Restoration of the poor oxygen transport function of ACD-stored blood by pyridoxal 5′-phosphate

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Summary. Pyridoxal 5′-phosphate is easily incorporated into ACD-stored erythrocytes without decrease of ATP, and restores the poor oxygen transport function with a similar effect to 2,3-diphosphoglycerate.

ACD(acid-citrate-dextrose)-stored blood is still commonly used for blood transfusion. However, the oxygen transport function decreases gradually during storage at 4 °C[1], due to the decrease of 2,3-diphosphoglycerate (2,3-DPG) in the erythrocytes[2,3]. There have been many attempts to improve or restore the oxygen transport function (Valeri[4]), usually by attempting to stimulate 2,3-DPG generation in erythrocytes, because the erythrocyte membrane is not permeable to 2,3-DPG. In this communication, the restoration of the oxygen transport function of ACD-stored blood by pyridoxal 5′-phosphate (PLP), a recently discovered allosteric effector[5–7], is described.

1 ml of ACD(NIH-A)-blood stored at 4 °C for various periods was centrifuged at 3000 rpm for 5 min, and 0.5 ml of plasma was replaced by 0.5 ml of isotonic phosphate-buffered PLP solution (50 mM Na-phosphate, 3 mM KCl, 7 mM Na-lactate and 150 mM glucose with various concentrations of PLP, of which the isotonicity was adjusted by NaCl; pH 7.0). After incubation at 37 °C for 4 h with gentle mixing, 0.5 ml of the mixture was washed with cold isotonic buffer 3 times at 4 °C (during washing, incorporated PLP was not released in to the medium). Incorporated PLP and adenylates were extracted by trichloroacetic acid, as described by Bartlett[8], and analyzed by high pressure liquid chromatography (Hitachi, type 634 A) with an anion exchanger (Hitachi, No. 2632), and simultaneously monitored at 295 nm and 254 nm, respectively. The rest of the incubation mixture was used for measuring the oxygen dissociation curve in isotonic Na-K-phosphate-buffered saline (pH 7.4) by the method of Imai et al.[9]. 2,3-DPG content was determined by the enzymatic method[10].

The changes in 2,3-DPG and ATP concentrations in erythrocytes during storage of ACD-blood showed very similar patterns (figure 1, a) to those reported[3,11–14]. The intracellular concentrations of PLP incorporated into stored erythrocytes after incubation with 2 different concentrations of PLP were shown in figure 1, b. Although PLP was easily incorporated into ACD-stored erythrocytes, as observed for various erythrocytes[15–19], the degree was lower in the 2,3-DPG-rich erythrocytes than in the 2,3-DPG-poor ones, and was constant for erythrocytes stored for 10 to 30 days. It seemed that 2,3-DPG content and the amount of incorporated PLP were in an inverse relation, as if both allosteric effectors competed. However, the degree of PLP incorpora-

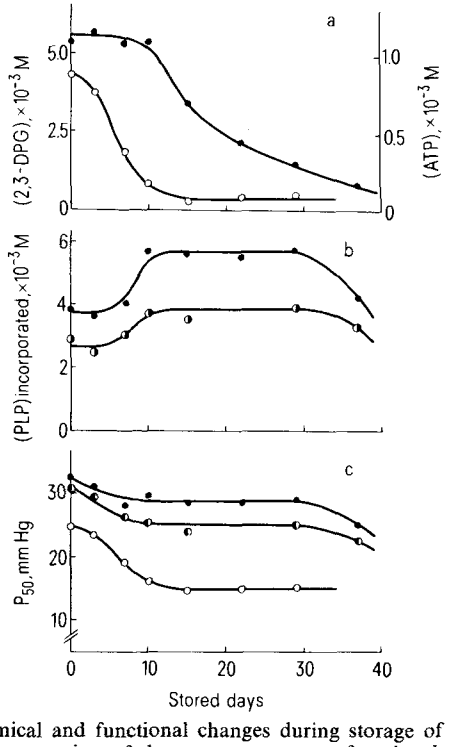

Fig. 1. Chemical and functional changes during storage of ACD-blood, and restoration of the oxygen transport function by PLP. *a* Changes of intracellular 2,3-DPG and ATP during storage. ○, 2,3-DPG; ●, ATP. *b* Incorporation of PLP into erythrocytes stored in ACD for various days. Performed in the extracellular PLP concentrations of 23.0 mM (●) and 11.5 mM (◐), as described in the text. *c* Oxygen affinity of stored and PLP-treated erythrocytes. Measured at 37 °C in the absence of CO_2. ○, stored erythrocytes; ● and ◐, erythrocytes treated as in (b). P_{50}, oxygen tension at half oxygenation.

