THE EVOLUTIONARY RELATIONSHIPS OF RODENTS SUGGESTED BY THE IMMUNOLOGICAL CROSS REACTIVITY HAPTOGLOBIN

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Abstract

In view of the tendency to study the evolutionary relationship between organisms at molecular level, informative serum protein and haptoglobin, was chosen from four species of rodents, black rat, albino, mouse, and small albino.

The protein was extracted from serum of each of those subjects, purified and identified by using sephadex column and disc polyacrylamide gel electrophoresis. Anti-haptoglobin for each sample was produced in hyperimmunized rabbits. The purity was examined by using immunoelectrophoresis.

Quantitative microcomplement fixation test was used to measure the index of dissimilarity (I.D.) which indicates the degree of relatedness between these species.

There were close evolutionary relations between black rat and albino, and between mouse and small albino only.

Introduction

In many recent taxonomic and evolutionary studies emphasis has moved from anatomical characters to the principal building units of the animals body at the molecular level of organization and the protein molecule.

It is generally accepted that certain proteins such as those of serum may be regarded as highly informative molecules since their synthesis is under direct genetic control.
control and is little influenced by environmental variations, such as transferrin, ferritin and haptoglobin etc.

In fact, there are few studies on phylogenetically and ontogenetically informative serum proteins available such as those of Mao (1977) who used transferrin and Mohammad (1983) who used ferritin. The approach used in the present investigation was an immunological analysis of proteins present in the sera of four species of rodents. Special references have been placed on the utilization of antibodies directed towards individually purified haptoglobin from black rat, albino, mouse, and small albino.

Haptoglobin (HP) is a glycoprotein, present usually in the a2 - globin fraction. This protein is derived from erythrocyte breakdown. It forms specific stable complexes with hemoglobin in a one-to-one ratio yielding a high molecular weight complex. This prevents under loss of iron through urinary excretion. As an informative protein, it is much recommended for use in studies of evolution at the protein level.

The antigenic relationships of haptoglobins from these species of rodents have been investigated by using the microcomplement fixation test according to the modification of Champion et. al. (1974).

Materials and Method

28 animals (mixed sexes) of each species and nearly of the same age (about 3 months) were used in this work.

Blood was collected from the heart or caudal vein of each animal. Haptoglobins have been isolated from the plasma according to Conuel and Smithies (1959) and a modification of Jayle and Moretti (1962) methods. Fractions containing haptoglobin were collected, concentrated, and dialyzed against 0.08M tris buffer, PH7.8, and loaded on to a sephadex column (900MM x \gamma) in the same buffer. For the assessment of the purity of haptoglobins, disc polyacrylamide gel electrophoresis was used following the method of Clark (1964) and the modification of Urnstein (1964) and Davis (1964).

Antihaptoglobin serum for each sample was produced in hyperimmunized rabbits. The purity and specificity of each rabbit antihaptoglobin serum was examined using
immunoelectrophoresis. The method of Scheidegger (1955) and the modification of Feinberg (1956) was used. Antigen and antihaptoglobin sera titrated using the quantitative microcomplement fixation test (Champion et al., 1974). To stabilize complement, 0.1% solution of bovine plasma albumin was added to all diluents.

Microcomplement fixation cross-reactivity served as a measure of the index of dissimilarity (I.D.). This is the extent to which an antihaptoglobin serum concentration must be raised for a particular haptoglobin to give a microcomplement fixation reaction equal to that of the reference antigen. This was calculated in the following way:

\[
\frac{A_{413\text{ (as control)}} - A_{413\text{ (experimental tube)}}}{A_{413\text{ (as control)}} - A_{413\text{ (cell control)}}} \times 100
\]

(equation 1)

A 413 (as control): Absorbance of antihaptoglobin control at 413 nm.

The antihaptoglobin serum control is usually taken as the upper limit of lysis. The controls of antihaptoglobin serum, antigen and complement are very similar.

Fig. 1
: Immunoelectrophoretic pattern of:
Fig. A 1- Black rat haptoglobin challenged against antiblack rat haptoglobin.
   2- Black rat haptoglobin challenged against antialbino haptoglobin.
Fig. B 1- Black rat haptoglobin challenged against antimouse haptoglobin. (no reaction)
   2- Black rat haptoglobin challenged against antismall albino haptoglobin.
Results

The haptoglobins of experimental animals and their corresponding rabbit antisera were both of high purity. Cross-reactivity was observed when small albino haptoglobin was challenged immunoelectrophoretically with rabbit antimouse haptoglobin, and vice versa. Same results were obtained when albino haptoglobin was challenged with rabbit antiblack rat haptoglobin and vice versa (Fig. 1). No reaction occurred when albino haptoglobin or black rat haptoglobin were challenged with rabbit antismall albino haptoglobin, or rabbit antimouse haptoglobin.

Quantitative measurements of reactions between isolated haptoglobin from black rat, albino, small albino and mouse against their respective rabbit antihaptoglobins sera were obtained by using the microcomplement fixation test.

