SOME PROPERTIES OF GLYCOGEN PHOSPHORYLASE B FROM MYOMAS OF THE HUMAN UTERUS

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Two forms of glycogen phosphorylase were detected in unpurified extracts of myomas of the human uterus and the surrounding myometrium. The active form of phosphorylase A (PA) accounted for 2-3% of the total phosphorylase activity. Activity of phosphorylase B (PB) in the myometrium was rather higher than in the myoma tissues. The content of PB in the uterus was 100 times less than in striated rabbit muscles. The uterine PB activity was increased about 10 times after purification of the enzyme by absorption on glycogen. Maximal PB activity from the myoma and myometrium was observed at pH 6.6. In the degree of its inhibition in the presence of glucose-6-phosphate and ATP inhibitors, PB from the myoma, myometrium, and striped muscles of the rabbit was virtually indistinguishable. However, in the presence of glucose, PB from the human uterus (myoma and myometrium) was inhibited to a somewhat greater degree than PB from rabbit striated muscle. The enzyme PB kinase from rabbit striated muscles catalyzed the conversion of PB from the myoma into PA, the active form.

The glycogen phosphorylase from striated muscle (α-1,4-glucan:orthophosphateglucosyltransferase, 2.4.1.1) is one of a well studied group of enzymes [1]. However, there is only limited information on the phosphorylases of smooth muscle. Few investigations of this enzyme have been made and all on homogenates or extracts. Differences have been found in the immunological properties of phosphorylases from striated muscles and from the smooth intestinal muscle of guinea pigs [5]. The smooth muscle of the rat uterus has been shown to contain two forms of phosphorylase, phosphorylase A (PA) and phosphorylase B (PB), with molecular weights of 230,000 and 200,000 respectively [9]. The object of the present investigation was to partially purify the glycogen phosphorylase from myomas of the human uterus and the surrounding normal tissue and to study some of its properties.

EXPERIMENTAL METHOD

Uterine myomas from women in the childbearing age, together with the myometrium, were obtained from the pathological laboratory (Head, Professor B. I. Zheleznov) of the All-Union Research Institute of Obstetrics and Gynecology. The tissue was frozen to −20°C, passed through a mincer, and homogenized in a tissue blender in the presence of 3 ml of a solution ($5 \cdot 10^{-2}$ M NaF and 10^{-3} M EDTA, pH 7.0) per gram tissue for 10 min at 4°C. After extraction for 1.5 h in the cold the homogenate was centrifuged at 35,000 g for 30 min. The extract was used for further purification or for determination of activity. Phosphorylase activity was measured by the method described by Yunis [10]. The protein concentration was determined by Lowry's method. Chromatographically pure glucose, glucose-6-phosphate, and ATP in concentrations of $2 \cdot 10^{-2}$ M and $4 \cdot 10^{-2}$ M respectively were used as inhibitors. The PB from rabbit striped muscles was purified by the method of Lisovskaya et al. [2]. The preparation of PB kinase was obtained by the method of Krebs and Fisher [7].

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TABLE 1. Effect of Inhibitors on Activity of PB (in %) from Human Uterus and Rabbit Muscles

Inhibitor	PB from myoma	PB from myometrium	PB from rabbit muscles
Glucose	64	62	77
Glucose-6-phosphate	57	60	60
ATP . . .	28	29	30

Note. Activity of PB measured at pH 6.8. Activity of PB in mixtures not containing inhibitors taken as 100%. Discrepancy between results in experiments 2-3%, not exceeding 5.5% in individual cases.

EXPERIMENTAL RESULTS

Activity of PA and PB in the extracts was measured at PA 6.3 in the presence of NaF to inhibit the action of phosphatases converting PA into PB [10]. The ratio between the two forms of phosphorylase in the original extract was determined after passing it through Sephadex G-25 gel to free the PB from endogenous AMP adsorbed on it [8]. After gel-filtration the phosphorylase activity of the extracts from the myomas and myometrium measured in the absence of AMP was reduced by several times compared with that of the untreated original extract, and it accounted for 2-3% of the total phosphorylase activity measured in the presence of AFP. Activity of PB in the extract from the myometrium and from the myomas had mean values of 0.27 and 0.23 μmole inorganic phosphate/mg protein respectively. The content of PB in the myomas and myometrium was two orders of magnitude lower than its content in rabbit striped muscles.

To concentrate the enzyme preparation a method of adsorption on glycogen was developed. Glycogen powder was added to the transparent tissue extract up to a final concentration of 1.5% and left to stand for 2.5 h. The suspension was centrifuged for 1 h at 88,000 g in the cold, the supernatant was discarded, the residue was suspended in $5 \cdot 10^{-2}$ M NaF and 10^{-3} M EDTA, pH 7.0, and NaCl was added to a final concentration of 1 M. After recentrifuging for 1.5 h at 88,000 g and 20°C the supernatant was collected, dialyzed against NaF and EDTA, pH 7.0, and its phosphorylase activity determined. A tenfold increase in the specific activity of the phosphorylase preparation from the myomas and myometrium was observed. Subsequently the properties of the PB were investigated, for the content of pH remained low.

The relationship between the activity of the partially purified preparations of PB and the pH of the medium was studied in β-glycerophosphate buffer. Optimal PB activity from the myomas and surrounding myometrium occurred at pH 6.6. According to data in the literature [4, 6], the optimal pH for PB from rabbit striped muscles is 6.7, and for pig liver phosphorylase, activated by AMP, it is 6.6.

A decrease in the activity of the partially purified phosphorylase preparations from the myoma and myometrium was observed under the influence of classical inhibitors of PB from rabbit muscles [1]. For comparison, the action of glucose, glucose-6-phosphate and ATP on rabbit PB was studied under the same conditions (Table 1).

The degree of inhibition of rabbit PB by glucose agreed with data in the literature obtained under similar conditions [3].

Changes in PB activity from the myoma and normal myometrium through the action of these inhibitors were practically identical. No differences likewise were found in the degree of inhibition of PB from the uterus and PB from the rabbit muscles under the influence of glucose-6-phosphate and ATP.

Some differences in the degree of inhibition of PB from the uterus and rabbit muscles were found only in the presence of glucose.

The possibility of conversion of PB from smooth muscle into its active form (PA) under the influence of PB kinase from striated muscle was investigated. For this purpose a sample of partially purified phosphorylase from the myoma was incubated at 30°C in $4 \cdot 10^{-2}$ M tris-β-glycerophosphate buffer, pH 8.6, containing $2 \cdot 10^{-2}$ M cysteine, 10^{-2} M magnesium acetate, and $5 \cdot 10^{-3}$ M ATP in the presence of a sample of rabbit PB kinase for 1 h. After incubation the mixture was diluted 1:10 with distilled water and the phosphorylase activity determined in the presence and absence of AMP. The results showed that phosphorylase activity in the absence of AMP was 95% of its activity in the presence of AMP. Consequently PB kinase from rabbit muscles almost completely converts PB from human uterine myomas into the active form, PA.

No qualitative differences in the properties of the phosphorylase from myoma and myometrium were thus observed. However, compared with rabbit PB, human uterine PB was inhibited somewhat more by glucose.

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