HEAT STABILITY OF AMP PYROPHOSPHORYLASE IN DIFFERENTIATING INTESTINAL EPITHELIAL CELLS

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SUMMARY

AMP Pyrophosphorylase occurs as a more heat labile variant in tip cells of rat jejunum. Treatment of the animals with actinomycin D resulted in greater heat lability in the enzyme from the cells of the villus tips. Cycloheximide treatment resulted in greater heat stability. The results suggest that two enzyme forms exist - labile and stable and that the labile form is short-lived in vivo.

A variety of investigators have shown that by a number of techniques that changes occur in the relative amounts of various enzymes present in cells as they mature or differentiate (1,2,3,4). It has previously been shown that such changes take place in the epithelial cells of the intestine of the rat which provides a unique model system for the study of cell maturation. The question has been raised as to whether or not the increases in the amounts of various enzymes found in the fully mature cells of the villus tip are due to greater synthesis or decreased inactivation of the same enzymes found in the immature crypt cells or whether new or different enzymes are produced in the cells of the villi (5). One parameter which could be examined is heat stability of the enzymes. Variations in heat stability have been shown to be correlated with half-life of enzymes in peripheral erythrocytes (6) and it is not unreasonable to assume that similar differences could be relevant to greater half-life of enzymes in the intestinal epithelium. Such changes could result in alterations in the amount of apparent enzyme activity. Furthermore it might be possible to detect enzyme variants by evaluating

differences in heat stability and to learn something about the regulation of the production of these molecules in developing cells.

In order to pursue this line of reasoning we studied the heat stability of several enzymes in various fractions of cells of the rat jejunal mucosa. Though some enzymes showed no difference in the rate of thermal inactivation in cells of various stages, the enzyme AMP pyrophosphorylase (ARPTase) which has previously been shown to increase in the amount per cell as the cells matured (4) did show different heat stabilities in various positions on the villus. Surprisingly the enzyme which is several folds higher in the villus tip was more heat labile in tip cells. Thus, the greater amount of activity was probably not due to a mechanism that resulted in stabilization of the enzyme in the upper cells of the villi. The possibility that stabilizers or destabilizers were responsible was examined by mixing upper and lower fractions. The inactivation of the mixture was the arithmetic mean of the values for the individual fractions.

The possibility was further considered that the reason for the greater heat instability of the APRTase of these cells was that the partial maturation that occurred during the life of the cell - from mitosis in the crypt to the present position further along the villus - made it more susceptible to inactivation. An alternative explanation, of course, is that the heat labile protein is a different enzyme. In order to check the validity of these hypotheses, animals were given cycloheximide and actinomycin D, and sacrificed one, three, and six hours after the administration of the drugs. Actinomycin, which caused about a 20-40 per cent reduction in enzyme activity (Table I), had little effect of the heat stability of any fraction except that from the tip which was more heat labile. Cycloheximide on the other hand which inhibits protein synthesis and resulted in reduced levels of AMP pyrophosphorylase (Table 1) caused a pronounced decrease in the rate of

TABLE 1

INHIBITION OF AMP PYROPHOSPHORYLASE BY ACTINOMYCIN D AND CYCLOHEXIMIDE

Values are percent of moles of AMP synthesized per minute per mg DNA treated rats compared to saline injected control rats. Values are average of two experiments with treated rats and three for control values. Conditions were the same as those given in legend to figure 1. In each experiment 4 animals were used for each time point.

