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Adenosine uptake in pyrimidine 5′-nucleotidase deficient human erythrocytes via a high affinity transport system

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Summary. Using a pulse-labeling technique, ^{14}C-adenosine uptake into pyrimidine 5′-nucleotidase (P5N) deficient erythrocytes (RBC) was found to be impaired. The Lineweaver-Burk plot showed K_m values of 2.0×10^{-3} mM and 0.2×10^{-3} mM for normal RBC and P5N deficient RBC, respectively. These results indicate that P5N is one of regulators of the adenosine transport system and/or is associated with adenosine carrier protein.
Key words. Erythrocytes; pyrimidine-5′-nucleotidase; ^{14}C-adenosine uptake.

Valentine et al.[1] have described a chronic hereditary hemolytic anemia with a deficiency of pyrimidine 5′-nucleotidase (P5N) and with an accumulation of glutathione and total nucleotides. However, the mechanism(s) of the accumulation of total nucleotides is still unclear, even though some characteristics of variant P5N in human RBC were demonstrated[2-7]. The uptake and transport of adenosine have been studied in various cell types[8-19], revealing some of the uptake mechanisms. Even though adenosine can be phosphorylated by adenosine kinase and can be deaminated by adenosine deaminase, its transport was not inhibited by the addition of Erythro-9-(2-hydroxy-3-nonyl) adenine, a potent adenosine deaminase inhibitor, to neuroblastoma cells deficient in adenosine kinase[10]. In cultured P388 murine leukemia cells, however, adenosine deaminase and nucleoside phosphorylase were found to have no role in adenosine transport; the adenosine deaminase inhibitor 2-deoxycoformycin (2dCF), which markedly inhibits adenosine phosphorylation and its deamination, had no effect on adenosine transport[14]. On the other hand, compared to normal fibroblasts, cultured nucleoside phosphorylase deficient cells could incorporate ony 2% and 4% of ^{14}C-inosine and ^{3}H-guanosine, respecitively[20].

All of these various mechanisms of nucleoside transport suggest important differences between the variant enzyme and the normal enzyme with or without the presence of inhibitor in the adenosine transport system. On the other hand, adenosine deaminase is associated with the nucleoside transport system in the human RBC membrane[21]. Kraupp et al.[12] have suggested the existence of an 'adenosine carrier protein' in the erythrocyte membrane, associated with P5N. If such a protein is present, it might be very valuable to investigate the molecular basis of adenosine uptake by this 'carrier protein' in RBC where variant P5N is present, in order to clarify the relationship between the 'carrier protein' and the enzyme.

Materials and methods. P5N deficient RBC. Leukocytes and platelet-rich plasma were removed by centrifugation from heparinized blood drawn from patients of two families[22-24] with P5N deficient hemolytic anemia. RBC were washed three times with saline and suspended in 10 mM-Tris HCl-0.15 M-NaCl (pH 7.4) at a cell concentration of 10^7/ml. All experiments were performed within 2–4 h after drawing the blood and within a period of 1–3 h after washing with saline. Reticulocytes comprised 0.2% of the normal RBC and 7.2% of the P5N deficient RBC. The RBC suspensions from a patient with hereditary spherocytosis whose reticulocytes were from 0.9 to 5.0% were also used as a control.

^{14}C-adenosine uptake to RBC. In ^{14}C-adenosine uptake studies[18], the RBC suspension was warmed to 37 °C for 5 min, and ^{14}C-adenosine (7.5 n mole) was then added to the suspension and rapidly mixed. After incubation at 37 °C for various time intervals, 0.17 ml of RBC suspension was layered into an Eppendorf microfuge tube containing 0.04 ml of 10% perchloric acid as the bottom layer, and 0.17 ml of layer of mixed oil (corn oil:n-butyl phthalate, 3:10). The tube was centrifuged at 15,000 rpm for 30 sec using an Eppendorf microfuge. Cellular material (15 ml) which passed through the oil layer and into the acid layer was removed and the radioactivity was determined in a scintillation counter.

(8-^{14}C) Adenosine was purchased from Amersham, all nucleotides were from Sigma Chemical Co., St. Louis, Mo., and all other chemicals were from Wako Pure Chemical Co., Japan.

Results. The time course of adenosine uptake by RBC is shown in figure 1. The maximal peak of the uptake was from 7 to 9 min in normal human erythrocytes. On the other hand, adenosine uptake was reduced in P5N deficient RBC with a shift of the maximal peak to the left and an asymmetrical curve. The total adenosine uptake of P5N deficient RBC was 31.7% that of normal RBC. These results indicate that the adenosine carrier system(s) is affected by P5N and the enzyme may be one of the regulator(s) of the adenosine transport system. Figure 2 represents the adenosine uptake of normal and P5N deficient RBC; both cell groups showed hyperbolic curves. The

apparent K_m for normal human RBC was 5×10^{-3} mM of adenosine per 3 min per 10^7 cells and that for P5N deficient RBC was 0.5×10^{-3} mM.

