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PROPERTIES OF THE INTERSPECIES HYBRIDS BETWEEN HAPTOGLOBIN $\alpha$ AND $\beta$ SUBUNITS

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1. Subunits $\alpha$ isolated from human haptoglobin were recombined with $\beta$ subunits of equine haptoglobin, and vice versa. Both hybrid proteins were separated on electrophoresis in polyacrylamide gel into four bands with mobilities corresponding to tetramers $2\alpha\cdot2\beta$, trimers $2\alpha\cdot\beta$, and dimers $\alpha\cdot\beta$, in addition to free subunits $\beta$.

2. The binding ability of haemoglobin and the antigenic specificity of tetramers depended on the origin of $\beta$ subunit.

3. Reduction of native and hybrid proteins with 2-mercaptoethanol led to gradual formation of $\alpha\cdot\beta$, $\alpha$, and $\beta$; the components $2\alpha\cdot\beta$ and $2\alpha$ appeared in trace amounts.

Human haptoglobin ($Hp^H$)\(^1\) of the 1-1 type is a tetramer composed of two light subunits $\alpha$ (mol. wt. 9100) and two heavy subunits $\beta$ (mol. wt. 40 000), (Malchy et al., 1973). The tetrameric structure of the Hp molecule is maintained by the non-covalent bonds and disulphide bridges, joining subunits $\alpha$ and $\beta$, and subunits $\alpha$ and $\alpha$. Haptoglobin forms with haemoglobin a stable Hp-Hb complex which shows catalytic properties of "true" peroxidase (Jayle, 1951).

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\(^1\) Abbreviations used: Hp, haptoglobin; Hb, haemoglobin; $Hp^H$, human haptoglobin; $Hp^E$, equine haptoglobin; $2\alpha^H\cdot2\beta^E$, the hybrid containing 2 subunits $\alpha$ of human haptoglobin and 2 subunits $\beta$ of equine haptoglobin; $2\alpha^E\cdot2\beta^H$, the hybrid containing 2 subunits $\alpha$ of equine haptoglobin and 2 subunits $\beta$ of human haptoglobin; PHMB, $p$-hydroxymercuribenzoate; SDS, sodium dodecyl sulphate.
Haptoglobin isolated from mammalian blood exhibit usually distinct structural similarity to Hp 1-1 (Fraser & Smith, 1971), it is, therefore, possible to construct hybrid proteins composed of subunits ζ and β from haptoglobins originating from various mammalian species (Dobrzanyska & Osada, 1977).

The aim of our present work was to determine biological properties of the hybrid proteins $2\alpha^H.2\beta^E$ and $2\alpha^E.2\beta^H$ formed from human and equine haptoglobins. The ability to form active complexes with haemoglobin and antibody, and dissociation of native and hybrid haptoglobin were studied.¹

**MATERIALS AND METHODS**

*Materials.* The reagents used were from the following sources: Sephadex G-200 (Pharmacia, Uppsala, Sweden); DEAE-23 cellulose (Serva, Heidelberg, F.R.G.); Coomassie Brilliant Blue, R 250 (B.H.D., Poole, England); p-hydroxymercuribenzoate, Na salt (Serva, Heidelberg, F.R.G.); N,N-methylene-bis-acrylamide (Bio-Rad, Richmond, Calif., U.S.A.); dodecyl sulphate, (Intern. Enzymes Ltd, Windsor, England); 2-mercaptoethanol (Fluka, Buchs, Switzerland). The standard proteins used for molecular weight determination were pure preparations of human haptoglobin and its subunits (Dobrzanyska & Osada, 1977), chymotrypsinogen A (mol. wt. 25 000) and bovine serum albumin (45 000) obtained from Serva. Other reagents were from P.O.Ch. (Gliwice, Poland).

Human haptoglobin, 1-1 type, was isolated from ascitic fluid by the method of Smith *et al.* (1962). Equine haptoglobin was isolated from blood serum as described by Dobrzanyska & Krawczyk (1979). The preparations used for the experiments were of 100% purity, as checked spectrophotometrically (Mouray, 1966) and by disc electrophoresis in polyacrylamide gel.

The p-hydroxymercuribenzoate derivatives of subunits ζ and β were prepared according to Bernini & Borri-Voltattorni (1970). Haptoglobin hybrids were obtained by the method of Dobrzanyska & Osada (1977) from the PHMB derivatives of the human and equine haptoglobins.

The antisera against HpH and HpE were obtained from the immunized rabbits as described previously (Dobrzanyska *et al.*, 1979).

*Methods.* Haptoglobin was determined by the peroxidase method of Jayle (1951). Immunodiffusion was performed according to Ouchterlony (1949). Electrophoresis in SDS-polyacrylamide gel and molecular weight determinations were carried out according to Weber & Osborn (1969).

