HEXOKINASE ISOZYMIC PATTERN DURING RED CELL AGING

Mauro MAGNANI, Vilberto STOCCHI, Marina DACHÀ, Franco CANESTRARI and Giorgio FORNAINI
Istituto di Chimica Biologica, Università degli Studi di Urbino, Via A. Saffi, 2, 61029 Urbino PS, Italy

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1. Introduction

It has been proposed that hexokinase (EC 2.7.1.1) plays an important role as a metabolic cause for red cell senescence because of its low activity and its marked decrease during cell aging [1,2]. This age-related hexokinase decline is a complex phenomenon, the enzymic activity appearing to have a biphasic decay pattern [3–5], followed by changes in the kinetic [5,6] and electrophoretic [4,6–10] properties of the enzyme. Recently, two distinct molecular forms of hexokinase (Ia,Ib) have been purified and characterized from rabbit reticulocytes, but only one of these was consistently present in mature rabbit red cells [11,12]. This predominant form corresponds to hexokinase type I present in the brain tissue, while the minor component (hexokinase Ib) does not correspond to any hexokinase previously described. We report here the age-related decay pattern of these two hexokinase isozymes and propose an explanation of the age-dependent kinetic and catalytic modifications previously observed during red cell aging.

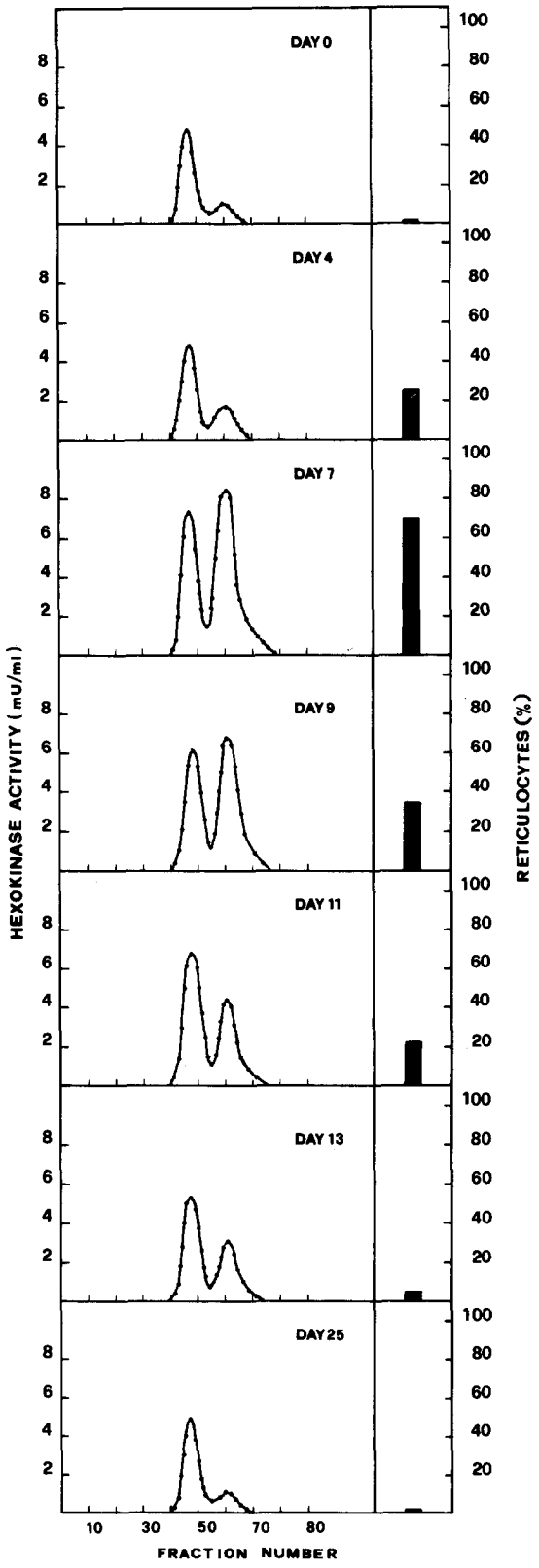
2. Materials and methods

Reticulocytosis was induced in rabbit by phenylhydrazine administration as in [5]. The reticulocytes and the red cells from untreated rabbit were washed twice in 0.16 M KCl solution and haemolysed in equal volumes of 0.4% (w/w) saponin solution. The cell stroma was removed by centrifugation at 13 000 × *g* for 30 min. Rabbit red cells were also separated into 5 fractions of increasing mean age by ultracentrifugation through gradients of Ficoll-Triosil [5]. Hexokinase isozymes separation was achieved by chromatography of 1 ml haemolyzates applied to DE-52 cellulose columns (0.7 × 20 cm) equilibrated in 5 mM sodium potassium phosphate buffer (pH 7.5) containing 3 mM KF, 3 mM 2-mercaptoethanol, 5 mM dithiothreitol and 3 mM glucose, and operated at 15–20 ml/h. The columns were developed with 280 ml linear gradients of KCl from 0–0.4 M in the equilibrating buffer. Fractions of 1.5 ml were collected and assayed for hexokinase activity as in [12]. The source of the materials have already been described [5,11,12].

3. Results

Hexokinase activity measured in blood samples, rich in reticulocytes, is several times higher than in normal red cell population [5]. To clarify the molecular basis of this phenomenon we have investigated in vivo the modification of the hexokinase isozymic pattern as a function of the presence of reticulocytes. As shown in fig.1, this high level of hexokinase in reticulocytes is mainly due to the presence of hexokinase type Ib. Moreover, the fast decrease of hexokinase activity observed during reticulocyte maturation is due predominantly to the decay of this isozymic from while hexokinase Ia shows a slow decay rate. However, hexokinase Ib appears not to be a peculiarity of the reticulocyte, in fact fig.1 also shows that if the number of reticulocytes is low (as at the 13th day from phenilhydrazine administration) this isozyme is still present.

