HIGH REACTIVITY OF KIDNEY PREPARATIONS FROM MAN, PIG, BEEF, DOG AND RAT FOR CARBAMYL PHOSPHATE HYDROLYSIS

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1. Introduction

Acyl phosphatase (EC 3.6.1.7) is widely distributed in animal tissues [1]. The ratio of activity with acetyl phosphate is 10 to 11 times that shown with carbamyl phosphate for preparations from beef brain, pig heart, and horse skeletal muscle and the ratio remains unchanged during all stages of purifications [2]. Interestingly, it has been shown recently that acyl phosphatase from human erythrocyte preparations which are very reactive with acetyl phosphate and 1,3 diphosphoglycerate do not attack carbamyl phosphate [3]. This communication shows that kidney preparations have a relatively higher reactivity with carbamyl phosphate than acetyl phosphate. Furthermore, the results described suggest that two types of acyl phosphatases are present in pig kidney.

2. Experimental

Fresh kidneys were obtained from either a local slaughterhouse or from laboratory animals, and human kidney was obtained as soon as possible after postmortem. Tissues were immediately processed or frozen at -20^0C until used. Reaction mixtures contained in 1 ml, 8 μmoles of acetyl or carbamyl phosphate, 100 μmoles of sodium acetate buffer (pH 6.0) and kidney extract. Twenty minutes incubation at 27^0C. One unit of enzyme activity is defined as the hydrolysis of one μmole of acetyl or carbamyl phosphate under these conditions. Acetyl phosphate was determined by the hydroxamic method of Lipmann and Tuttle [4]. Carbamyl phosphate was determined as hydroxyurea by the method of Levine and Kretchmer [5].

3. Results and discussion

In contrast to acyl phosphatase from other tissues which, as previously mentioned, have acetyl phosphatase/carbamyl phosphatase ratios of 10–11/1, the enzyme from kidney of all species thus far tested showed from $\sim$ 3/1 to nearly 1/1 ratio of activities. This is illustrated in table 1.

Centrifugation of homogenates from pig kidney at 15 000 *g* for 30 min results in a greater ratio of acetyl phophatase/carbamyl phosphatase in the supernatant than in the pellet (table 2). Therefore, an attempt was made to further separate the activities into a soluble and pellet fraction with a high specificity for carbamyl phosphate by successive washings and centrifugations. However, as shown in table 2 little resolution between the acetyl phosphatase and carbamyl phosphatase activities

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Table 1
Phosphatase activity with acetyl phosphate and carbamyl phosphate of kidney from several species

Source	Units per gram of kidney with		Ratio $\frac{\text{acetyl phosphate}}{\text{carbamyl phosphate}}$
	Acetyl phosphate	Carbamyl phosphate	
Beef	320	380	0.8
Pig	440	440	1.0
Dog	315	100	3.2
Rat	400	160	2.5
Man	150	90	1.6

Tissues were homogenized in a Waring blender with 8 vol of water.

Table 2
The effect of centrifugation and washing on activity of pig kidney acyl phosphatase

Preparation	Total units with		Ratio $\frac{\text{acetyl phosphate}}{\text{carbamyl phosphate}}$
	Acetyl phosphate	Carbamyl phosphate	
Homogenate	5800	4000	1.5
1st supernatant	4200	2850	1.47
Pellet	2350	2120	1.08
Pellet washed once	1740	1600	1.09
2nd supernatant	520	400	1.3
Pellet washed twice	1600	1350	1.19

Ten grams of frozen pork kidney were homogenized in 5 vol of 0.15 M KCl using a Potter Elvehjem homogenizer. All centrifugations were at 15 000 *g* for 30 min. The pellets were washed with 140 ml of 0.15 M KCl.

was obtained. Moreover, after the second washing little additional activity became soluble. Attempts to prepare a fraction rich in carbamyl phosphatase by either acetone drying or ethanol fractionation resulted in variable inactivation of the carbamyl phosphatase activity. The activities of acetone powders and ethanol fractions prepared from both whole pork kidney and from the pellet are illustrated in table 3. The table also typifies the effect of alkaline treatment of beef kidney. As shown, when beef kidney water homogenate was brought to pH 11–12, stirred gently for 20 min and then re-adjusted to pH 5.5 there was a two-fold increase of activity with acetyl phosphate, but the activity with carbamyl phosphate remained the same. Beef brain, pig heart and chicken breast enzymes showed no such effect.

The variations in the acetyl/carbamyl phosphatase ratio in the kidney fractions by such procedures contrasts with that observed in other tissues where a constant ratio of activity with acetyl phosphate to carbamyl phosphate activity is observed at all stages of purification. Furthermore, relatively larger amounts of activity with carbamyl phosphate are present in kidney than in any other tissue thus far tested. This, together with the recent discovery of the erythrocyte acyl phosphatase which is inactive with carbamyl phos-

Table 3
Effect of acetone, ethanol and alkali treatment on kidney acyl phosphatase

Experiment	Preparation	Units per gram of tissue with		Ratio $\frac{\text{acetyl phosphate}}{\text{carbamyl phosphate}}$
		Acetyl Phosphate	Carbamyl Phosphate	
1	Homogenate	277	207	1.3
1	Acetone powder	70	189	0.37
2	Pellet	94	100	0.94
2	Acetone powder of pellet	125	27	4.61
3	Supernatant	250	162	1.51
3	Ethanol fraction	44	0	
4	Beef kidney homogenate	327	211	1.55
4	Beef kidney homogenate after alkaline adjustment	580	190	3.05

In experiment 1, frozen pig kidney was ground. A portion was homogenized in 4 vol of water. An acetone powder [6] was made from the remainder, and then extracted with 7 vol of water. In experiment 2, frozen pig kidney was ground and then homogenized in 7 vol of 0.15 M KCl. After spinning at 15 000 *g* for 30 min, a portion of the pellet was removed and an acetone powder [6] was made of the remainder. In experiment 3, pig kidney was homogenized in 5 vol of 0.15 M KCl and centrifuged at 10 000 *g* for 10 min. An equal vol of ethanol was added to a portion of the supernatant (at 0°) centrifuged at 15 000 *g* for 30 min and the precipitate taken in 1 vol of 0.15 M KCl. In experiment 4, the pH of a 1:10 water homogenate of beef kidney was adjusted to 10.5–11 with 1 M NaOH and after 20 min in the cold re-adjusted to pH 5.5 with 1 N acetic acid.

phate [3], suggests that there may be several types of acyl phosphatases in tissues. Whether an enzyme specific only for carbamyl phosphate exists in kidney and in other tissues remains to be elucidated.

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