ELIMINATION OF BOVINE HAPTOGLOBIN FROM RAT CIRCULATION

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The rate of elimination from rat circulation of bovine or rat haptoglobin, of their complexes with homologous haemoglobin, and of preparations deprived of the terminal sialic acid (asialo-haptoglobin), was studied.

The rate of elimination was identical for bovine haptoglobin and its complexes with bovine or rat haemoglobin (the half-life time, $t_{1/2}$, was 10 h). The half-life time of complexes of rat haptoglobin with bovine or rat haemoglobin was about 2 h, and it was much shorter than $t_{1/2}$ for rat haptoglobin (13 h).

The shortest half-life time was observed for bovine and rat asialo-haptoglobin, 35 and 15 min, respectively.

The elimination curves showed a biphasic or triphasic character, depending on the rate of elimination.

The haemoglobin liberated intravascularly from erythrocytes, becomes bound to haptoglobin, a glycoprotein of blood. The complex formed is rapidly removed from circulation by endocytosis in liver [1-4]. According to Wada et al. [2] formation of the haptoglobin-haemoglobin complex is the first step of haemoglobin metabolism in liver. Kino et al. [5] suggest that the hepatic receptor of the complex recognizes the conformational changes of haptoglobin molecule resulting from its binding with haemoglobin, but that the mechanism of the receptor-haptoglobin binding differs from that operating in the case of receptors for asialo-glycoproteins [6] or for the haem-haemopexin complex [7].

In the previous work [8] it was found that bovine haptoglobin, although it forms a complex with haemoglobin and is a typical “acute-phase”

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protein, appearing in cow serum only in inflammatory and post-surgery conditions, differs distinctly in its physico-chemical properties and quaternary structure from the so far described mammalian haptoglobins.

It seemed, therefore, interesting, to compare the elimination from rat circulation of this protein as well as its complex with haemoglobin, with the elimination of homologous haptoglobin.

MATERIALS AND METHODS

Bovine haptoglobin was prepared from sera obtained from animals with an inflammatory condition as described previously [8]. Rat haptoglobin was isolated from sera of animals which 48 h before bleeding were given a subcutaneous injection of turpentine (0.5 ml/100 g body weight). Haptoglobin was isolated by affinity chromatography on a Sepharose 4B-rabbit haemoglobin column, as described by Haugen et al. [9]. Bovine, rat and rabbit haemoglobin was prepared according to Woźniak [10].

The complexes of bovine or rat haptoglobin with bovine or rat haemoglobin were obtained in the following way: to 10 mg of haptoglobin dissolved in 3 ml of 0.1 M glycine buffer, pH 8.6, was added a twofold excess of haemoglobin. After 15 min the excess of haemoglobin was removed by filtration through a Sephacryl S-300 column equilibrated with glycine buffer containing 0.5 M NaCl.

Sepharose 4B was activated with bromocyanide and coupled to protein according to March et al. [11]. Protein was determined according to Lowry et al. [12].

Rat haptoglobin was de-sialized with use of Clostridium perfringens neuraminidase (1.25 U/mg, Boehringer, F.R.G.) at pH 6.5 according to Jones et al. [13].

Since bovine haptoglobin is insoluble at pH values lower than 8.0, its de-sialization was performed as follows: 10 mg of the purified preparation was dissolved in 0.1 M glycine/NaOH buffer, pH 8.6, and added to 10 ml of a suspension of Sepharose 4B - bovine apohaemoglobin equilibrated with the same buffer [8]. After stirring the mixture for 2 h, the gel was washed with 0.05 M acetate buffer, pH 6.5, containing 154 mmol/l NaCl and 9 mmol/l CaCl₂, then it was suspended in the same buffer and 1 mg of neuraminidase was added. The suspension was stirred for 24 h at 37°C, then the gel was washed with 0.1 M glycine buffer, pH 8.6, and the protein eluted from the gel with 6 M urea was dialysed against 0.05 M glycine buffer, pH 8.6, and concentrated by Amicon ultrafiltration to about 2 mg protein/ml.

