Occurrence of Phosphodiesterase and Ribonuclease Activities in the Microsomes from Cow's Milk

Sir:

Occurrence of RNase in cow's milk was reported and that of PPDase was also suggested by E. W. Bingham and C. A. Zittle¹⁾. In wheat seedlings^{2,3)}, PPDase was found in the microsomes, and the metabolism of nucleic acids in microsomes was thought to be interesting, considering from the roles of microsomes in cells. The distribution of PPDase activity in milk was examined, and the PPDase activity was found to be concentrated in the microsomes.

Fresh cow's milk was centrifuged at 1,500×g for 15 min. and cream was separated from skim milk⁴⁾. The cream was washed twice by dispersion in water, and the washings were collected by use of separating funnel and centrifuged at 1,500×g to remove the

cream as much as possible (butter milk). Microsomes were precipitated by centrifuging the butter milk at 100,000×g for 120 min. and supernatant was obtained. Whey was prepared from skim milk by the elimination of casein with adjusting pH to 4.6. The distribution of hydrolytic activity for RNA and DNPP in each fraction separated from cow's milk was estimated. Generally, DNPP is considered to be the specific substrate for PPDase⁵⁾.

As shown in Table I, a good amount of the PPDase activity was considered to be contained in the microsomal fraction of milk, and the activity was completely inhibited by the addition of EDTA. The pH optimum of the PPDase activity in the microsomes was found around 8.0 when DNPP was used as

TABLE I. DISTRIBUTION OF HYDROLYTIC ACTIVITY FOR RNA AND DNPP
IN COW'S MILK

Fractions	Total units (in 100 ml milk)						Total nitrogen (mg)
	RNA			DNPP			
	None	MgCl ₂	EDTA	None	MgCl ₂	EDTA	
Whey	31,300	21,400	28,100	52	62	0	149
Butter milk							
supernatant	430	320	400	5	6	0	10
microsomes	350	280	290	47	55	0	11

RNase activity was measured by the method described in the previous paper⁶⁾ using the reaction mixture containing, in a total volume of 2 ml, 5 mg of yeast RNA, 20 μ moles of MgCl₂ (or EDTA), 200 μ moles of Tris-HCl (pH 8.0) and enzyme. PPDase activity was measured as follows: The reaction mixture containing, in a total volume of 4 ml, 2 μ moles of Ca (DNPP)₂, 40 μ moles of MgCl₂ (or EDTA), 400 μ moles of Tris-HCl (pH 8.0) and enzyme was incubated at 37°C for 20 min. The reaction was stopped by the addition of 1 ml of 1 N NaOH. Optical density of the supernatant was measured at 420 m μ and corrected by the method described by Ogata et al⁷⁾. One unit of the enzyme was defined as the amount of enzyme which catalyzed the formation of 1 μ mole of *p*-nitrophenol per ml of reaction mixture per hour.

Abbreviations: RNA, ribonucleic acid; RNase, ribonuclease; PPDase, phosphodiesterase; DNPP, bis (*p*-nitrophenyl) phosphate; EDTA, ethylenediaminetetraacetic acid (disodium salt).

1) E.W. Bingham and C.A. Zittle, *Biochem. Biophys. Res. Comm.*, **7**, 408 (1962).

2) F. Ibuki, A. Aoki and S. Matsushita, *This Journal*, **27**, 316 (1963).

3) F. Ibuki, A. Aoki and S. Matsushita, in contribution.

4) M.J. Bailie and R.K. Morton, *Biochem. J.*, **69**, 35 (1958).

the substrate (Fig. 1).

RNase activity was also observed in the

5) H.G. Boman and U. Kaletta, *Biochim. Biophys. Acta*, **24**, 619 (1957).

6) S. Matsushita, *Mem. Research Inst. Food Sci., Kyoto Univ.*, **17**, 23 (1959).

7) Y. Nakao and K. Ogata, *This Journal*, **27**, 291 (1963).

FIG. 1. pH-Activity Curve of the Microsomal PPDase Activity.

pH 5.5~6.0, acetate buffer; pH 7.0~9.0, Tris-HCl buffer.

microsomes though the total units in the microsomes were less compared with those in the whey (Table I). Since the PPDase activity in the microsomes was completely inhibited with the addition of EDTA, all of the activity obtained with adding EDTA was considered to be due to RNase activity when RNA was used as the substrate. Its pH optimum was thought to be 8.0 (Fig. 2). From the results, milk microsomes contained PPDase and RNase activities as similarly as in the microsomes of wheat roots cells^{2,3}. However, the pH optimum of the PPDase activity in the milk microsomes was found in alkaline region, while that of the PPDase activity in the microsomes from wheat roots

FIG. 2. pH-Activity Curve of the Microsomal RNase Activity.

pH 5.5~6.0, Acetate Buffer; pH 7.0~9.0, Tris-HCl Buffer.

cells was found in acidic region.

When RNA was digested with milk microsomes at pH 9.0, purine nucleotides formed were dephosphorylated by phosphatases and only pyrimidine nucleotides could be found by column chromatography.

The studies on the products by this enzymatic reactions are in progress.

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