A C T A B I O C H I M I C A P O L O N I C A
Vol. 35 1988 No. 3

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ELIMINATION FROM RAT CIRCULATION OF GOAT AND SHEEP HAPTOGLOBIN AND THEIR COMPLEXES WITH RAT HAEMOGLOBIN*

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Received 4 December, 1987

The rate of elimination from rat circulation of ^{125}I-labelled human, goat and sheep haptoglobin, and their complexes with rat haemoglobin was studied.

The clearance of human haptoglobin-rat haemoglobin complex from rat circulation was about 5 times faster than that of free haptoglobin.

For sheep and goat haptoglobin and their complexes with rat haemoglobin the rate of elimination was identical, the half-life time $t_{1/2}$ ranging from 8 to 9 h.

The haemoglobin-haptoglobin complex formed *in vivo* during intravascular haemolysis or administered intravenously is incorporated into liver parenchymal cells through specific receptors for the molecule and in this way is rapidly eliminated from mammalian circulation [1, 2, 3]. Accelerated metabolism of chicken haptoglobin or human haptoglobin in complex with homologous haemoglobins was also observed in birds [4].

Osada [5] purified and partially characterized the haemoglobin binding protein of bovine blood (corresponding to haptoglobin) which differs distinctly in physico-chemical properties from the so far known mammalian haptoglobins. The author has also found that bovine haemoglobin-haptoglobin complex is eliminated from rat circulation at the same rate as free bovine haptoglobin, which suggests that this complex is not metabolized in the same way as other haemoglobin-haptoglobin complexes [6].

* This work was supported by the Central Research and Development Program 3.13.6.1.1.

Therefore, an investigation was undertaken on the rate of elimination from rat circulation of goat and sheep haptoglobin and of their complexes with rat haemoglobin. These animals were chosen because they also belong to the Bovidae family [7] and their haptoglobins closely resemble bovidae haptoglobin.

MATERIALS AND METHODS

Goat (*Caprus hircus*) and sheep (*Ovis aries*) were injected subcutaneously with 5 ml of turpentine; since in the blood of healthy animals haptoglobin appears only in trace amounts, it was necessary to induce an inflammatory condition to obtain a higher amount of haptoglobin. The blood was withdrawn from the jugular vein 48 h later.

Human haptoglobin of 2 - 2 type, and goat and sheep haptoglobins were isolated by affinity chromatography on Sepharose 4B CL coupled with chicken cyan-methaemoglobin according to Delers *et al.* [8]. Preparations of haptoglobins dissolved in 0.06 M phosphate buffer, pH 6.8 were further purified on a hydroxyl-apatite (Calbiochem, U.S.A.) column equilibrated with the same buffer. The protein was eluted from the column with a linear NaCl concentration gradient up to 0.3 M. Fractions of 5 ml were collected at a flow rate of 10 ml/cm^2/h and those containing haptoglobin, as estimated according to Jayle [9] were dialyzed against distilled water and then lyophilized.

Chicken and rat haemoglobin was prepared according to Woźniak [4].

Complexes of human, goat and sheep haptoglobin with rat haemoglobin were obtained in the following way: to 2 mg of haptoglobin dissolved in 1 ml of sterile 15 mM phosphate-buffered 0.15 M saline, pH 7.2 (Wytwórnia Surowic i Szczepionek, Lublin, Poland) was added 0.4 ml of 1% rat haemoglobin. The excess of haemoglobin was removed from the complex formed by filtration through a column (1.2 × 35 cm) containing Ultrogel AcA 44 and equilibrated with the same solution. Fractions of 2 ml were collected at flow rate of 12 - 14 ml/h.

The preparations were labelled with ^{125}I by the method of Salacinski *et al.* [10] with the use of Iodogen. Specific activity of the preparations was 8.5 - 9.2 × 10^7 cpm/mg.

The rate of elimination from and half-life times ($t_{1/2}$) of the preparations in the rat circulation were determined as described previously [6].

RESULTS

The rates of elimination from rat circulation of goat and sheep haptoglobin and their complexes with rat haemoglobin were compared with those for the analogous preparations of human haptoglobin type 2 - 2, which due