Determination of sialic acid by the resorcinol method [14] revealed that desialization of rat haptoglobin amounted to 93% and of bovine haptoglobin to 80%.
For $^{125}$I labelling of rat haptoglobin and its complexes with haemoglobin, as well as of rat asialo-haptoglobin, the method of Greenwood et al. [15] at pH 6.2 was applied. Specific activity of the preparations obtained ranged from 9.12 to $9.78 \times 10^7$ cpm/mg.

Bovine haptoglobin, its complexes with haemoglobin and bovine asialo-haptoglobin were $^{125}$I-labelled as follows: all reagents were prepared in 0.05 M glycine/NaOH buffer, pH 8.6. To 0.5 ml of the solution of the preparation (about 1 mg protein) 10 $\mu$l of Na$^{125}$I (20 MBq) and 0.25 ml of chloramine-T solution (5 mg/ml) was added. The reaction was stopped after 30 s by adding 0.5 ml of Na$_2$S$_2$O$_5$ (2.6 mg/ml), and the excess of non-reacted $^{125}$I was discarded by separation on a Sephadex G-25 column (1.5 x 30 s). Specific activity of the preparations obtained was 4.1 - 4.65 $\times 10^7$ cpm/mg.

Half-life times ($t_{1/2}$) of the preparations studied were determined in the blood of 3-month-old male Buffalo rats weighing 230 - 250 g. The rats were injected, under ether anaesthesia, with 130 - 150 $\mu$g of $^{125}$I-labelled preparation into the thigh vein. At determined time intervals, 0.1 ml of blood was withdrawn from the tail vein into tubes containing 10 $\mu$l of heparin. After centrifugation, 50 $\mu$l of plasma was taken and added to 1 ml of 20% trichloroacetic acid. The supernatant after centrifugation was discarded and the radioactivity of the sediment was read in a Gamma-Automat NRG-603 (C.S.S.R.). The number of impulses observed about 30 s after the injection was taken as 100%, and the other results were expressed as percentage of radioactivity decrease. The half-life times were calculated from straight parts of the curves presented in a semilogarithmic plot. The data are mean values for three determinations, the range not exceeding 1.5%.

Kinetic analysis was carried out according to Dobrszycka et al. [16]. Plasma radioactivity was calculated from the equation $b(t) = c_1 e^{-b_1 t} + c_2 e^{-b_2 t} + \ldots \ldots$ with half-life $t_{1/2} = \ln 2/b$ and the fractional turnover rate $= (c_1/b_1 + c_2/b_2 + \ldots \ldots)^{-1}$ where b is the slope and c the intercept.

RESULTS

The rate of clearance from rat circulation of bovine haptoglobin, its complexes with bovine or rat haemoglobin, and of bovine asialo-haptoglobin, was compared with the elimination rate of the corresponding preparations of rat haptoglobin. As it may be seen in Fig. 1 there was no difference in the rate of elimination from rat circulation between bovine haptoglobin and its complexes either with bovine or rat haemoglobin. The determined half-life time, 10 h, was identical for the three preparations. The clearance of rat haptoglobin complexed with homologous or hetero-
logous haemoglobin was more rapid than that of rat haptoglobin alone
(2 h versus 13 h, Fig. 2).

Bovine asialo-haptoglobin was eliminated from rat circulation after a time
twice as long as that observed for rat asialo-haptoglobin (t_{1/2} 35 min and
15 min, respectively).

The calculated fractional turnover rates are summarized in Table 1. They were high for preparations of short half-life time (0.25 - 2 h) and
biphasic character of the curves while low in the course of elimination
of triphasic character.

**DISCUSSION**

Haptoglobin, a protein that has the capacity to bind haemoglobin
appears in the blood of animals with inflammatory condition, but not
in that of healthy ones. The bovine haptoglobin, a high molecular glyco-
### Table 1

Elimination of bovine and rat haptoglobins, their complexes and asialohaptoglobins from rat circulation. A kinetic analysis