The Lineweaver-Burk plot of adenosine uptake is shown in figure 3 where K_m for normal RBC is 2.0×10^{-3} mM and that for P5N deficient RBC is 0.2×10^{-3} mM. The differences of V_{max} in figure 2 are not statistically significant.

All of these experiments were also performed using RBC from a patient with hereditary spherocytosis with the same percentage of reticulocytes as that of P5N deficient RBC; as well as with those from people heterozygous for the P5N deficiency whose reticulocytes were 0.8%. The results from the studies using hereditary spherocytosis and the heterozygous individuals were the same as those for normal RBC. For simplicity, all of these are represented by the same symbol (open circle) in all of the figures.

These results indicated that the difference in adenosine uptake was not due to the number of reticulocytes in the prepared RBC suspension but to the relation between the variant P5N and 'adenosine carrier protein'. Furthermore, 'adenosine carrier protein' in P5N deficiency has a high affinity for adenosine (fig. 3).

Discussion. The curves in figure 1 are essentially similar to those reported in other mammalian cells over the period studied[12, 14, 16]. The time course of labeled adenosine uptake conformed well to a simple, completely symmetrical, transport model with a carrier in normal human erythrocytes. The results in figure 2 are comparable with those of guinea pig RBC[12], where adenosine uptake follows Michaelis-Menten kinetics. However, the uptake curve of P5N deficient RBC is steeper than that of normal RBC, which seems slightly sigmoidal.

It is very interesting to compare these observations with the oxygen saturation curve of the tetramer and monomer of hemoglobin or myoglobin. As shown in general work on the genetic mutation of hemoglobins and enzymes, if the enzyme protein of P5N in P5N-deficient RBC has an abnormality in the primary structure due to a change in structure gene, such an abnormality may induce a conformational change in the enzyme protein and hence may affect the binding site of P5N to the 'adenosine carrier protein'.

The results presented here demonstrate that P5N in RBC is associated with or closely related to 'adenosine carrier protein' as studied by Kraupp et al.[12], and that this 'variant P5N as studied by Fujii[2] and Torrance[6] cannot work with the 'adenosine carrier protein' owing to a conformational abnormality. The adenosine uptake into human RBC consisted of two components, facilitated diffusion and simple diffusion[16]. Nucleoside enters the cell by facilitated diffusion at the physiological concentration of adenosine and the major fraction is directly phosphorylated to adenosine monophosphate (AMP)[16]. According to our present knowledge, adenosine deaminase activity and adenosine kinase activity are within the normal range in P5N deficient RBC, and AMP is not a substrate of P5N. Thus, the metabolism of adenosine and AMP in P5N-deficient RBC should be indentical to that in normal RBC.

If 'uptake' can be defined as being the transfer of radioactivity in space or of components, regardless of metabolic conversion[14], it can be said that the adenosine 'uptake' had a low capacity (fig. 1) or was impaired in P5N deficient RBC; even though the affinity for adenosine was higher than in normal RBC (fig. 3). These dissociated results were also revealed in studies of glutathione transport using inside-out vesicules from human erythrocytes[25], where a low affinity for glutathione and a high transport velocity were shown. Furthermore, Kondo et al.[26] demonstrated that oxidized glutathione (GSSG) transport was inhibited by CTP or UTP which was elevated in P5N defi-

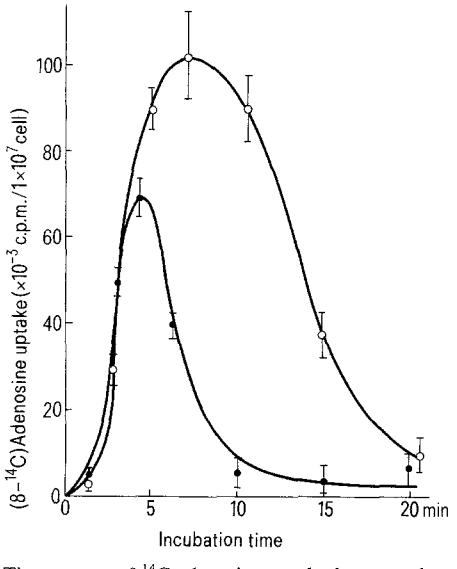

Figure 1. Time course of ^{14}C-adenosine uptake by normal and hereditary spherocytosis (HS, open circle) and pyrimidine 5'-nucleotidase deficient (closed circle) erythrocytes. Results are from five independent experiments.

