HEAT LABILITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN SOME SENESCENT HUMAN CULTURED CELLS. EVIDENCE FOR ITS POSTSYNTHETIC NATURE

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SUMMARY : The nature of heat lability of glucose-6-phosphate dehydrogenase in senescent cells has been studied in human liver-derived cultured cells and in human fetal lung fibroblasts. The main data obtained were the followings : 1. When purified, glucose-6-phosphate dehydrogenase from either young or senescent cells exhibited similar heat stability. 2. In some cases, previous dialysis of the crude extracts from senescent cells improved heat stability of glucose-6-phosphate dehydrogenase. 3. The crude extracts from senescent cells deprived of endogenous glucose-6-phosphate dehydrogenase by specific immunoneutralization were able to alter heat stability of exogenous pure enzyme secondarily added to the extracts. 4. Some proteic fractions purified from senescent cell extracts were also able to alter heat stability of exogenous enzyme.

From these results it appeared that heat lability of enzymes in senescent cells could be due to postsynthetic, medium-dependent, phenomena rather than to age-dependent misincorporation of aminoacids into the enzyme molecules.

INTRODUCTION : Several authors have reported the presence of heat labile forms of various enzymes in senescent cultured cells (1, 2, 3) or in some organs of old animals (4, 5, 6, 7). The studies of stability were performed in crude extracts in some cases (1, 2, 3, 6) and in purified preparations in other cases (4, 5, 7). Glucose-6-phosphate dehydrogenase has been especially

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studied, by Holliday and Tarrant in human senescent lung fibroblasts (MRC 5) (3), by Pendergrass et al in human skin fibroblasts (8), by Goldstein and Singal in fibroblasts from subjects with Werner's syndrome (1) and by Wulf and Cutler in organs of old mice (6). With the exception of Pendergrass et al, all other authors found an increase of heat lability in senescent cells. These authors used crude homogenates of cultured cells or tissues. Their interpretation of the presence of heat labile fractions of glucose-6-phosphate dehydrogenase was that it reflected the accumulation of altered proteins due to faulty protein synthesis. Consequently these authors assumed that such a heat lability of glucose-6-phosphate dehydrogenase in senescent cells supports the "error catastrophe theory" originally postulated by Orgel (9). The aim of our work was to elucidate the nature of heat lability of glucose-6-phosphate dehydrogenase in various types of cultured cells.

PROCEDURE

Cell cultures : We used two different types of cultured cells :
1) Liver cell strains obtained from explant cultures of human liver. The culture conditions and the characteristics of these cells were described previously (10,11). They become senescent after passage 15.
2) Fetal lung fibroblast strain obtained from "Le Centre d'Etudes de Biologie Prénatale (CEBIOP)*.

The young cells referred to cells in phase II and old cells to cells in phase III, according to the criteria of Hayflick(12,13). The cells in phase II were studied before the 9th passage for the liver-derived strains and the 35th passage for the fibroblasts. The cells in phase III were studied after the 15th passage for the liver strains and the 50th passage for the fibroblasts.

Heat stability. The cells were lysed by three successive cycles of freezing and thawing. Stability to heat was appreciated at 52° in 50 mM tris chloride buffer pH 8, containing 20 µM $NADP^+$, 2 mM β mercaptoethanol, 1 mM EDTA and 2 mM ε aminocaproïc acid (10). The dilutions used had enzyme activity of 0.06 IU/ml and contained 1 mg/ml bovine albumin.

Purification of glucose-6-phosphate dehydrogenase. The cell extracts in 10 mM sodium phosphate buffer pH 6 were applied to a CM Sephadex column equilibrated with the same buffer, as described previously (14). The column was washed with a 50 mM phosphate buffer without $NADP^+$, and the proteins (devoided of all glucose-6-phosphate dehydrogenase activity) were collected. The enzyme was specifically eluted by adding 1 mM $NADP^+$ to the washing buffer (14). Then the protein remaining bound to the column were eluted by a 200 mM phosphate buffer pH 7.5. 1 mg/ml bovine albumin was added to the glucose-6-phosphate dehydrogenase-containing fraction, and the enzyme was co-precipitated

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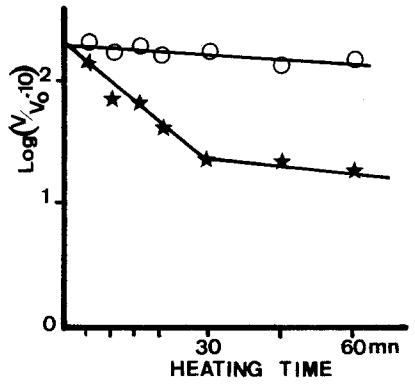

Fig. 1 : Heat lability of glucose-6-phosphate dehydrogenase from young and old liver-derived cultured cells.

