal of these peptides leads to increased affinity of Hp molecule to Hb. The mature human Hp molecule is a tetramer composed of two short and two long polypeptide subunits, two α and two β chains, linked with disulfide bonds, respectively. The carbohydrate component of avian Hp is bound only to the β chain (Delers *et al.*, 1983). From its part, α chains are three types, separated electrophoretically (Sadrzadeh & Bozorgmehr, 2004): α1 (8.9 κDa) – fast chain (α1F) and slow chain (α1S) and α2 (16 κDa). Alpha chains possess a domain, called complement-controlling protein (CCP) (Kato & Enjyoju, 1991), that has been detected in many proteins involved in the regulation of complement factors H and the C1R receptor. Further, this subunit possesses

mannose-binding lectin (MBL) and MBL-associated serine proteases (MASPs). There is a significant homology (28–33%) between amino acid sequences in these chains and serine-containing proteases (Kurosky *et al.*, 1980).

Although Hp is present in all mammals, polymorphism is discovered only in men (Sadarzadeh & Bozorgmehr, 2004). Hp 1-1 consists of one α1β dimer with molecular weight of 86 κDa. Hp 2-2 consists of multiple α2β chains, with numerous disulfide bonds and molecular weight of 170–900 kDa. Hp 2-1 is heterozygous and is composed of one α1β dimer and numerous α2β units with molecular weight of 90 to 300 kDa (Sadrzadeh & Bozorgmehr, 2004).

It has been found out that the synthesis of α and β chains is controlled by two genetic loci (Smithies *et al.*, 1962). The locus responsible for the production of α subunit is on chromosome 16q 22. The three different α chain types (α1F, α1S and α2) are controlled by three alleles – Hp alfa 1 fast, Hp alfa 1 slow and Hp alfa-2. The α1–chain is composed of 84 amino acids whereas the β-chain – of 245 amino acids. The α1 variants differ in the amino acid at position 54: lysine in α1 fast, glutamic acid in α1 slow. The α2 chain is a product of unequal crossing over. The β-chain with molecular weight of about 35–40 kDa is similar to chymotrypsin serine proteases but it does not possess amino acid residues, essential for the catalytic activity of the enzyme (Kurosky *et al.*, 1974).

The synthesis of Hp occurs mainly in the liver, although in mice, the Hp gene is expressed in other tissues (lungs, skin, spleen, kidneys and adipose tissue) as well (D'Armiento *et al.*, 1997). Hp formation is enhanced by growth hormone, insulin, bacterial endotoxins, prostaglandins, cytokines (interleukin-1, interleukin-6, tumour necrosis factor (Raynes, 1991). In adult men, Hp concentration is higher (from 38 and 208 mg/dL) as compared to children. The half-life of Hp is 3.5 days and that of Hp:Hb complex – approximately 10 min (Bowman, 1993). The Hp:Hb complex is removed by binding to the CD 163 receptor on the surface of macrophages and monocytes. The expression of the macrophageal CD 163 receptor is enhanced by glucocorticoids, as well as by interleukin-6 and interleukin-10 (Sadrzadeh & Bozongmehr, 2004). Wicher & Fries (2006) proved that CD 163, that is a monocytic receptor for the Hp:Hb complex in men, is a member of the same protein family as PIT 54 (haemoglobin-binding protein in chickens). The available information about PIT 54 is limited.

Wicher & Fries (2006) were the first to provide evidence that the Hb-binding homologue of the β-chain of mammalian haptoglobin appeared at an early evolutional stage at the time of bony fish emergence.

The data of Wicher (2006) and Wicher & Fries (2006) analyzing gene sequences and comparing them to those of other animals have shown that bony fish possessed a gene, coding a protein homologous to that of mammalian Hp. It was also shown that this protein was bound to Hb. The evolutional aspects of Hb scavengers were investigated by Wicher & Fries (2009). The authors demonstrated that systems for Hb disposal appeared in fish. Apart from Hp, this role is also played by haemopexin and α1-macroglobulin. It should be stated that later, Hp disappeared in some vertebrates and reappeared again in chickens as a soluble receptor, resembling Hp. In mammals, Hp polymorphism appeared independently at two different time periods, providing the advantage of improving the

resistance to infections. These data, together with phylogenetic analyses prove that chicken haptoglobin (Hpc) belongs to proteins involved in complement activity regulation. These proeins are able to bind the monosaccharide mannose as they contain lectin – a plant protein used to detect a carbohydrate component on the surface of membranes. It is also similar to serine-containing proteases (MASPs). It was surprisingly found out that the gene coding Hp was absent in chickens and frogs. In chicken plasma, Wicher & Fries (2006) detected a different Hb-binding protein and called it PIT 54. The experiments of Schaefer *et al.* (2006) also affirmed that PIT 54 existed only in chickens and had the same function as mammalian Hp, but the amino acid sequence of its protein component was different from that of mammalian haptoglobin molecule.