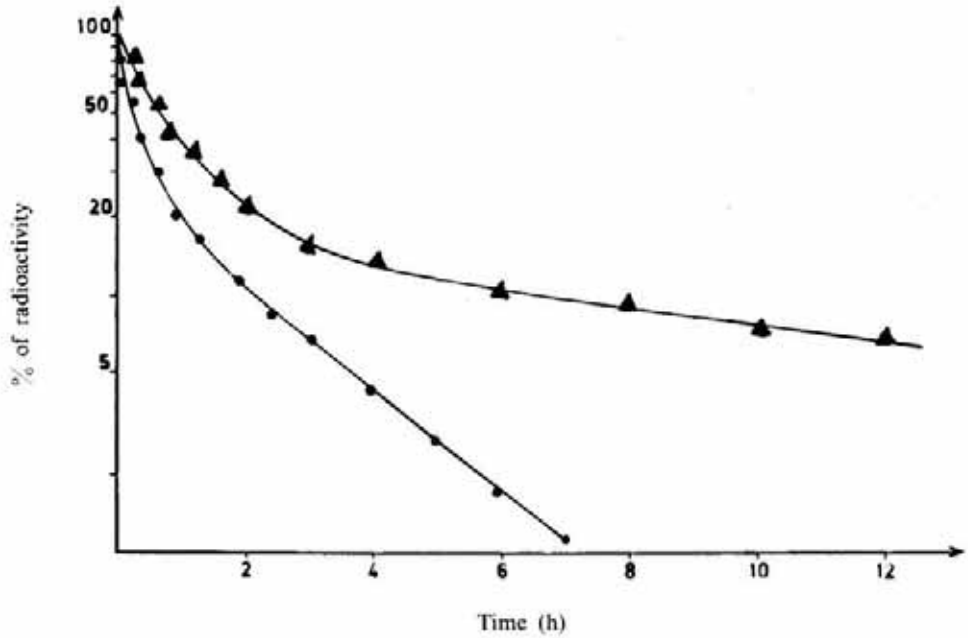

Fig. 1. Elimination curves of ^{125}I-labelled preparations of human haptoglobin from rat circulation. Human haptoglobin 2 - 2 type (▲), rat haemoglobin-human haptoglobin complex (●).

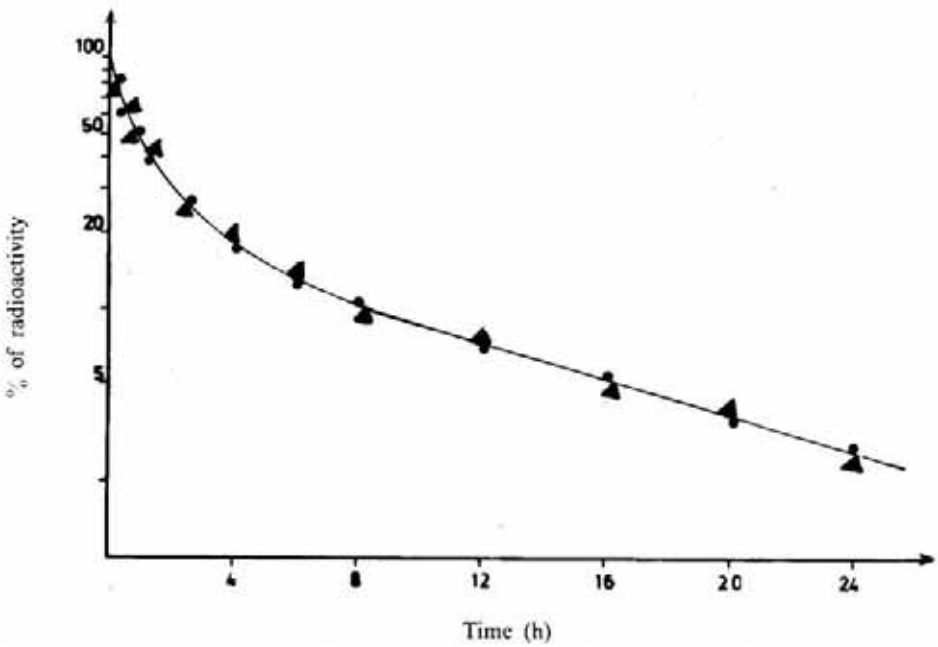

Fig. 2. Elimination curves of ^{125}I-labelled preparations of sheep haptoglobin from rat circulation. Sheep haptoglobin (▲), rat haemoglobin-sheep haptoglobin complex (●).

to its polymorphic character corresponds to heterologous haptoglobins of animals from the Bovidae family.

The clearance of human haptoglobin and its complex with rat haemoglobin had a typical course; metabolism of the complex being more rapid than of haptoglobin alone, and the half-life times $t_{1/2}$ being 1.75 and 9.0 h, respectively (Fig. 1).

The binding of sheep and goat haptoglobin with rat haemoglobin did not bring about a similar effect, and the rate of elimination from rat circulation of those haptoglobins and their complexes with rat haemoglobin was identical (Fig. 2 and 3).

