A UNIQUE ELECTROPHORETIC PATTERN OF TRIOSEPHOSPHATE ISOMERASE IN HUMAN CULTURED FIBROBLASTS

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1. Introduction

Electrophoretic studies on triosephosphate isomerase (D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1., TPI) have shown in most cases three bands migrating close to one another; such results have been obtained in man [1], rabbit [1, 2] and other animals [3]. According to a recent paper by Krietsch et al. [4] the intermediary band is the result of hybridization between the two others. Sometimes faster bands have been observed [3] but they are fainter than the three major ones.

In preliminary work [5], we observed that for several enzymes the isozymic pattern of human cultured fibroblasts was different from that of adult or fetal tissues. The present paper reports a unique pattern for TPI, showing discrete fast-migrating active bands.

2. Methods

Human tissues were obtained through biopsies or at autopsy; embryonic tissues were from 2 and 4 months old embryos. Fibroblasts were cultured from the same embryos or from skin biopsies taken on children and adults.

Tissues were extracted in a glass homogenizer with water, and homogenates were centrifuged at 27,000 *g* for 15 min. A final dilution of 1:50 or 1:100 was used. Red cells were washed, then hemolyzed with 50 vol. water.

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Electrophoresis was performed according to Kaplan et al. [1] on starch gel, in tris 0.1 M, EDTA 4.5 mM pH 9.4. Buffer was undiluted for the electrode vessels and was diluted 1:10 for the gel; electrophoresis was run for 18 h in the cold at 12 mA and 10 V/cm.

Staining used the following reactions:

$$\text{glyceraldehyde-3-Pi} \xrightleftharpoons{\text{TPI}} \text{dihydroxyacetone-Pi (DHAP)}$$

$$\text{DHAP} + \text{NADH} \xrightleftharpoons{\text{glycerol-3-Pi DH}} \alpha\text{glycerol-3-Pi} + \text{NAD}$$

The staining mixture is as follows: DL-glyceraldehyde-3-Pi 6 mM, NADH 0.5 mM; EDTA 5 mM, α glycerol-3-Pi DH 14 units per ml, tris buffer pH 8.0 0.1 M. TPI activity appears as defluorescing bands within 5 to 20 min, which show a tendency to diffuse afterwards.

3. Results and discussion (fig. 1)

Adult tissues: In agreement with previous results three bands close to one another were found. The two first bands are equally strong in red cells, while the first band predominates in all tissues; the third is markedly weaker. All these bands represent the slow zone of activity (S zone). In most tissues, but not in extracts of blood cells (RBC, WBC or platelets), a rather faint band in a more anodic position appears (fast band, F).

Embryonic tissues: A greater predominance of band 1 over band 2 is observed while band 3 seldom appears. The F band is more marked than in adult tissues. No sig-

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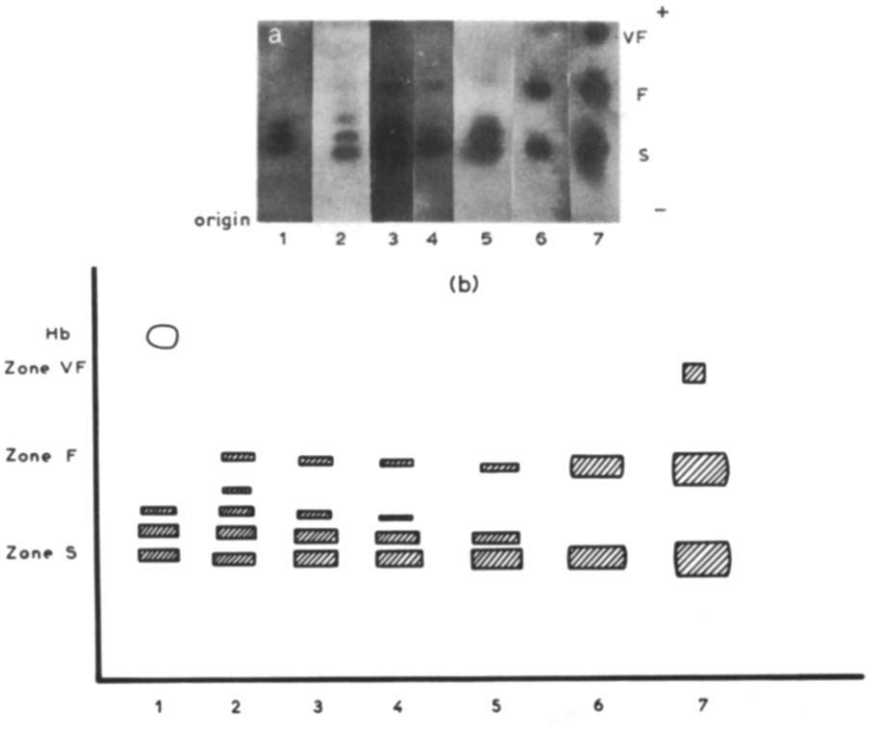

