UDC 577.155.2 : 591.434.1

56. Jun Okuda : Metabolism of Flavin Nucleotides. III. Distribution of Enzymes in the Cells of the Mucosa of Small Intestine causing Dephosphorylation of Flavin Mononucleotide.

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In the earlier papers of this series,[1,2] it was reported that flavin mononucleotide (FMN) is not decomposed in saliva and stomach juice, but slightly decomposed in bile and pancreatic juice, and strongly in the small intestine.

From the histochemical point of view, the author and others[3] studied the dephosphorylation of FMN in the small intestine and it was found that FMN-dephosphorylating enzyme was concentrated in the epithelial cells of the mucosa of the small intestine.

The distribution of FMN-dephosphorylating enzyme in the cells of the mucosa of the small intestine was studied and the present report deals with the distribution of FMN-dephosphorylating enzyme in the nuclei, mitochondria, and cytoplasm fractions of the cells of the mucosa of the duodenum, jejunum, and ileum.

Methods

Preparation of Homogenate of the Mucosa of the Duodenum, Jejunum, and Ileum—Dog's small intestine was collected from a slaughter house immediately after killing and the mesenteric membranes were removed. About 15 cm. of the duodenum, 18 cm. of the jejunum, and 20 cm. of the ileum were cut out separately, the canal was opened, and incubated in physiological saline solution for 1 hr. at 0° to remove the viscous substance as much as possible[4] (physiological saline solution was changed during this treatment). Each mucosa (4.2 g.) was obtained by scraping it with the edge of a plastic spatula. The mucosa was homogenized with 10 volumes of sucrose solution (0.25*M*).

Preparation of Subcellular Fraction—The procedure of Schneider and Hogeboom was used as basis for the fractionation.[5] Using the above homogenate, the nucleus fraction was separated by centrifugation (520*g*, 0°, 20 min.), resuspended in the same sucrose solution, and separated by the same centrifugation. The first upper layer was further treated by centrifugation (11,000*g*, 0°, 20 min.) to obtain the mitochondria fraction, and the supernatant was designated as the cytoplasm fraction.

Incubation—Incubation mixture (total, 1.0 cc.) contained FMN (final concentration, $1.0\times10^{-3}M$), $MgCl_2$ (final concentration, $1.0\times10^{-3}M$), and 0.1 cc. of the cell fraction preparation in monoethanolamine buffer (pH 9.5). Incubation was made at 37° for 10 min. in a dark room, in order to prevent photodecomposition of flavins.

Determination of Flavins—The separatory determination of flavin compounds was made by the measurement of flavins on paper strips as described in the preceding paper.[6]

Determination of Nitrogen—Nitrogen of subcellular fraction was estimated by indophenol method[7] after mineralization.

Experimental Results and Discussions

Distribution of FMN-dephosphorylating enzyme in the small intestine was first studied. In experiments with the homogenate, the jejunum was found to contain the highest activity for dephosphorylation of FMN, among three parts of the small intestine (duodenum, jejunum, and ileum), and the lowest activity was observed in the ileum as shown in Table I.

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2) J. Okuda : *Ibid.*, **6**, 665(1958).
3) K. Yamada, J. Okuda : Paper presented at the Chubu Local Section of the 16th Annual Meeting of the Japanese Anatomical Society, 1957.
4) I. Ishibashi : Unpublished data.
5) W. C. Schneider, G. H. Hogeboom : J. Biol. Chem., **176**, 259(1948).
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Table I. Distribution of FMN-Dephosphorylating Enzyme in the Cell Fraction of the Small Intestine

Organ	Fraction	Total vol. (cc.)	Total N (mg.)	Total dephosphorylated FMN (mol.)	Dephosphorylated FMN (mol./N mg.)
Duodenum 4.2 g. wet wt.	Homogenate	42	25.2	1.9×10^{-4}	7.5×10^{-6}
	Nucleus	10	9.5	2.7×10^{-5}	2.8×10^{-6}
	Mitochondria	2	1.0	8.0×10^{-6}	8.0×10^{-6}
	Cytoplasm	33	13.0	1.6×10^{-4}	1.2×10^{-5}
	Recovery (%)		93.0	98.0	
Jejunum 4.2 g. wet wt.	Homogenate	42	26.2	2.2×10^{-4}	8.5×10^{-6}
	Nucleus	10	9.8	2.5×10^{-5}	2.5×10^{-6}
	Mitochondria	2	1.0	8.4×10^{-6}	8.4×10^{-6}
	Cytoplasm	33	13.0	1.8×10^{-4}	1.4×10^{-5}
	Recovery (%)		92.0	96.0	
Ileum 4.2 g. wet wt.	Homogenate	42	20.2	1.3×10^{-4}	6.4×10^{-6}
	Nucleus	10	7.3	1.9×10^{-5}	2.6×10^{-6}
	Mitochondria	2	1.0	6.0×10^{-6}	6.0×10^{-6}
	Cytoplasm	33	10.9	1.2×10^{-4}	1.1×10^{-5}
	Recovery (%)		95.9	106.1	

Then the distribution of this enzyme was examined in each cell fraction of three parts of the small intestine. When the total amount of the enzyme activity was tested in each subcellular fraction, about 80% of it was contained in the cytoplasm fraction.

The ratio of dephosphorylation of FMN(per mg. N) was calculated and the highest activity was found in the fraction of cytoplasm as shown in the same table. The activity of the mitochondria fraction was higher than that of the nucleus fraction. It is clear, therefore, that FMN-dephosphorylating enzyme is present in each subcellular fraction and most intensively in the supernatant of the homogenate, i.e., cytoplasm fraction.

From these results and from histochemical results,[3] it will be supposed that FMN-dephosphorylating enzyme is present mostly in the cytoplasm portion of the epithelial cells in the small intestine.

The author expresses his deep gratitude to Prof. Dr. T. Takahashi of the University of Kyoto and Dr. K. Yagi of the Nagoya University for their guidance and encouragement throughout this work.

Summary

Distribution of FMN-dephosphorylating enzyme in the cells of the small intestine was studied.

1) In the case of a homogenate, the highest activity was found in the jejunum. The activity of the ileum was lower than that of the duodenum.

2) In the case of subcellular fraction, about 80% of the activity was found in the cytoplasm fraction. In each case, the highest activity was found in the cytoplasm fraction and that in the mitochondria fraction was higher than that of the nucleus fraction.

(Received September 30, 1958)