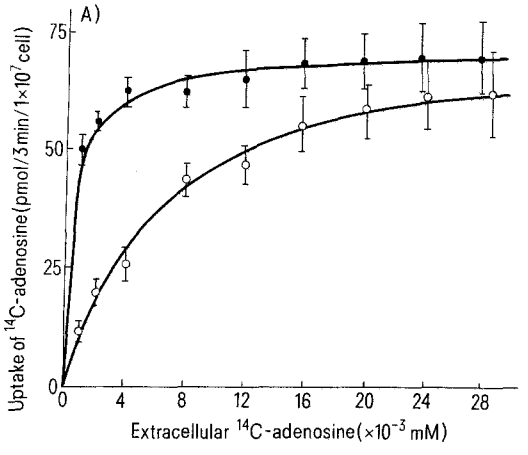

Figure 2. Uptake of adenosine by normal erythrocytes and those of hereditary spherocytosis (HS, open circle), at various concentrations of adenosine in suspension medium at 37 °C for 3 min. Each point is the mean of five experiments. Vertical brackets represent standard deviation.

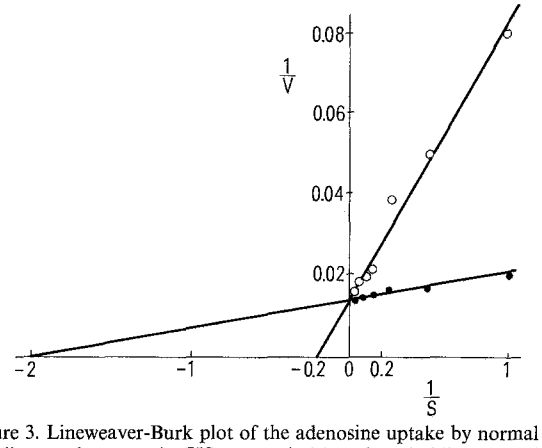

Figure 3. Lineweaver-Burk plot of the adenosine uptake by normal and herediatry spherocytosis (HS, open circle) and pyrimidine 5'-nucleotidase deficient (closed circle) erythrocytes. The reciprocal of the uptake velocity (pmol/min/1 × 10^7 cell) was plotted against the reciprocal concentration of ^{14}C-adenosine from data in figure 2A.

ciency. The impairment of adenosine uptake in P5N deficient erythrocytes is of interest, even though the results cannot lead to an understanding of the main reason for hemolysis of the erythrocyte.

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Morphology of the intercapsular segment of the oviduct in the golden hamster with special reference to ovum-transit from ruptured follicles to the ampulla

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Summary. The intercapsular segment of the oviduct in the golden hamster is not a simple duct which has constant outer and inner diameters. After penetrating the bursa ovarica the ICS has a circular constriction and the corresponding oviductal lumen is narrowed.
Key words. Hamster, golden; oviduct, hamster; intercapsular segment, morphology; oviductal lumen.

The oviduct is not a simple duct with a uniform luminal width and homogeneous functional features through the whole length, but is complex. For example, in the isthmic portion the lumen is narrow, the luminal surface is lined with relatively sparsely-ciliated epithelium and the oviductal wall is thick[1,2], while in the ampulla the lumen is ample, the surface epithelium has abundant with cilia and the muscle layer is not so highly developed[3,4]. Therefore, investigators think that ova are transported by ciliary action in the ampulla and are propelled by oviductal contraction in the isthmus. One cannot always deal with the morphology and functions of the oviduct as a whole. In the present study, we demonstrate that the intercapsular segment (ICS)[5] of the oviduct in the golden hamster has a constricted region at its root, and discuss its functional significance in the transport of ova.

Materials and methods. Animals. Female golden hamsters with b.wt varying from 90 to 120 g were used. They were kept in an air-conditioned room with automatic illumination switched on at 05.00 h and off at 19.00 h. Under these lighting conditions adult females showed a viscous vaginal discharge in the morning of day 1 of the estrus cycle and ovulated between 01.00 and 04.00 h of day 1[6-8].

Endocast of the ICS. A 21-gauge needle attached to a tuberculin syringe containing 50 µl of metacrylate resin was inserted into the ampulla through an incision in the ampullary wall and 20 to 40 µl of the resin was injected toward an ovarian end of the oviduct. Then the oviduct with the bursa ovarica and ovary was isolated and put into a 1% NaOH solution. The tissue was completely eroded after incubation at 37 °C for 24 h. The endocast was rinsed in a distilled water, air-dried, gold-coated and observed under a scanning electron microscope. Endocasts on day 1, 2, 3, 4 and during ovulation were made.

Injection of a dye into the bursal cavity. Under ether anesthesia 20 µl of 1% alcian blue 8GX (AB) dissolved in 0.9% NaCl was injected into the bursal cavity through the fat pad surrounding the bursa. 30 min later the ICS with part of the ampulla was removed and histological sections were made. Hamsters in the non-ovulatory period and in the ovulatory period were used.

Specimens for photomicroscopy (PM), scanning and transmis-