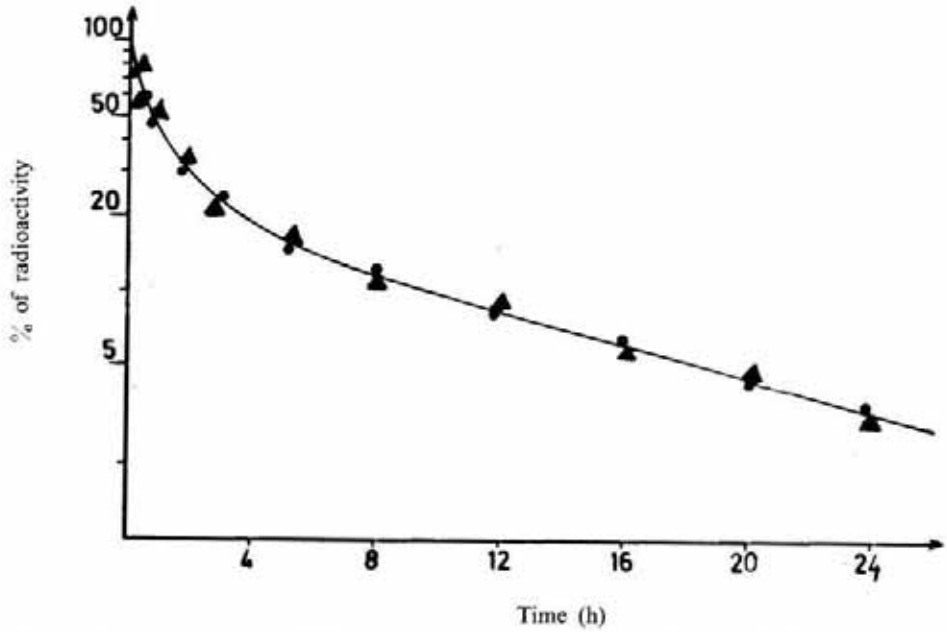

Fig. 3. Elimination curves of ^{125}I-labelled preparations of goat haptoglobin from rat circulation. Goat haptoglobin (▲), rat haemoglobin-goat haptoglobin complex (●).

From the kinetic analysis of the results obtained (Table 1) it may be seen that the biphasic character of the elimination curves was observed only in the case of the human haptoglobin-rat haemoglobin complex.

For all the other preparations studied the elimination curves had a triphasic character and the fractional turnover rates were 5 - 6 times lower than that for the human haemoglobin-haptoglobin complex.

Table 1

Elimination of human, sheep and goat haptoglobin and their complexes from rat circulation – kinetic analysis

Kinetic analysis was carried out according to Dobryszycka *et al.* [18]

^{125}I-labelled preparations	n	Half-life time (h)	Slopes (h^{-1}) b_1	b_2	b_3	Intercepts (c) c_1	c_2	c_3	Fractional turnover rate (h^{-1})
Human haptoglobin type 2-2	3	9.0	0.0770	0.4620	1.1552	0.18	0.60	0.22	0.2613
Sheep haptoglobin	4	9.2	0.0753	0.3466	0.6931	0.22	0.58	0.20	0.2048
Goat haptoglobin	3	8.4	0.0825	0.4332	0.8664	0.20	0.60	0.20	0.2475
Human haptoglobin-Hb complex	4	1.75	0.3961	3.4657	—	0.25	0.75	—	1.1798
Sheep haptoglobin-Hb complex	4	9.3	0.0745	0.3300	0.8664	0.23	0.59	0.18	0.1967
Goat haptoglobin-Hb complex	4	8.2	0.0845	0.3961	0.9241	0.19	0.62	0.19	0.2488

DISCUSSION

Haptoglobin, α_2-acid glycoprotein of serum, belongs to the "acute-phase" proteins which means that its level is much increased in various pathological conditions both in human and animal blood [11, 12].

Haptoglobin has not been found in many sera of healthy cow, sheep and goat [13]. This was probably due to insufficiently sensitive techniques used for its detection [13] as haptoglobin circulation in normal sera of Bovidae family members is extremely low [16] and rises to detectable level only as a response to stress, e.g. gamma radiation [14] or subcutaneous injection of turpentine [15, 16].

Haptoglobin has the capacity to form with haemoglobin stable complexes both *in vivo* and *in vitro* but it is not a typical transport protein as it is not released from the complex into circulation. It is sometimes called a "suicide protein" as its complex with haemoglobin is eliminated rapidly from the circulation and undergoes endocytosis in the liver reticuloendothelial system [2, 4].

For bovine haptoglobin and its complexes with either homologous or heterologous haemoglobin the rate of elimination from rat circulation was found to be identical, and their half-life time $t_{1/2}$ was 10 h [6].

In present studies it was demonstrated that also goat and sheep haptoglobin-rat haemoglobin complexes were eliminated from rat circulation at the same rate as were free haptoglobins, the half-life times being 8 - 9 h.

Cow, goat and sheep haptoglobins are heterogeneous and they form a series of polymers of increasing molecular weight, in that they resemble human haptoglobin of 2 - 2 type with polymorphic fractions of M_r 170000 - - 600000 [17].

Goat and sheep haptoglobin-rat haemoglobin complexes are probably recognized as "foreign proteins" as it was suggested in the case of bovine haptoglobin-haemoglobin complex [6]. In conclusion, catabolism of haptoglobins isolated from the blood of Bovidae family animals and their complexes with haemoglobins differs distinctly from that so far reported for others mammals.

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