HAPTOGLOBIN (PIT 54) IN CHICKENS

Literature data about Hp in infectious poultry diseases are very scarce. The first serous attempts for investigation of chicken Hp are carried out by Musquera *et al.* (1979), who detected Hp in chicken serum by cellulose acetate gel electrophoresis. At pH 6.4, chicken Hp moves towards the cathode, whereas the Hp:Hb complex – towards the anode. The authors investigated also the separation of chicken Hb on acrylamide gel and found out one bigger and two smaller fractions. The motility of the Hp:Hb complex is very similar to that of pure Hb, but when comparing the relative intensity of the three fractions in both cases, it was found out that they were different. By means of more contemporary methods as fluorescence titration, Musquera *et al.* (1979) demonstrated the lack of binding between chicken Hp and mammalian Hb. This fact made the authors believe that Hp was not present in chicken plasma. The species specificity of the binding of Hp and Hb in chickens is rather significant, due to the differences between mammalian and avian haemoglobin. Human Hp could bind avian Hb, but the avian Hb-binding protein could not bind human Hb. This demonstrates that avian and human Hp bind in a different manner and that the specificity of binding to Hb is determined by the haptoglobin part that is involved in binding (Musquera *et al.*,1979).

Hpc binds chicken Hb and this is connected to insignificant increase in its peroxidase activity as compared to that of free Hbc (Musquera *et al.*, 1979). The affinity of binding between Hpc and Hbc is lower than that of mammalian Hpm:Hbm complex. This is evidence by the fact that the Hpc:Hbc complex is dissociated by 5M urea or 1M $MgCl_2$, whereas the complex of Hpm and Hbc is dissociated by a higher concentration of urea, i. e. 8M (Delers *et al.* 1981). Furthermore, the Hpc molecule is more compact, as shown by the Stokes' molecular radius – 35.5 nm vs 45.3 nm in mammals. Wozniak (1984) reports that the half-life of Hpc is 12.9 hours and that it is longer compared to that of Hpc:Hbc, (3.1 hours) and believes that it is internalized by the chicken liver by means of receptor-mediated exocytosis.

While the publications of Musquera & Planas (1977), Wozniak (1984) and Mazur-Gonkowska *et al.* (2004) described the presence of haptoglobin in chicken plasma, contradictory data were published by Wicher (2006), who claimed that haptoglobin was lacking in chickens. This new biomarker, analogous to mammalian haemoglobin-binding protein, was called by the author PIT 54. It is not still confirmed whether this protein is constantly

present in the blood of chickens or is an APP, produced only after challenge.

In order to reveal some of the secrets about Hpc or PIT 54, as it is currently called, some issues of the discussion throughout the years should be summarized from historical point of view. So, one of inflammation biomarkers in chickens, homologous to mammalian Hp, is described as Hpc until 2004 and as PIT 54 after 2006.

The first doubt about the existence of Hp in chickens was expressed by Reich (1956). His attempts to discover a blood constituent, homologous to mammalian Hp, have been unsuccessful. Later, Darcel & Bide (1969) and Musquera & Planas (1977) provided evidence about the presence of Hp:Hb complex in chicken serum, but their investigations were based only on electrophoretical mobility and the staining pattern of Hp:Hb complex. Soon after that, Musquera *et al.* (1979) demonstrated the existence of Hp in chickens. Unlike mammalian Hp that is able to bind to various animal haemoglobins, including chicken haemoglobin, chicken Hp discovered by Musquera *et al.* (1979) had no such properties and bound only avian and reptilian Hb.

By using gel electrophoresis, it was shown that Hpc has a high molecular weight and that when chicken Hb is added, a high-molecular complex is formed with peak absorption at 410 nm. The electrophoretic mobility of this complex on cellulose acetate gel corresponds to that mobility of Hp:Hb complex, formed when human or mammalian serum containing Hp is applied. The fluorescence signal in these studies also evidences the formation of a complex between chicken Hp and chicken Hb and hence explains why authors, who used mammalian Hp and chicken Hb and obtaining no complex concluded that haptoglobin was lacking in chicken serum. It was initially assumed that chicken and human Hp bind haemoglobin in a different manner and that the specificity of this binding depends on the Hb-binding part of chicken Hp. Benesch *et al.* (1976) discovered that the interaction between one of haemoglobin chains and the bivalent Hp is reversible in the initial fast binding step. In the next slow stage, this product polymerizes, adds new Hb molecules and in excess of Hp combines with a second Hp molecule. Thus, two haptoglobin molecules are involved in the complex, bridged by the Hb tetramer. This complex could still bind normal αβ Hb dimers at the vacant Hp sites. The very low O_2 affinity of Hb is shifted by the very high stability of the Hp:Hb complex. Later, Delers *et al.* (1983) continued to investigate the structural traits of chicken Hp and its binding capacity. The authors tried to explain why did avian Hp bind only species-specific Hb whereas mammalian Hp was able to combine all haemoglobin types, including that of chickens. They provided an evidence that chicken Hp was a new variant of the already known mammalian Hp and found out that its molecular weight was 68 kDa in non-dissociated state and 62–64 kDa in dissociated state. When in non-dissociated state, is has a monocatenary structure, as avian liver lacks the step of peptides removal during maturation, that occurs immediately after its biosynthesis. This explains the fact that chicken Hp is more compact than human Hp, with lower sedimentation coefficient of 3.9 Sv (as compared to that of human Hp – 4.2 Sv).