Section			
	1	57	42
Tip (24.1)	3		45
	6	61	50
Upper	1	71	60
Mid (14.5)	3		63
	6	68	59
Lower	1	82	72
Mid (13.1)	3		75
	6	80	70
Upper	1	78	74
Crypt (12.8)	3		80
	6	83	63
Lower	1	80	94
Crypt (8.1)	3		90
	6	82	93

Values in parentheses are mμmoles AMP synthesized/min/mg DNA in control experiments.

synthesis of heat labile pyrophosphorylase so that the extracts from cells of various positions of the villus were equally susceptible to heat inactivation one hour after administration of cycloheximide. The

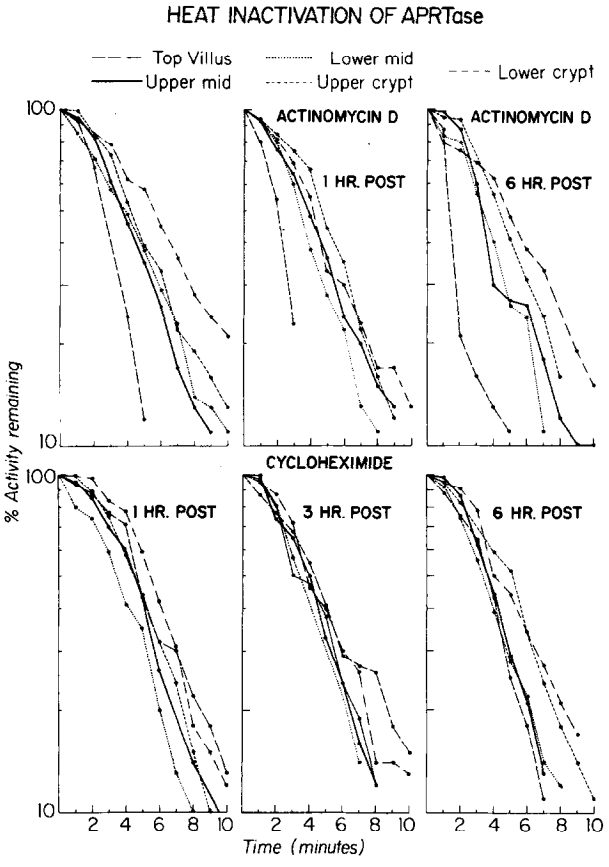

Fig. 1. Heat Inactivation of AMP: Pyrophosphorylase in Extracts of Intestinal Cells

Animals were killed by cervical dislocation, a portion of the jejunum removed and slit lengthwise and the mucosal epithelial cells separated with a planing apparatus (4). Successively deeper cuts were made at 125 μ intervals to obtain tip, upper med and lower mid villus and upper crypt. Lower crypt was obtained by scraping the remainder with a glass slide. Tissues were homogenized in ice-cold Tris-HCl buffer (pH 7.4). Enzyme assays were carried out as previously described (4). Activity was based upon radioactivity of adenine which was converted to a form not washed from DEAE cellulose paper with 10^{-3} M $HCOONH_4$. The values are those of a typical experiment. The mucosa from 4 animals was pooled. The control experiment was done three times. The values at each point were with 15% of each other. The treated experiment was done twice. The values agreed within 20%.

Cycloheximide (1.5 mg per kilo) and actinomycin D (1 mg per kilo) were injected intraperitoneally. The rats were 150-180 g males.

Volumes of extracts were adjusted to contain equal protein concentrations. Equal volume aliquots were heated at 55° for the indicated periods of time and then assayed. Results are expressed as fractions of initial values which were from 10 to 20 mμmoles/minute/mg protein and resulted in activities in excess of 1200 cpm/assay.

sensitivity to heat inactivation did not return to the normal pattern by six hours though there was evidence that reversal was beginning to occur at this time. The simplest explanation, in view of the known actions of cycloheximide and actinomycin D, is that there is a new heat labile form of the enzyme in the cells of the villi and this is regulated by a relatively stable m-RNA. Since the tip enzyme is a rapidly renewed protein it is more sensitive to cycloheximide inhibition than the other proteins. This explanation may be too facile and overlook alternative explanations based upon other known actions of cycloheximide and actinomycin. Nevertheless these data conform to the concept that development or maturation is effected or accompanied by the synthesis of specific proteins which are functionally the same as those formed in less differentiated cells but are unique in other properties. They further suggest that it is advantageous to the animal to produce less stable enzymes for cells of very short life expectancy.

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