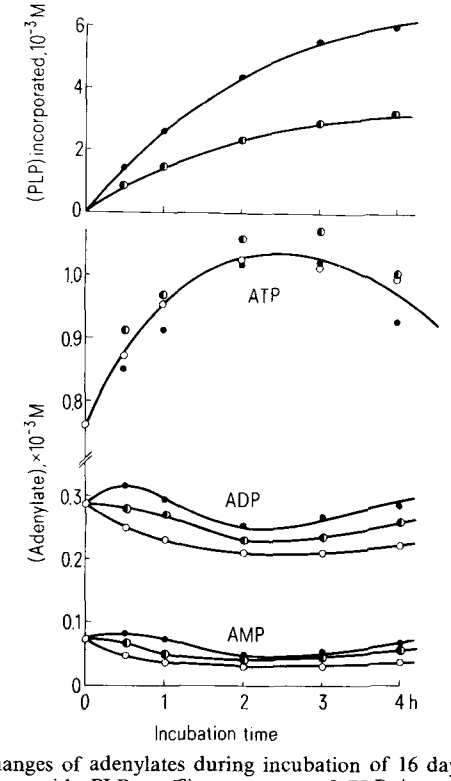

Fig. 2. Changes of adenylates during incubation of 16 days-stored erythrocytes with PLP. *a* Time courses of PLP incorporation. Incubated at 37 °C in the extracellular PLP concentrations of 5.59 mM (◐) and 21.0 mM (●). Hematocrit of the reaction mixture was 29%. *b* Adenylates changes during incubation. ○, incubated without PLP; ◐ and ●, incubated with PLP as in (a).

tion for erythrocytes stored more than 30 days decreased. During incubation, 2,3-DPG content did not change significantly, and the content of ATP, an important substance for 24 h-posttransfusion survival of erythrocytes[20,21], also did not change or rather increased slightly in these experimental series. Furthermore, the degree of hemolysis during incubation for 4 h was less than 3%.

The time courses of PLP incorporation and the changes of adenylates in erythrocytes, which were performed for the 16 days-stored erythrocytes, are shown in figure 2. Although the ATP-content increased in the initial stage of the incubation, there were no systematic differences between the control experiment and the PLP experiments, and between the amounts of PLP incorporated, within the experimental error. On the other hand, it seemed that more ADP and AMP, systematically, accumulated in erythrocytes as they were incubated with more PLP. Therefore, systematic changes in ATP content (perhaps incubation with more PLP might result in a greater decrease of ATP) may be concealed in the experimental error, and ATP may partly participate in the incorporation of PLP. In this sense, the decreased incorporation of PLP by erythrocytes stored for more than 30 days in figure 1, b may be explained by the marked decrease of ATP in the erythrocytes. Furthermore, the changes in the membrane properties of erythrocytes during storage may partly affect the degree of PLP incorporation.

The oxygen affinity of erythrocytes treated by PLP (represented by P_{50}) after various periods of storage, is shown in figure 1, c. It is clear that the oxygen transport function of the ACD-stored blood was perfectly improved by PLP, as reflected by the oxygen affinity. Furthermore, it was proved for ACD-stored erythrocytes that the change of the oxygen affinity due to PLP was almost same as that with 2,3-DPG, as already observed for hemoglobin solution[6,7]. However, the Hill's coefficient (n) of erythrocytes containing 5 mM PLP was 2.2, while that of fresh ACD-erythrocytes containing 5 mM 2,3-DPG was 2.6. The decreasing degree of the heme-heme interaction was dependent on the amounts of PLP incorporated, as previously observed[19].

In conclusion, PLP is easily incorporated into ACD-stored erythrocytes without any decrease in ATP content, restores the poor oxygen transport function with a similar effect to 2,3-DPG, and may be applicable for blood transfusion.

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Spherule cells in *Drosophila* species

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Summary. Spherule cells are restricted to the larval stage and make up 5–16% of cells in the hemolymph. Their morphology varies between species, mainly due to the shape of their inclusions which may be oval ('spheroidocytes'), polyhedral ('crystalloid cells'), or clearly crystalline ('crystal cells'). These inclusions are very rich in tyrosine. They liquefy rapidly in vitro, whereby the cells become hyaline (coagulocytes).

Known since 1864 under the name of 'Körnchenkugeln'[2], and subsequently designated as 'granules mûriformes'[3], or 'sphérules de granules'[4–6], spherule cells seem to be of common occurrence in insects[7–10]. There is however some controversy concerning the Diptera: In *Calliphora erythrocephala*, spherule cells are absent according to certain authors[11,12] but have been recognized by others [10,13]. Dennell[14] did not find them in *Sarcophaga falculata*, whereas in *S. bullata* they are reported to be present[15–17]. Concerning the various species of *Drosophila* which have been investigated, most of the authors [17–27] have overlooked this type of cell, while Rizki[28] reported it from *D. willistoni* under the designation 'spheroidocyte'. Spherule cells have more recently been identified in *D. hydei*[29]. We examined several species of *Drosophila* and one species of *Zaprionus* in order to see whether spherule cells are generally present, and whether there are species-specific differences in their morphology.

Material and methods. Fresh hemolymph was collected on refrigerated slides and observed under phase contrast. In *D. hydei*, all phases of larval development from hatching to pupation were examined, in other species selected stages only. Following immediate pre-fixation in 45% acetic acid which is essential for preserving the cells, smears were fixed in either 100% ethanol followed by orcein staining for direct observation, or Carnoy for May-Grünwald Giemsa staining, or 10% neutral formaldehyde for the detection of proteins with a high tyrosine content according to the technique of Glenner and Lillie, modified by Morel and Sisley as quoted by Thompson[30].