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Short communication

Serum haptoglobin in clinical biochemistry: Change of a paradigm

L'haptoglobine sérique en biochimie clinique : changement d'un paradigme

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ABSTRACT

Serum haptoglobin (Hp) was discovered by Max Fernand Jayle as a young assistant professor in the Department of Biochemistry of the Paris Medical Faculty, headed by Professor Michel Polonovski. Jayle showed that Hp was an acute phase glycoprotein and worked out its routine determination in the blood-serum, based on its complex formation with hemoglobin, using the increased peroxidase activity of the hemoglobin–haptoglobin (Hb–Hp) complex, for routine determination in clinical biochemistry for the characterisation of inflammatory processes, together with other acute phase glycoproteins as orosomucoide. Later Smithies described the genetic control of human Hp-isoforms and quite recently Andersen et al. reported the elucidation of the crystal structure of the porcine Hb–Hp complex. In that article there was no mention of the discovery of Hp, neither of its determination in clinical biochemistry as an inflammatory marker. The only biologically significant role assigned to Hp by Andersen et al. is its (hypothetical) role to prevent or minimize the harmful effects of Hb during intravascular hemolysis, by the generation of reactive oxygen species (ROS) and complexing it. This shift of paradigm, not at all exceptional in medical biochemistry, will be described and discussed with its pitfalls and consequences.

Mots clés:
Haptoglobine
Hémoglobine
Peroxydase
Glycoprotéines de la phase aiguë
Inflammation

RÉSUMÉ

L'haptoglobine (Hp) a été découverte dans le sérum humain par M.F. Jayle avec M. Polonovski dans le département de biochimie de la faculté de médecine de Paris, ainsi que son rôle comme marqueur de processus inflammatoires en tant que glycoprotéine de la phase aiguë. Jayle a mis au point sa détermination basée sur l'activité peroxydasique de son complexe avec l'hémoglobine (Hb–Hp), utilisée au cours des décennies suivantes dans les laboratoires cliniques pour caractériser les processus inflammatoires, ensemble avec le dosage d'autres glycoprotéines de la phase aigüe comme l'orosomucoïde. Un travail récent (Andersen et al.) a décrit la structure par diagramme aux rayons X du complex Hb–Hp porcin en grand détail. Dans cet article la seule fonction biologique de l'Hp a été attribuée à sa capacité de protéger les tissus contre les effets nocifs des produits réactifs de l'oxygène (ROS) lors d'hémolyse intravasculaire. Dans cet article nous nous proposons d'analyser ce changement de paradigme, déplacement d'interprétation d'un concept biomédical, avec ses erreurs et conséquences.

1. Abbreviations

Hb	hemoglobin
Hp	haptoglobin
ROS	reactive oxygen species
RNS	reactive nitrogen species

2. Introduction

The recent publication by an international team of the detailed three-dimensional structure of the porcine hemoglobin–haptoglobin (Hb–Hp) complex [1] reminded me decades of research in the Department of Biochemistry of the Paris Medical Faculty, under the direction of the discoverers of haptoglobin, M. Polonovski and M.F. Jayle. The routine use of Hp determination as an acute phase glycoprotein, together with some other inflammatory markers such as orosomucoid or alpha1-acid glycoprotein [2,3] was practiced for decades in clinical biochemistry

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laboratories. As every innovation in medical biochemistry, this one was also accompanied by speculations on the biological "roles" of acute phase glycoproteins. Since the discovery by Polonovski and Jayle in 1938 of Hp and of its complex formation with Hb as well as its determination [4,5], speculations were directed to the nature of the acute phase reaction, its diagnostic value as well as towards the origin of acute phase glycoproteins. As they are rich in carbohydrates, some authors, such as Catchpole and Pirani at the University of Illinois in Chicago, proposed the "depolymerisation" of ground substance "mucopolysaccharides" as a possible source of acute phase, carbohydrate rich glycoproteins [6]. This hypothesis had to be abandoned after the demonstration by Miller [7] and Sarcione [8] of the hepatic origin of acute phase glycoproteins. These arguments were completed and the Catchpole–Pirani hypothesis abandoned after the clarification of the molecular composition of connective tissue matrices, renamed extracellular matrix by Hay [9] and more correctly intercellular matrix by Balazs [10]. Remained the question concerning the function of acute phase glycoproteins, the role of their rich carbohydrate content and their pathological significance. We propose a succinct analysis of these problems in the light of the recent description of the three-dimensional structure of the porcine Hb–Hp complex [1].