Delers *et al.* (1988) have isolated three molecular variants of Hcc, on the basis of its reaction with concanavalin A. These variants have a different molecular weight, determined by means of polyacry-

lamide gel electrophoresis in the presence of Na dodecyl sulfate. The analysis of glycopeptides after protease degradation has also shown various structures, but the degree of glycosylation was not correlated with the inflammation.

Chicken Hp is a single chain and is bound to a carbohydrate component, unlike all other identified haptoglobin types that possess a tetramer structure (Delers *et al.*, 1983).

Hpc has 12 internal disulfide bonds, whereas human Hp 1-1 has a lower number. The proportion of the carbohydrate component of Hpc is smaller, and peroxidase activity of avian Hb increases slightly after binding to the isospecific Hp, whereas the binding of human Hp to homologous Hb results in considerably increased activity. This way, Delers *et al.* (1983) concluded that the avian "novel haptoglobin" was very different from other mammalian Hp types. Therefore, arises the question whether it belongs to mammalian Hp family, but the answer is in deciphering its amino acid sequence, that was recently performed.

In polyacrylamide disc gel electrophoresis, Iwasaki *et al.* (2001) reported the appearance in front of albumin of a new protein, member of scavenger receptor cysteine-rich (SRCR) family of proteins in chickens injected with turpentine. It was isolated, characterized and cDNA cloned. After 50% saturation and ammonium sulfate precipitation of chicken serum and subsequent fractionation of the supernatant by means of chromatography and DEAE-Toyopearl 650S column gel filtration, it was discovered that this glycoprotein contained mannose and that its N-terminal amino acid sequence was not homologous to those of other chicken APPs. The physiological function of this protein was determined in two test systems: chemiluminescence and electron spin resonance spectroscopy. It was shown that the protein was an antioxidant and suppressed the superoxide ion released throughout the receptor activation. It was called 18-B and it was shown that its complete amino acid sequence was homologous to that of SRCR family of proteins involved in the regulation of leukocyte function. Furthermore, it is composed of four SRCR domains and participates in cell function regulation via inhibition of the excessive production of the reactive oxygen species.

Wicher (2006) extended its research on PIT 54 and established that in chickens, the gene previously described to code Hp, was now identified as coding PIT 54. The protein has a molecular weight of approximately 68–69 kDa, and in the literature is identified not only as PIT 54, but also as the 18-B protein discovered by Iwasaki *et al.* (2001). It has the same function as Hp, i. e. Hb-binding properties, and therefore is detected with the non-specific assay kit for Hp. The studies of Wicher (2006) on chicken genome did not reveal a gene, coding a protein resembling mammalian Hp. The proteins, most homologous to PIT 54, as is the case with *X. tropicalis* frogs, are orthologues of C1r, C1s, MASPs and other serine proteases. Thus, the author concludes that chickens lack Hp gene and possess a gene for PIT 54 instead. However, the fact that a Hb-binding protein was previously described, prompted Wicher & Fries (2006) to identify it. Using affinity chromatography with immobilized chicken Hb followed by SDS/PAGE, a protein band and mass of 69 kDa was detected. Then, mass spectroscopy revealed that the band contained PIT 54 or 18-B, a soluble member of the SRCR protein family. From domestic goose plasma, the

same investigators have isolated a 75 kDa Hb-binding protein by liquid chromatography. By mass spectroscopy, it was proved to be homologous to PIT 54. By means of immobilized Hb, Wicher & Fries (2006) have isolated three polypeptides of 70 κDa, 35 kDa and 15 kDa from another paleognathous bird species – the ostrich. The 70 kDa polypeptide has been identified as equal to PIT 54 (69kDa), whereas the N-terminal sequence of the 35 kDa polypeptide was similar to that of the β-chain of mammalian Hp. Under conditions that do not allow reduction, the 15 and 35 kDa polypeptides form a 100 kDa complex by disulfide bridging, similar to mammalian Hp. The N-terminal amino acid sequence of the 35 kDa polypeptide was 66–80% identical to the same region of the β-chain of mammalian Hp. To summarize, all these data confirm that throughout the evolution of chickens, PIT 54 has taken over the function of Hp and has replaced it completely (Wicher & Fries, 2006). From the 70 kDa polypeptide, the authors have obtained an amino acid sequence, similar to that of chicken PIT 54.

