Transformations of Glycolysis Control Characteristics in Human Erythrocytes during Blood Storage

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With 4 Figures

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The increase of the storage time of preserved donors blood represents an important practical problem. The limitation of the blood storage time is conditioned by the decline of erythrocyte viability during storage. This viability of erythrocytes will largely depend on the state of the glycolytic system providing the cell with ATP and reducing equivalents. The performance of glycolysis as an energy supply system is determined by the glycolysis control characteristics, i.e. by the dependence of the glycolytic flux on the ATP concentration [4–6, 12, 15, 16]. In native erythrocytes the relationship is represented by the bell-shaped curve with a steeply descending part at physiological ATP concentrations. The glycolysis control characteristics in erythrocytes of different donors will coincide being plotted in normalized form [5, 6, 12]. The invariance of normal glycolysis control characteristics enables data obtained by examining different donors to be compared.

This paper deals with investigating changes in glycolysis control characteristics of erythrocytes during the storage of preserved donors blood.

Materials and Methods

Erythrocytes from 6 normal healthy blood donors were studied. Donors blood was collected in 250 ml glass flasks containing 50 ml of standard glucose-citrate preservative solution. One liter of this solution contained 30 g of anhydrous glucose and 20 g of disodium citrate monohydrate. After collecting the blood from each donor the flask was aseptically distributed into four equal portions, and then the blood was stored at 4 °C. Before measurements the contents of the storage flasks were shaken. [ATP] was measured in the whole blood immediately after opening the flask. Then, the erythrocytes were separated by centrifugation for 10 min at 1,000 g. Plasma and upper layer of packed cells were
removed. Packed erythrocytes were resuspended in 2–3 volumes of isotonic buffer solution, suspension was centrifuged for 10 min at 1,000 g and the supernatant was removed. This procedure was repeated three times for removing the glucose-containing conservant completely. The isotonic buffer solution containing 125 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 1.2 mM Na₂HPO₄, 50 mM Tris-HCl (pH 7.5 at 37 °C) was used for washing and incubating erythrocytes. The washed erythrocytes were resuspended in equal volume of isotonic buffer solution and glucose was added to the final concentration of 4–6 mmol per l of suspension. The suspension of erythrocytes was distributed into several fluoroplast cells and incubated at 37 °C during 6 hours by stirring it constantly. The erythrocyte suspension in one of these cells was used for control, different quantities of sodium arsenate were added into the other cells at the beginning of incubation to decrease the erythrocyte ATP concentration [5, 6]. After 3 hours of incubation the steady state values of glycolysis rate, ATP and glucose-6-phosphate (G6P) concentrations were established. These values were used for plotting glycolysis control characteristics according to [5, 6]. The glycolysis rate was determined by glucose decline. Those values of rates and concentrations obtained in the control suspension will be termed as physiological ones. For determining glucose and G6P, 0.2 ml of samples of erythrocyte suspension were fixed with 0.5 ml of 0.5 M perchloric acid. The protein precipitate was removed by centrifugation and the supernatant was neutralized with a saturated solution of K₂CO₃ to pH 6.0–8.0. Glucose concentration in neutralized supernatant was measured by the hexokinase method [8]. The concentration of G6P was measured by the glucose-6-phosphatedehydrogenase method [9] with fluorometric determination of NADPH. The concentration of ATP was measured by the modified luciferase-luciferin method [2].

**Results**

Great individual variations could be observed in all measured parameters and in its kinetics during blood storage. However, the qualitative picture of erythrocyte metabolism during blood storage was found to be the same for all donors studied.

The level of [ATP] decreased monotonously during blood storage (Fig. 1). After washing off and incubating erythrocytes for several minutes under physiological

![Fig. 1](image-url) Change in ATP concentration in erythrocytes of two donors (A and B) during blood storage

○ — ATP concentration in erythrocytes of preserved blood;
● — ATP concentration in erythrocytes after washing off and incubation under physiological conditions.

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Fig. 2. Change in the physiological rate of glucose consumption in erythrocytes during blood storage.

Fig. 3. Changes in the dependence of the glucose consumption rate on ATP concentration (glycolysis control characteristics) in erythrocytes of two donors (A and B) during storage. The solid line indicates glycolysis control characteristics in native erythrocytes. Physiological points of glycolysis are marked by black symbols. Storage time is indicated under the curves.
conditions the ATP concentration increased to some extent, and then remaining constant for 6–7 hours of incubation. The value of this [ATP] increase depended on the storage time.

The physiological rate of glucose consumption in erythrocytes increased during the first weeks of storage and in some cases it exceeded more than twice that value obtained on the first day of storage. Then, the physiological rate of glucose consumption began to decrease (Fig. 2).

Figure 3 demonstrates the process of glycolysis control characteristics developing in the erythrocytes of two donors. Two stages of evolution can be distinguished. At the first stage the glycolysis control characteristics maintain their initial shape, change will only occur at the physiological point on the curve. Changes of the physiological rate of glycolysis and ATP concentration cause the physiological point of glycolysis to move towards the maximum of the curve. At the second stage the characteristics themselves begin to change. The whole curve is lower in comparison with the initial one. The maximum on the curve is less evident and in some cases it may disappear altogether.

The physiological concentration of G6P in erythrocytes decreased during storage (Fig. 4). The dependence of G6P concentration on ATP concentration remains qualitatively the same at all periods of storage.

Discussion

The results obtained demonstrate the existence of significant changes in regulatory properties of the erythrocytes energy metabolism during storage.