Results of microcomplement fixation reactions between dilutions of haptoglobin and dilutions of antihaptoglobin arranged in a checker-board pattern were obtained. When (results of these microcomplement fixation test) were plotted as a percentage of complement fixed (calculated as shown in equation 1) against concentrations of tested antigens (haptoglobins), bell shaped curves were obtained. The peak of each individual curve varied with the antihaptoglobin serum concentration. It was shown in all samples tested that the maximum fixation of complement occurred when the amount of antigen per tube was in the order of 90 ng, regardless of the concentration of antihaptoglobin. The maximum percentage of complement fixed varied between those four species. In case of black rat haptoglobin the figures were 89% and 87% for albino, 81% for small albino and 78% for mouse.

When the heights of peaks in each set of complement fixation curve is plotted against the respective antihaptoglobin concentration, straight lines are obtained. The slope (m) of these lines is defined by the equation:

\[ Y = mX + b \]

where: \( Y = \% \) complement fixed
\( X = \) antihaptoglobin concentration
\( b = \) the \( Y \) intercept

\text{equation 2}

When black rat haptoglobin was titrated against rabbit antialbino haptoglobin and vice versa, bell shaped curves were produced (Fig. 2). Straight lines were produced when peak heights of the complement fixation curve were plotted against antihaptoglobin concentration (fig. 3). Same results were obtained when mouse
haptoglobin was titrated against antismall albino haptoglobin and vice versa (Figs. 4, 5).

When black rat haptoglobin and antismall albino haptoglobin or antimouse haptoglobin were reacted, no complement fixation occurred (Fig. 6). This also applied when albino haptoglobin was similarly reacted with antismall albino haptoglobin or antimouse haptoglobin, (Fig. 7).

The lines calculated from heterologous reactions when compared with those from homologous reactions provided a basis for measuring the immunological relatedness between the albino and black rat in terms of the index of dissimilarity, in this case the figure was 1.45. Similar results were obtained for the immunological relatedness between mouse and small albino with an I.D. of 1.2 (calculated as in equation 3).

\[
\text{I.D.} = \frac{YH - Yh}{m} + \frac{XH}{XH}
\]

The effect of antismall haptoglobin concentration on complement fixation. The antigen (mouse haptoglobin) varies in concentration from 40 ng. in tube 1 to 130 ng. in tube 10.
Fig. 3

The dependence of peak heights of the complement fixation curve on the concentration of antismall albino haptoglobin when titrated against mouse haptoglobin.
Fig. 4

The effect of antialbino haptoglobin on complement fixation. The antigen (black rat haptoglobin) varies in concentration from 40 ng. in tube 1 to 130 ng. in tube 10.
The dependence of peak heights of the complement fixation curve on the concentration of antialbino haptoglobin when titrated against black rat haptoglobin.

Fig. 5
Black rat haptoglobin titrated against anti small albino haptoglobin.
Fig. 7

Albino haptoglobin titrated against antismall albino haptoglobin.
where \( m = \text{slope} \)

\[
YH = \text{maximum percent complement fixed with homologous antigen}
\]

\[
Yh = \text{maximum percent complement fixed with heterologous antigen}
\]

\[
XH = \text{antiserum concentration producing maximum percent complement fixed with the homologous antigen.}
\]

\[
Xh = \text{antiserum concentration producing maximum percent complement fixed with the heterologous antigen.}
\]

\text{equation 3}

Similar results were obtained previously by using other protein and ferritin (Mohammad, 1983).

\textbf{Discussion}

Microcomplement was chosen because it is capable of detecting minor structural differences between antigens (George and Dessauer, 1970). Thus, it is possible to define a quantitative measure of cross-reactivity known as the index of dissimilarity. Several investigators such as Prager & Wilson (1971) and Champion \textit{et. al.}, (1974), have demonstrated that in general there is a close relationship between the value of maximal fixation and the concentration of antiserum employed. The complement fixation data obtained in this study shows a close relationship between black rat and albino haptoglobins as well as between those of mouse and small albino.

Straight lines produced provide basis for measuring immunological relatedness between haptoglobins by comparing their homologous and heterologous reactions with rabbit antihaptoglobins sera. It is surprising to discover that the variations between black rat haptoglobin and both mouse and small albino haptoglobins, as well as between albino haptoglobin and both mouse and small albino haptoglobins were so large that no fixation occurred. It is expected that the structure of antigenic sites for haptoglobin in various species of rats would diverge. It seems likely that the great dissimilarity between black rat and albino haptoglobins, and those of mouse and small albino is due to variations in their primary structure. Further studies are necessary to determine the difference in amino acid sequences of haptoglobin from various species of rodents. The protein molecules that determine form and function are intricately folded chains of amino acid units. The primary
structure of each protein is governed by the sequence of nucleotides in the genetic material. The proteins of an organism are therefore the direct manifestation of its genetic endowment.

Mutation and natural selection are two fundamental interacting processes in evolution. Thus, the degree of divergence between species may differ when judged on the one hand by an ultimate criterion such as amino acid sequences, and on the other hand by techniques which investigate features of the protein structure such as charge or antigenic character.
References