Gradual reduction of disulphide bonds was performed using a 1200-fold excess of 2-mercaptoethanol with respect to protein. At the defined time intervals, 100 μl portions of the incubation mixture were withdrawn, and

¹ A preliminary report has been presented at the XI International Congress of Biochemistry (Toronto, Canada, July 1979; *Abstr. Comun.* 03-2-5167, p. 178).
20 µl of 0.6 M-iodoacetamide was added to block free sulphydryl groups. After 10 min the mixture was subjected to electrophoresis in polyacrylamide gel containing SDS, then the gels were left for 15 h in 15% trichloroacetic acid, and stained with 0.05% Coomassie Blue in 15% trichloroacetic acid. The amount of the particular fractions was determined densitometrically with a Carl Zeiss (Jena) densitometer.

RESULTS

Subunits αHp^H^ were hybridized with subunits βHp^E^ and vice versa. In both cases, four bands appeared on SDS-polyacrylamide-gel electrophoreograms, and were evaluated quantitatively (Fig. 1). Composition of the hybrids was established by measuring molecular weight (Fig. 2) according to Weber & Osborn (1969).

![Fig. 1. Densitograms of the SDS-polyacrylamide gel electrophoreograms of the hybridization mixtures: αHp^H^ plus βHp^E^ (gel 1), and αHp^E^ plus βHp^H^ (gel 2). The percent content of the components is indicated.](image)

The following hybrids of subunits α and β were obtained: (a) on mixing subunits α^H^ with β^E^- : 2α^H^-2β^E^, 2α^H^-β^E^, α^H^-β^E^, β^E^-; (b) on mixing subunits α^E^- with β^H^- : 2α^E^-2β^H^, 2α^E^-β^H^, α^E^-β^H^, β^H^-. In both cases, tetramers constituted 75% of total hybrids, whereas contribution of other products differed depending on the origin of the subunits.

The tetramers were separated from other hybridization products by Sephadex G-200 gel filtration, then their ability for complexing haemoglobin and antibody was assayed (Table 1).

The Hb^E^-Hp^H^ complex showed a higher peroxidase activity than the homologous complex with equine haptoglobin. The hybrid containing human β-chain had a higher ability to bind equine haemoglobin than 2α^H^-2β^H^- tetramer. In the double-immunodiffusion assay with rabbit antiserum against human and equine haptoglobin, the hybrid proteins showed precipitation
Fig. 2. Molecular weight of the components of the hybridization mixtures: \( \alpha \text{Hp}^E \) plus \( \beta \text{Hp}^E \) and \( \alpha \text{Hp}^E \) plus \( \beta \text{Hp}^H \), determined according to Weber & Osborn (1969). The standard proteins used (not shown): chymotrypsinogen A (mol. wt. 25 000), albumin (45 000), human haptoglobin (98 000), \( \alpha \) subunit of human Hp (9100) and \( \beta \) subunit of human Hp (40 000).

### Table 1

**Binding of haemoglobin by human, equine and hybrid haptoglobins**

The peroxidase activity was determined according to Jayle (1951).

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Peroxidase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native human haptoglobin 1-1, (2\alpha^H.2\beta^H)</td>
<td>100</td>
</tr>
<tr>
<td>Native equine haptoglobin, (2\alpha^E.2\beta^E)</td>
<td>78.9</td>
</tr>
<tr>
<td>Human haptoglobin*, (2\alpha^H.2\beta^H)</td>
<td>90.9</td>
</tr>
<tr>
<td>Equine haptoglobin*, (2\alpha^E.2\beta^E)</td>
<td>69.4</td>
</tr>
<tr>
<td>Hybrid (2\alpha^H.2\beta^E)</td>
<td>70.0</td>
</tr>
<tr>
<td>Hybrid (2\alpha^E.2\beta^H)</td>
<td>78.0</td>
</tr>
</tbody>
</table>

* Control preparation obtained by recombination of homologous subunits.
lines pointing to full antigenic compatibility with that haptoglobin from which derived subunit β, and only partial antigenic compatibility with the haptoglobin from which subunit α originated (Table 2).