—○—○— young cells
—✶—✶— old cells

A : whole homogenate
B : purified preparations of glucose-6-phosphate dehydrogenase
Temperature : 52° C

with albumin by ammonium sulfate at 80% saturation. The enzyme preparation and the other protein fractions (also precipitated by 80% saturated ammonium sulfate) were extensively dialysed agains the tris chloride buffer described above before being used.

Immunologic methods. In some experiments, enzyme from both young and old cells was specifically neutralized by a monospecific anti-human glucose-6-phosphate dehydrogenase serum from rabbits (14). Then pure human glucose-6-phosphate dehydrogenase from either leukocytes or platelets (14,15,16) was added to the cell extracts, and its stability was studied as described above.

RESULS AND DISCUSSION : Four experimental approaches have been used to determine the nature of heat lability of glucose-6-phosphate dehydrogenase in senescent cells.

1) Heat stability of glucose-6-phosphate dehydrogenase purified from old or young cells.

We have pooled the cells from different liver-derived strains in phase II and in phase III. The whole homogenate of old cells contained a fraction of heat labile glucose-6-phosphate dehydrogenase amounting to about 35% of the total activity (3) (fig. 1). By contrast, the extract of young cells seemed to contain less than 10% of heat-labile enzyme. After purification,

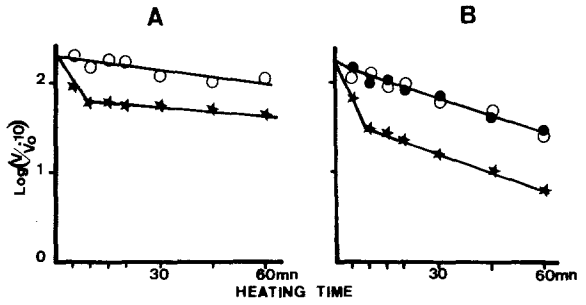

Fig. 2 : Influence of the crude homogenates from old or young fetal lung fibroblasts on the heat lability of exogenous glucose-6-phosphate dehydrogenase.
-O-O-Homogenate of cells at the 34th passage
-X-X-Homogenate of cells at the 61th passage
-●-●-Pure leukocyte glucose-6-phosphate dehydrogenase diluted in the same tris chloride buffer as the cell extracts.
A : stability of the endogenous enzyme of the cell homogenates. In this experiment serum of a non immunized animal was added to the homogenate, at the same concentration as that of the antiserum used in "B".
B : stability of pure leukocyte enzyme, added to the homogenates of old or young cells, or diluted in the buffer.
Endogenous own enzyme of the cell homogenates has been totally neutralized by the addition of specific anti human glucose-6-phosphate dehydrogenase serum (1 µl per 20 IU of enzyme activity). After incubation for 1 hour at 37° and 6 hours at 4° the extracts were centrifuged for 30 mn at 20,000 g. Then pure human glucose-6-phosphate dehydrogenase from leukocytes was added to the cell homogenates in such a manner that final enzyme activity was identical with that measured before immunoneutralization.

stability of glucose-6-phosphate dehydrogenase from either old or young cells was similar (fig. 1). The yield in enzyme activity of this purification procedure was about 50% for both cell extracts.

The same result was obtained with the fetal lung fibroblast strain.

2) Influence of dialysis.

The influence of dialysis of the crude homogenates against the tris chloride buffer described above was variable : in some cases the amount of heat-labile enzyme in the old cell extract seemed to be greatly reduced whereas, in other cases, dialysis remained without any noticeable influence.

3) Influence of the crude homogenates from either old or young cells on the stability of exogenous glucose-6-phosphate dehydrogenase.