The decrease of [ATP] during blood storage at positive temperature is a well known fact [1, 7, 11]. Erythrocyte ATP level could be shown to be restored after transfusion in vivo [20]. However, partial restoration of [ATP] under quasi-physiolog-
gical conditions in vitro without adding purines, seems to be not mentioned previously. The decline of [ATP] observed during the earlier period of storage can be explained by the greater depression of glycolysis in comparison with the depression of ATPases at low temperature. Incubation of stored erythrocytes under physiological conditions leads to a restoration of balanced metabolism and normalization of the ATP concentration. The shift of the physiological point towards the maximum on glycolysis control characteristics observed during the earlier period of storage can be explained by the activation of the erythrocyte ATPases [6, 10, 12, 17]. Such activation is evidently caused by an increase of erythrocyte membrane permeability and disturbance of intracellular ions composition during storage [19, 21]. The observed shift of the physiological point cannot be explained by the activation of glycolysis produced by the decrease of erythrocyte 2,3-diphosphoglycerate level during storage, because this activation would lead to the upward shift of all glycolysis characteristics.

The decrease of the [ATP] in erythrocytes during storage is accompanied by a rise of [AMP] [18]. Storage of erythrocytes with high AMP concentration will cause the adenylate pool to decline. This decrease of the adenylate pool prevents the complete restoration of the normal ATP level during incubation under physiological conditions. The decrease of ATP concentration in human erythrocytes leads to a sharp fall of the maximal rate of pentosephosphate pathway [3], disturbing the antioxidative protection of erythrocytes. During prolonged storage the key glycolytic enzymes may be distorted in their functions i.e. hexokinase and phosphofructokinase. Indeed, the activity of erythrocyte phosphofructokinase can be observed to decrease considerably during blood storage [14]. As can be seen from the data presented in Figure 3 B and Figure 4 glycolytic rates correspond to the same concentrations of ATP and G6P decrease in the period between 2 and 4 weeks of storage. This indicates a change in hexokinase kinetic parameters which may be due either to an increase of the hexokinase Michaelis constant for ATP or to an decrease of the hexokinase inhibition constant for G6P because virtually no alteration of the erythrocyte hexokinase can be observed during blood storage [13, 14].

The decrease of the adenylate pool and the impairment of key glycolytic enzymes, which take place at the latest periods of storage, lead to a significant modification of erythrocyte glycolysis regulation manifested by a shift down of its characteristics and disappearance of its maximum. This seems to be one of the main causes for erythrocyte viability to be decreased during storage because those erythrocytes with flat characteristics are unable to maintain the stable ATP level, thus being unable to maintain normal functions of important ATPases.

It should be noted that a separation of donors with erythrocytes keeping normal regulation of metabolism for a relatively long period of storage would permit the time of blood storage to be extended without modifying hemoconservants and storage conditions.

Summary

Changes in glycolysis control characteristics (dependence of glycolysis rate on ATP concentration) in erythrocytes were studied during the storage of donors blood with glucose citrate hemo-
conservant. During the first two weeks of storage the shape of glycolysis control characteristics in the erythrocytes could be shown to remain practically unchanged, which was represented by a bell-shaped curve such as in fresh erythrocytes. During this period the physiological point of glycolysis will move along the glycolysis control characteristics towards the maximum of the curve. Once the maximum of the physiological point has been reached, the shape of the curve can be seen to change. The maximum on the curve becomes less evident, moving down and to the left from its initial position. These changes will occur after two to four weeks of storage. In some cases the maximum on glycolysis control characteristics will disappear at the latest stages of storage. The changes observed will occur in blood of different donors at different moments of storage. The nature of the changes observed and their influence on erythrocyte viability are discussed.

Zusammenfassung


Résumé

On a étudié les modifications des valeurs de contrôle caractéristiques pour la glycolyse (dépendance du taux de glycolyse de la concentration d’ATP) dans les érythrocytes pendant le stockage du sang des donneurs dans un conservant citrique de glucose. Il s’est montré que pendant les premières deux semaines du stockage la courbe de la glycolyse dans les érythrocytes est restée pratiquement inchangée et s’est présentée sous forme d’une courbe en cloche comme dans les érythrocytes frais. Dans cette période la valeur physiologique de la glycolyse s’est dirigée le long des valeurs caractéristiques de contrôle vers le maximum de la courbe et dès que la valeur physiologique a atteint le maximum la forme de la courbe a changé. Le maximum était moins marqué, il était plus bas et plus vers la gauche de sa position d’origine. Ces modifications ont apparu après deux à quatre semaines de stockage. Dans quelques cas le maximum des valeurs de contrôle de la glycolyse a disparu dans le stade final du stockage. Les modifications observées ont apparu dans les sangs de donneurs différents à des périodes différentes de stockage. On discute sur la nature des modifications observées et sur l’influence qu’elles ont sur la viabilité des érythrocytes.

Резюме

Исследованы изменения регуляторной характеристики гликоглизы (зависимость скорости гликоглизы от концентрации АТФ) в эритроцитах в процессе хранения донорской крови в глюкозо-цитратном консервирующем растворе 76. Показано, что в течение первых двух недель хранения форма регуляторной характеристики гликоглизы эритроцитов практически не изменяется и имеет, как и у свежезакупленных эритроцитов, вид колоколообразной кривой. В это время физиологическая точка гликоглизы перемещается вдоль регуляторной характеристики гликоглизы по направлению к максимуму на кривой. Когда физиологическая точка приближается к максимуму начинает меняться форма характеристики гликоглизы. Максимум на кривой становится менее
выражен и сдвигается вниз и влево от исходного положения. В некоторых случаях на поздних стадиях хранения максимум на регуляторной характеристике гликогеназа перемещается и он становится фитофобной. Эти изменения происходят после двух-четырех недель хранения. Наблюдаемые изменения нарушают в крове разных доноров в разные сроки хранения. Обсуждаются причины наблюдаемых изменений и их влияние на жизнеспособность эритроцитов.

References


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