**Table 2**

Cross-reactions of human, equine and hybrid haptoglobins
The double-immunodiffusion method of Ouchterlony (1949) was applied.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Antiserum against haptoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>human, 1-1</td>
</tr>
<tr>
<td>Human haptoglobin</td>
<td>+</td>
</tr>
<tr>
<td>Equine haptoglobin</td>
<td>−</td>
</tr>
<tr>
<td>Hybrid 2α²β²E</td>
<td>±</td>
</tr>
<tr>
<td>Hybrid 2α²β²H</td>
<td>+</td>
</tr>
</tbody>
</table>

The time-course of reduction of native and hybrid haptoglobins in the presence of 2-mercaptoethanol given in a 1200-fold excess with respect to protein, was followed for 90 min; the appearance of reduction products was detected by SDS-polyacrylamide gel electrophoresis (Figs. 3, 4). It has been found that 85% of the tetramers 2α²β²H and 2α²β²E disappeared after 15 and 20 min, while hybrid tetramers 2α²β²H and 2α²β²E after 13 and 34 min, respectively. In all hybrids, dimer αβ was the main product of partial reduction. Trimers 2αβ were present in trace amounts. Reduction of Hp²E and the 2α²β²E hybrid produced an insignificant amount of 2α dimer.

**DISCUSSION**

Equine haptoglobin, like human, rat and rabbit haptoglobins, but unlike dog Hp (Kurosky et al., 1979) does not dissociate to dimers in 6mM urea. Reduction results in liberation of free subunits α and β which may recombine with the analogous subunits of haptoglobins from other mammalian species to form hybrids. Hybridization of human and equine haptoglobins, similarly as hybridization of human and porcine haptoglobins (Dobryszycza & Osada, 1977), resulted in the formation of a four-component mixture in which the hybrid tetramer predominated while the contribution of other components in the mixture differed slightly, depending on the origin of the subunits. The hybrids formed from Hp² and Hp²E, similarly as the proteins obtained from human and porcine haptoglobins (Dobryszycza & Osada, 1977), showed antigenic properties characteristic of that haptoglobin from which originated subunit β, a carrier of most of the antigenic determinants (Javid & Fuhrman, 1971). Peroxidase activity of the Hb²E-Hp² and Hb²E-Hp² complexes as well as that of hybrid tetramers also depends on the origin
Fig. 3. Time-course of dissociation of human haptoglobin 1-1 (A), and equine haptoglobin (B). For details see Methods. The components obtained: △, αβ; ○, β; □, α; ▲, 2α; Δ, 2αβ; ○, 2α-2β.

of subunit β. It seems of interest that the heterologous HbE-HpH complex shows higher peroxidase activity, measured by the method of Jayle (1951), than the homologous HbE-HpE complex.

During a gradual dissociation of native and hybrid haptoglobins by 2-mercaptoethanol, 2αβ, αβ and 2α appear, whereas no dimer 2β is formed.
Fig. 4. Time-course of dissociation of the hybrids: $2\alpha^H.2\beta^E$ (A), and $2\alpha^E.2\beta^H$ (B). For details see Methods. Designation of components as in Fig. 3.

Thus, the structure of hybrids resembles the structure of native human and equine haptoglobins in which disulphide bridges bind heavy and light subunits, as well as light and light subunits.

Virella & Parkhouse (1973) and Petersen & Dorrington (1974), on reduction and oxidation of human immunoglobulins, obtained as the main
products $\alpha\beta$ and $2\beta$ dimers and $2\beta\alpha$ trimer. Basing on the theoretical model proposed by the above-mentioned authors, and the data obtained in our experiments, the following scheme of the reduction of native and hybrid haptoglobins is suggested:

![Reduction Scheme](image)

In all cases, the reduction proceeds via reactions 1, 2 and 3, the $\alpha\beta$ dimer being the main intermediate. Disulphide bonds joining subunits $\beta$ with homologous or heterologous subunits $\alpha$ are more resistant to the action of reducing agents than the bonds linking subunits $\alpha$ and $\alpha$.

REFERENCES


WŁASCIWOŚCI HYBRYDOWYCH HAPTOglobIN POWSTAŁYCH
Z RÓŻNOgATUNKOWYCH PODJEDNOSTEK $\alpha$ I $\beta$

Streszczenie

1. Przeprowadzono hybrydyzację pomiędzy podjednostkami $\alpha$ wyizolowanymi z haptoglobiny ludzkiej i podjednostkami $\beta$ haptoglobiny końskiej i odwrotnie. W obu przypadkach elektroforeza w zelu poliakryloamidowym otrzymywanych białek hybrydowych wykazała obecność 4 pasm o ruchliwościach odpowiadających tetramerom $2\alpha\cdot2\beta$, trimerom $2\alpha\cdot\beta$, dimerom $\alpha\cdot\beta$ oraz wolnym podjednostkom $\beta$.

2. Zdolność wiążania hemoglobininy oraz specyficzność antygenowa tetramerów zależne były od pochodzenia podjednostek $\beta$.

3. Redukcja białek natywnych i hybrydów poddanych działaniu nadmiaru 2-merkaptopropanolu przebiegała ze stopniowym pojawianiem się składników $\alpha\cdot\beta$, $\alpha$ i $\beta$; składniki $2\alpha\cdot\beta$ i $2\beta$ pojawiały się w śladowych ilościach.

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