<table>
<thead>
<tr>
<th>125I-labelled preparations (n)</th>
<th>Half-life time (h)</th>
<th>Slopes (h⁻¹)</th>
<th>Intercepts (c)</th>
<th>Fractional turnover rate (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine haptoglobin (3)</td>
<td>10</td>
<td>0.0693</td>
<td>0.3648</td>
<td>0.8664</td>
</tr>
<tr>
<td>Rat haptoglobin (3)</td>
<td>13</td>
<td>0.0530</td>
<td>0.5776</td>
<td>1.7328</td>
</tr>
<tr>
<td>Bovine haptoglobin-Hb*complex (6)</td>
<td>10</td>
<td>0.0701</td>
<td>0.3643</td>
<td>0.8647</td>
</tr>
<tr>
<td>Rat haptoglobin-Hb*complex (6)</td>
<td>2</td>
<td>0.3466</td>
<td>2.7726</td>
<td>—</td>
</tr>
<tr>
<td>Bovine asialohaptoglobin (3)</td>
<td>0.58</td>
<td>1.1951</td>
<td>5.5452</td>
<td>—</td>
</tr>
<tr>
<td>Rat asialohaptoglobin (3)</td>
<td>0.25</td>
<td>2.7726</td>
<td>18.4839</td>
<td>—</td>
</tr>
</tbody>
</table>

* Elimination of haptoglobin complexes containing rat or bovine haemoglobins was very similar, therefore the results obtained were submitted jointly to the kinetic analysis.

Protein (sedimentation coefficient 16.4 S) is a polymer composed of a single type of subunits of $M$, 26000 [8], in contrast to human haptoglobins of most mammals, which are tetramers built of light subunits $\alpha$ and heavy subunits $\beta$, with molecular weight of about 100 000 [17]. Bovine haptoglobin contains about 6.8% of sialic acid and 8.2% of other carbohydrates.

Fig. 3. Elimination curves of rat 125I-asialohaptoglobin (○) and bovine 125I-asialo-haptoglobin (●) from rat circulation

The short, only 35-min half-life time in rat circulation of bovine asialohaptoglobin (Fig. 3) indicates that the galactose residues exposed by desialization are recognized by specific hepatic receptors (lectins) and that the

It seems however that both bovine haptoglobin and its complexes with either bovine or rat haptoglobin undergo endocytosis as "foreign proteins". This supposition is supported by the observed lack of any differences in the rate of disappearance from rat circulation between bovine haptoglobin and its complexes with haemoglobin (Fig. 1), whereas in the systems containing other haptoglobins and their complexes with haemoglobins these differences are significant. In mammalian liver a specific receptor eliminates rapidly from circulation the haptoglobin-haemoglobin complex [1 - 5].

Murray et al. [1] and Borel et al. [19] determined the half-life time of rabbit haptoglobin in rabbit circulation to be about 5 h, whereas the haptoglobin-haemoglobin complex was eliminated five times faster. A still more distinct difference was observed by Krauss & Sarcione [20]. They determined the half-life time of human haptoglobin and its complex with haemoglobin in rabbit circulation to be 19 and 2 h, respectively. Accelerated clearance of the haptoglobin-haemoglobin complex was observed also in humans [21], rats [4] and birds [10].

Bovine haptoglobin differs probably from haptoglobins of other mammals not only in physico-chemical properties but also in biological ones, and it may fulfill in cow organism other functions in which haemoglobin binding is not necessarily involved. Probably, the conformational changes occurring in a bovine haptoglobin molecule on its binding to haemoglobin are not of such a character that, according to Kino et al. [5], would permit recognition of the complex by hepatic receptor.

It seems rather improbable that cow liver contains a receptor specific only for the homologous haptoglobin-haemoglobin complex, as mammalian hepatic receptors show as a rule low specificity [3, 20, 22, 23], binding also complexes of haptoglobin and haemoglobin derived from other species.

The methods for labelling proteins with radioactive iodine involving the use of chloramine-T, are sometimes criticized [24 - 26]. It is believed that chloramine-T, by modifying proteins, shortens apparently their half-life time, leaving out of consideration the fact that incorporation of $^{125}$I into protein leads by itself to protein modification, irrespective of the method applied.

McCoy & Dixon [27] observed a direct relationship between the rate of protein elimination from circulation in vivo and the amount of chloramine-T used for iodination. Low chloramine-T concentration and short reaction time (such as used in the method of Greenwood et al. [5] applied in the present work) cause but very small modifications in proteins. It should be added that the present studies have a comparative character and an error inherent of the method would equally concern all the labelled preparations.
REFERENCES