3. Haptoglobin, its discovery, its determination and biomedical significance

The isolation and characterization of Hp by M.F. Jayle in M. Polonovski's laboratory was published in 1938 [4,5]. Its description, as a carbohydrate-rich serum glycoprotein, isolated from human blood was based on the discovery of its high affinity for hemoglobin and the formation of a 1 to 1 Hb–Hp complex (for the early literature, the monography of Jayle et al. can be consulted [11]). From this time on, the French authors realized the significant activation of the peroxidase activity of Hb, studied previously by the team of M. Polonovski. During his experiments to characterize Hp, Jayle realized the importance of the substrate to be used for the determination of the serum concentration of Hp by the estimation of the peroxidase activity of the Hb–Hp complex. He showed that ethyl-peroxide was a much better substrate than hydrogen peroxide, which was however not available commercially and had to be synthesized in the lab. One day during this process, ethyl peroxide exploded in the hands of Jayle who lost the sight of both his eyes. This important infirmity did not stop the carrier of Jayle who became chairman of the Department of Biochemistry of the Paris Medical Faculty after the death of M. Polonovski in 1953 and continued his research with his team which I joined, motivated by my interest in this enzymatically challenging subject as well as of its implication in the inflammatory processes and its pathological significance. With a reduced team and in close contact with Jayle, we could contribute to the understanding of some of the aspects of structure-function relationships at the Hb–Hp complex formation, of its enzymatic activity and pathophysiological role as an acute phase glycoprotein [12–14] We also contributed to the elucidation of the relationship of the acute phase reaction and connective tissue degradation in model and pathological processes in order to establish the (indirect) relation between the inflammatory process of connective tissues and Hp increase in the blood circulation [15,16].

4. A short incursion in medical epistemology: experimental facts and their interpretation

In the aforementioned remarkable description by the team of Andersen et al. [1] of the crystal structure of the porcine Hb–Hp complex there is no mention of the discovery of Hp and of its medicobiological significance, besides the mention that it is an acute-phase glycoprotein. Since the discovery of Hp and its intensive study as a marker of inflammatory processes, during the 1950s and 1960s, the emphasis for the interpretation of this early recognized pathological process, inflammation, recognized by the Greco-Roman physicians who described its five cardinal signs: heat, redness, swelling, pain and dysfunction (*Calor, rubor, tumor, dolor, functio lesa*) an important progress was accomplished in the understanding of its molecular mechanisms. The role of cytokines, receptors, mononuclear cells and other details led to the recent definition of the "inflammosome", subject of ongoing interest [17,18]. It has to be remembered that the importance of the inflammatory process in cardiovascular pathology, such as myocardial infarct, as well as in some malignant tumors was recognized and studied using the determination of acute phase glycoproteins in Jayle's time [11]. But let us return to the peroxidase activity of Hb–Hp. Work on free radicals, or better on reactive oxygen and nitrogen species (ROS, RNS) and its medical applications started also in the 1950s, as shown also by the free radical theory of aging, proposed by Harman in 1955 [19] and studied and cited since by a number of scientists as shown also by the book of Ingrid Emerit and Britton Chance on aging and the role of free radicals [20]. The pathological importance of these highly reactive molecular species is in sharp contradiction with their role as vitally important signal mediators in several physiological regulatory systems such as vasodilation by $NO^{\bullet}$ production and several other ROS- or RNS-mediated biological reactions. This is the so-called Jeckyll–Hyde aspect of this family of highly reactive molecular species, vitally important but also strongly destructive. One example of this ambivalent situation intensively studied in clinical and experimental cardiology is the role of ROS and RNS species in some cardiovascular diseases, based on the role of xanthine oxidoreductase as responsible for their release. Allopurinol, an inhibitor of this enzyme, is efficient in the treatment of some of such diseases [21–23].

Let us come to the physiological relevance of Hp attributed to its high affinity for Hb. Peroxidase activity and its potential implication in pathology, as mentioned, was studied from the time Hp was discovered and its strong affinity for Hb quantitatively assessed for its use in clinical chemistry and quantification. The statement in the above cited Andersen et al. report [1] concerning the essential role of Hp for protection against the peroxidase activity of Hb during intravascular hemolysis can be challenged. The work of the Polonovski–Jayle team, discussed in some details above, covered also peroxidase activity [24,25]. It clearly showed that the Hb–Hp complex has an increased peroxidase activity as compared to Hb alone. Therefore, it is hard to conceive that the essential biological role of Hp could be the protection against Hb's peroxidase activity, quite weak indeed, as compared to that, much stronger of the Hb-Hp complex, as shown by the early studies of the Paris Team [11]. This is another example of the difficulty to attribute biological "roles" for reactions which mostly follow their fate driven by conformational affinity and constraints without any "intention" to be "useful" for the organism. As proposed by François Jacob [26], Nature is tinkering without any teleological presuppositions of "usefulness". "Purpose" and "biological role" are the indirect result of evolutionary constraints, the result of Darwinian selection. We should remind in this respect a further complication. Human Hp comes in several isoforms, demonstrated originally by Smithies using starch gel electrophoresis [27,28]. This genetic heterogeneity of human Hp isoforms should result in heterogeneity of Hb–Hp complexes differing in their peroxidase activity, as studied already by the team of Jayle using then available methods, especially immunoelectrophoresis and polarography [29,30]. There remains, among

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others, to explore the role of the reticulo-endothelial system in the clearance of Hb–Hp complexes formed after intravascular hemolysis. This might well be the "biological role" of Hb–Hp complex formation. Clearly, further work is needed to understand in molecular detail what really happens during intravascular hemolysis.

Let us conclude by citing the essential principle of Karl Popper's philosophy for experimental sciences [31]: every experiment should provide further projects to control and eventually overthrow previous results, and so on. It certainly will be fascinating to further characterize at the molecular level human Hb–Hp complexes with their genetic heterogeneity, as was done by the team of Andersen et al. [1] for porcine Hb–Hp. Such studies could shed light on the variation of peroxidase activity of the genetic variants of human Hp complexed with their Hb, also subject of genetic control as well as further study of their biological significance.

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