The presence of Hp in non-mammalian vertebrates has been also studied by genetic methods. Comparing the available genome cards and databases, it was found out that the gene encoding a haptoglobin-like (HpL) protein existed in bony fish and neognathic birds but not in lower animals and amphibians. Chickens and ducks are representatives of two different neognath lines. The absence of Hp in their plasma confirms that in them, this gene was lost throughout the evolution. Ostriches are from the modern avian line (paleognaths). They appeared 80–90 million years before the neognaths (Wicher & Fries, 2006).

Based on available genome chart data, PIT 54 exists only in neognathous birds. Probably, this protein appeared for the first time in an ancestor of paleognaths and neognaths birds, which also possessed Hp. Then, paleognaths retained both proteins whereas neognaths lost the Hp gene and PIT54 remained the only Hb-binding protein.

On the basis of the similarity of amino acid sequences, Hp in mammals, HpL in fish and PIT 54 in neognathous birds (chickens) belong to complement-activating serine proteases: C1r, C1s, MASP-1, MASP-2, MASP-3. All of them consist of 8 domains: two C1r, C1s; Uegf -1; one epidermal growth factor-like protein domain, two CCP domains and one serine protease domain.

The authors explain the evolution of HpL and Hp in mammals with a number of events. After partial duplication of the gene encoding Hp, C1r, C1s, MASP-2, the prototype of Hp appeared. The organization of its domains is similar to that of mammalian Hp that consists of two CCP domains and one serine protease (MASP) domain, but still preserves the functions of an active protease. Next, serine and histidine residues essential for protease activity, are substituted with alanine and lysine or arginine in the active site. These events occur in the common ancestor of bony fish and mammals. In fish, the CCP domain of Hp suffers a deletion whereas an intron is inserted in the exon encoding the SP domain, finally resulting in the present HpL gene (Wicher & Fries, 2006).

The absence of the Hp gene in frogs and chickens motivated Wicher & Fries (2006) to investigate the corresponding areas of their genome. They found out that both frogs and chickens possessed a gene coding ATP-dependent DNA helicase (DHX38), located near to the dihydroorotate dehydrogenase (DHODH) gene. In mammals, the DHX 38 gene is also close

to that of DHODH, separated only by genes coding for Hp and thioredoxin-like protein 4B. All these data testify that in frogs and chickens, the HP gene has been removed by deletion from the locus between DHODH and DHX38. In the same report, Wicher & Fries (2006) provide evidence that in bony fish, the HpL protein possesses one or two domains similar to that of mammalian Hp. In ostriches, being a primitive type of birds, there is haptoglobin with domain structure almost equal to that of mammalian Hp. These investigations are continued by Huang *et al.* (2006), but on a completely new level, by analysis of chicken serum proteome.

In conclusion, chicken haptoglobin or PIT 54 is a single-chain polypeptide, bound to carbohydrate component, unlike the tetramer structure of mammalian haptoglobin. PIT 54 is the functional homologue of mammalian Hp. Human Hp could bind avian haemoglobin, but the contrary is not possible. Furthermore, the binding between Hpc and Hbc in birds is less strong. Avian Hp is more compact as compared to mammalian Hp, its binding to Hb occurs in two stages with the participation of 2 Hp molecules that bind to Hb tetramer. The molecular weight of chicken PIT 54 is 68 κDa, of goose –75 κDa, of ostrich – about 100 κDa, whereas mammalian Hp is much heavier (90 to 300 κDa) and consists of two types of chains: α (three subtypes) and β. The α1–chain of mammalian Hp is composed of 84 amino acids and the β-chain: from 245 amino acids. The variants of the α1- chain (fast and slow) differ by the amino acid on position 54. During the maturation of PIT 54, whose structure is similar to that of b-chain of mammalian Hp, its end residues are not removed from the polypeptide chain and thus, its affinity to Hb is smaller compared to mammals. The investigation of haemoglobin scavengers evolution show that they appeared in bony fish. They belong to the family of serine proteases, Further in evolution, haptoglobin disappears in frogs but reappears as PIT 54 in one order of neognath birds (*Gallus gallus domesticus*) to perform the same function as mammalian haptoglobin.

The development of precise and reliable assays for blood haptoglobin could make it an important biomarker for surveillance of poultry farms with regard to the presence of occult herd infections.

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