Identification of haptoglobin as an endogenous inhibitor of prostaglandin H synthase in the cytosol fraction of primary cells from sheep vesicular glands

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It was shown that cytosol of primary sheep vesicular gland cells inhibits peroxidase activity of prostaglandin H synthase (PGHS). The degree of the enzyme inactivation depends on cytosol concentration. It was established that cytosol contains glycoprotein haptoglobin that is one of the cytosol basic components responsible for its property to inhibit PGHS. Haptoglobin is supposed to participate in endogenous regulation of PGHS activity in sheep vesicular glands.

Prostaglandin H synthase; Haptoglobin; Cytosol; Endogenous regulation; Sheep vesicular gland

1. INTRODUCTION

Prostanoids are produced by bienzyme systems of prostanoid synthesis from polyunsaturated fatty acids [1]. The limiting enzyme of such systems is prostaglandin H synthase (PGHS) that reveals two activities - cyclooxygenase and peroxidase [1,2].

The regulation of the prostanoid level is of great importance for the organism. Nonsteroidal antiinflammatory agents widely used in clinical practice can be related to exogenous inhibitors [2,3]. The knowledge about endogenous regulation mechanisms of prostanoid synthesis is limited. Endogenous inhibitors of prostanoid synthesis were found to be present in blood serum [4-6], in placenta [7], human amniotic fluid [8], endometrium of bovine and sheep [9]. Haptoglobin was shown to be one of such inhibitors in the serum [6,10].

In this communication we report on: (i) the effect of cytosol from sheep vesicular gland primary cells on the PGHS activity, (ii) the presence of haptoglobin in cytosol and (iii) the effect of haptoglobin from cytosol on the peroxidase activity of PGHS.

2. MATERIALS AND METHODS

Solubilized preparations of PGHS were isolated from sheep vesicular glands [1]. The PGHS peroxidase activity was registrated spectrophotometrically as the rate of adrenalin oxidation by hydrogene peroxide [2] and was 53 units/mg·min. Haptoglobin was isolated from sheep blood serum [12].

The protein concentration was determined according to the method of Lowry. Primary cells were obtained from sheep vesicular glands stored at -20°C. Vesicular glands were thawed out in the physiological solution at 4°C overnight, then used for the isolation of primary cells [13]. The washed cells were subjected to isotonical shock to obtain cytosol. The suspension was centrifuged at 8000 $\times g$ for 15 min. The supernatant was kept at -10°C. The protein concentration of cytosol was 0.8-2.6 mg/ml.

Hemoglobin binding abilities of cytosol, of cytosol after the incubation with Hb-Sepharose and of the protein(s), eluated from the sorbent with 8M urea, were determined by the electrophoretic mobility [14] and by the level of peroxidase activity [15] of their mixtures with methemoglobin after 30 min incubation at 37°C.

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3. RESULTS AND DISCUSSION

The effect of the cytosol solution (protein 2.12 mg/ml) on the peroxidase activity of the PGHS was studied (Fig. 1). The decrease in the enzyme activity occurred practically linearly with the increase in cytosol concentrations. Haptoglobin, isolated from blood serum, inhibited the PGE_2 production, the oxygen consumption and the peroxidase activity of PGHS [6,10]. That enabled us to suggest that this glycoprotein may be responsible for the ability of cytosol from sheep vesicular gland cells to inhibit PGHS. Therefore, an analysis of the cytosol solution was performed to reveal the presence of haptoglobin. It is known [13] that haptoglobin has a specific property to quickly bind hemoglobin forming a stable complex (Hp-Hb). This complex, likewise hemoglobin, is stained with benzidine on the electropherogram but differs from it in its electrophoretic mobility. This complex is notable for a higher peroxidase activity in comparison with hemoglobin [13]. Methods of haptoglobin identification in different biological liquids are based on these properties. Fig. 2 shows that after cytosol incubation

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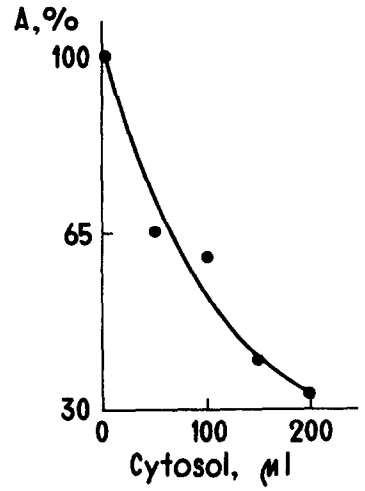

Fig. 1. The effect of cytosol concentration on PGHS peroxidase activity (A%).

with methemoglobin, on the electropherograms two heme-containing components are stained with benzidine (c): methemoglobin and new bands, which are absent in the case of cytosol (b) and methemoglobin. The plurality of new bands can be explained by sheep haptoglobin being polymorphous (d) [16].

Thus, results show the presence in cytosol of haptoglobin.

Haptoglobin binds with Hb-sepharose forming a stable complex that can be destroyed by 8 M urea [17]. The analysis of the eluate after its dialysis and concentration showed that it contained a protein revealing the property to bind hemoglobin into a stable complex (f). Cytosol after its incubation with Hb-sepharose didn't contain this protein (e).

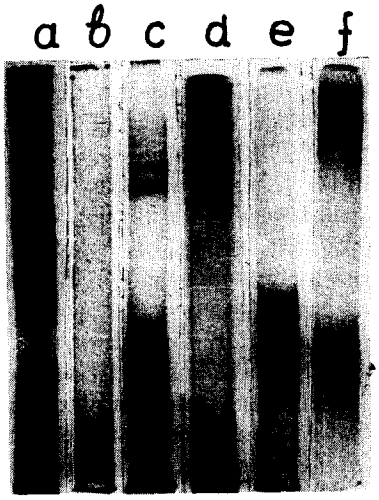

Fig. 2. The electrophoretical proof of the presence of haptoglobin in cytosol from primary cells of sheep vesicular glands. a,b - cytosol; c - cytosol + methemoglobin; d - haptoglobin + methemoglobin; e - cytosol after incubation with Hb-Sepharose + methemoglobin; f - protein eluated from Hb-Sepharose with 8M urea + methemoglobin. Staining with Benzidin (a,c-f) and with Amidoblack (b).

Table I

Effect of cytosol from primary cells of sheep vesicular glands and its components on the peroxidase activity of PGHS*

Additions	Final concentration of added protein (mg/ml)	Relative PGHS activity (%)
None	0	100
Cytosol	0.048	76
	0.095	31
	0.492	31
Cytosol after incubation with Hb-Sepharose	0.058	100
	0.087	93
Protein eluated from Hb-Sepharose with 8 M urea[a]	0.08	34

[a] Eluate was dialyzed and concentrated

Then the influence of haptoglobin removal from cytosol on its ability to inhibit the peroxidase activity of PGHS was investigated (Table I). From these data it may be concluded that the incubation of PGHS with the cytosol solution leads to a loss of its peroxidase activity. After the isolation of haptoglobin from cytosol the latter practically looses its ability to inhibit the enzyme. Haptoglobin isolated from cytosol retained the property to inhibit PGHS.

Thus, haptoglobin is a component of cytosol proteins in sheep vesicular gland cells and probably participates in the endogenous regulatin of the PGHS activity.

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