The approach reported (fig.1) does not permit one to obtain a homogeneous population of old red cells so as to study their hexokinase isozymic pattern. In order to overcome this problem we have separated the whole rabbit red cell population into 5 fractions of increasing mean age by ultracentrifugation through gradients of Ficol–Triosil and the results are shown in fig.2 where it can be seen as expected, that hexo-



kinase Ib is higher in the young red cells (fractions 1 and 2) and decreases as the cell age increases. A new and interesting result is that a small amount of hexokinase Ib is present also in the older red cells (fraction 5). From these findings we can conclude that the presence of hexokinase Ib is not related only to the reticulocytes, but is a normal component of the red cell with a faster decay than hexokinase Ia.

4. Discussion

The results reported show that the decay of hexokinase activity during red cell aging is a process that involves a decay of hexokinase Ia and Ib at different rates. Hexokinase Ib is the predominant form of the soluble glucose phosphorylating activity present in rabbit reticulocytes but becomes the minor component in the mature cell, and is also present in the older red cells. As we have reported [11] the Lineweaver-Burk plots of initial rate as function of glucose concentrations, show that the affinity constant of hexokinase Ia is 4×10^{-5} M while that of hexokinase Ib is 1.25×10^{-4} M. These differences and the fact that young red cells contain mainly hexokinase Ib, can account for the changes in kinetic properties of the glucose phosphorylating activities observed during cell aging [5]. In conclusion, in rabbit red cells, glucose phosphorylation is regulated during cell aging by two distinct enzymic forms with different kinetic and physical properties [11]. This fact when associated with the decline in adenosine triphosphate and other glycolytic intermediates, observed during cell aging, can contribute confirmation to the hypothesis of a metabolic cause for red cell senescence [1,2].

Fig.1. In vivo age dependence of rabbit red blood cell hexokinase isozymic pattern. Reticulocytosis was induced in a group of 5 rabbits by 3 days phenylhydrazine administration and blood was collected in EDTA at day 0, 4, 7, 9, 11, 13 and 25 after treatment. A sample of 1 ml was applied to a DE-52 cellulose column (0.7 × 20 cm) equilibrated in 5 mM sodium potassium phosphate buffer (pH 7.5) containing 3 mM KF, 3 mM 2-mercaptoethanol and 5 mM glucose and operated at 15–20 ml/h. The column was developed with 280 ml of a linear gradient of KCl from 0–0.4 M in the equilibrating buffer. Fractions of 1.5 ml were collected and assayed for hexokinase activity.

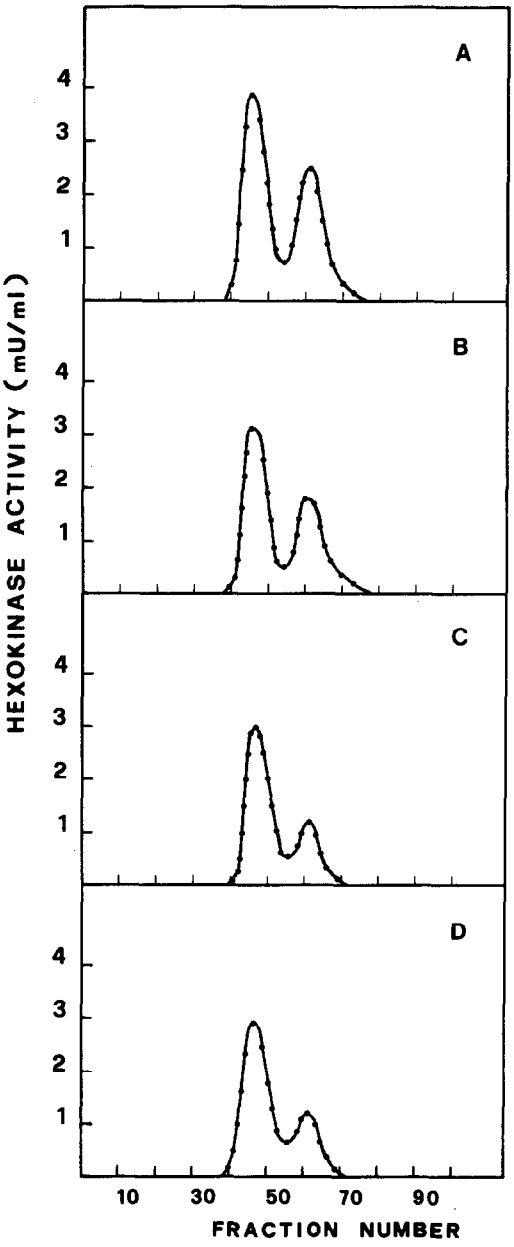


Fig.2. Hexokinase isozymic pattern in rabbit red cells of different ages. Rabbit red cells were separated into 5 fractions of increasing mean age by ultracentrifugation through gradients of Ficcoll-Triosil. Fractions 1 and 2, which contain the less dense cells, have been pooled. These cells and those of fractions 3,4 and 5 were collected in separate tubes, washed and haemolized in an equal volume of 0.4% (w/w) saponin solution. Samples of 1 ml were applied to DE-52 columns and chromatographed as reported in fig.1. Chromatographic profile of hexokinase: (A) from the pooled fractions 1 and 2 (young cells); (B) from fractions 3; (C) from fraction 4; (D) from fraction 5 (old cells).

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