Fig. 2 shows the thermal stability curve of pure leukocyte glucose-6-

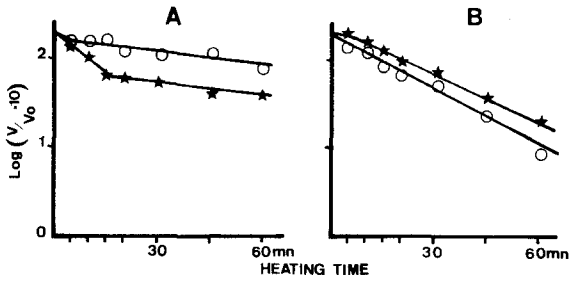

Fig. 3 : Influence of protein fractions from old and young liver-derived cultured cells on heat stability of pure human platelet glucose-6-phosphate dehydrogenase.

- The protein fractions from old and young cells were separated from glucose-6-phosphate dehydrogenase by CM Sephadex chromatography as described in the chapter "Procedure".
- Pure enzyme from platelets was diluted in solutions containing 0.3 mg/ml of proteins from either old or young cells, and its heat stability was checked.

The original cell homogenates were those used in the experiments described in fig. 1

—○—○— young cells
—×—×— old cells

phosphate dehydrogenase added to homogenates of old or young cells whose own enzyme was previously eliminated by specific immunoneutralization : the old cell extracts were able to induce the appearance of a "thermolabile fraction" from exogenous pure glucose-6-phosphate dehydrogenase whereas the young cell extract did not.

4) Influence of protein fractions from either old or young cells on the stability of exogenous glucose-6-phosphate-dehydrogenase.

The protein fractions eluted from the CM Sephadex column by the 50 mM phosphate buffer deprived of $NADP^+$ were mixed with the fractions eluted by the 200 mM phosphate buffer. The preparations from old or young liver-derived cells were then mixed with pure glucose-6-phosphate dehydrogenase (from human platelets) whose heat-stability was then studied. This experiment showed (fig. 3) that the protein fractions obtained from the old cells induced an apparent thermoinstability of glucose-6-phosphate dehydrogenase when compared with the fractions obtained from the young cells.

From these results it appeared obviously that, in the various cultured cell strains that we have studied, heat-lability of glucose-6-phosphate dehydrogenase did not result from the misincorporation of aminoacids into the enzyme molecules by a faulty protein synthesis machinery, but rather from the postsynthetic alteration of the enzyme by age-dependent factors.

These factors could be, in some cases, dialysable factors. Such a type of little molecule has been demonstrated to be responsible for the lability of this enzyme in polymorphonuclear cells from patients with chronic granulomatous disease (17). We have personally described another type of dialysable molecules, abundant in some leukemic cells, able to postranslationally alter the properties of glucose-6-phosphate dehydrogenase (18). These "modifying factors", however, did not seem to be involved here since, by contrast with the enzyme treated by such factors, isoelectric point of glucose-6-phosphate dehydrogenase is not modified in old cells (10).
Some protein fractions from old cells seemed also to be able to alter heat stability of glucose-6-phosphate dehydrogenase, as proved by the influence on the thermal stability of a pure exogenous enzyme of the cell fractions isolated by CM Sephadex chromatography. The nature of these proteins can only by hypothesized. Proteolytic enzymes, or $NADP^+$ glycolydrolases active on the structural $NADP^+$ of glucose-6-phosphate dehydrogenase (19) might be involved.

Some authors have recently purified enzymes from old animals ; in some cases (superoxide dismutase from rat liver (4), isocitrate lyase and enolase from Turbatrix aceti (5, 7)), the pure preparations showed an increased sensitivity to heat, while in other cases heat stability of the pure enzymes was normal (e.g. phosphoglycerate kinase and triose phosphate isomerase from Turbatrix aceti (20, 21)). The presence of heat-labile fractions (as well as the accumulation of inactive cross reacting material (4, 5, 7, 20)) in the enzyme preparations purified from old animals could result from the irreversible action on these enzymes of various substances accumulated in some old cells, as reported in our paper.

In conclusion, although these experiments did not directly test the hypothesis that senescence is associated with accumulation of faulty synthesized proteins, they question the validity of using increased heat-lability as a measure of such errors. Postsynthetic alterations of enzymes or modifications in the cell environment in old cells could result in a similar heat-lability.

Finally the question may be asked whether, in some cases, such post-translational events can play a role in the functional impairment of the senescent cells.

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