Fig. 1. Electrophoreses of TPI extracted from various tissues. (1) Hemolysate; (2) muscle adult; (3) thyroid adult; (4) placenta; (5) embryo; (6) fibroblasts 1:100; (7) fibroblasts 1:20. Fig. 1(b) is a diagram of fig. 1(a).

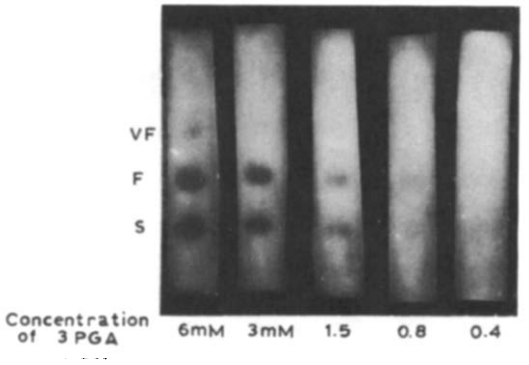

Fig. 2. Fibroblast extract: Reaction of the TPI with decreasing concentration of substrate.

nificant differences can be shown between different tissues. Placenta extracts resemble those of embryonic tissues.

Fibroblasts: Extracts from fibroblasts vary from all the others in three ways [2]: In the S zone only band 1 is visible. Diffusion occurs before any other band can be visualized. The F band stains as intensively as the S band. A new band appears in the anodal region which was never observed in tissue extracts (very fast band, VF). The distance between VF and F is the same as that between F and S.

Several control experiments have been performed which lead to following conclusions;

a) Isozyme patterns are reproducible and due to TPI activity. Suppression of substrate or the enzyme glycerol-3-Pi DH abolishes defluorescence.

b) Differences in migration are probably not due to major size differences or aggregation: elelctrophoresis in 10% or 15% starch gel does not change the relative rates of migration.

c) Decreasing amounts of substrate in the staining reaction weaken S and F bands equally, suggesting a similar affinity of S and F for glyceraldehyde-3-Pi (fig. 2).

d) In contracts, there is a marked difference in thermal stability; a much faster decrease of F than S bands is seen after exposure to 50° for 10 to 60 min. Band VF is still more heat labile and disappears within 5 min (fig. 3).

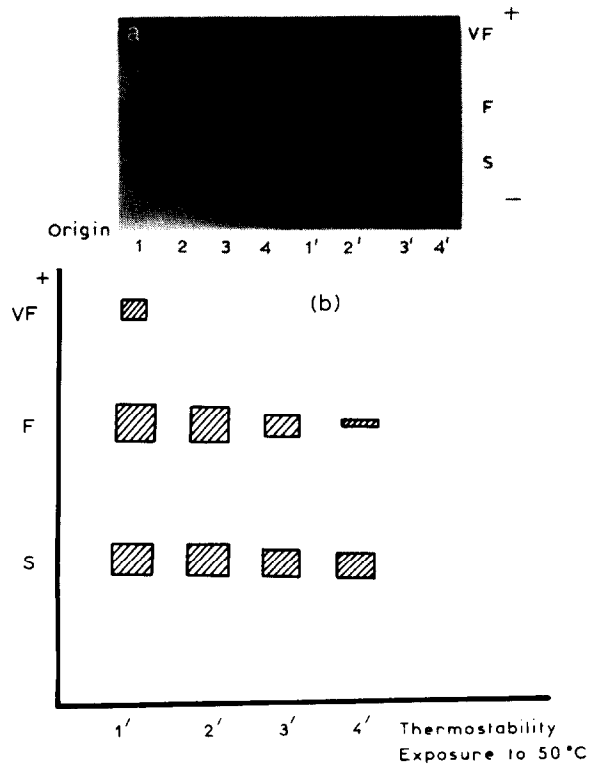

Fig. 3. Fibroblast extract: Thermal stability of TPI isozymes. 1 to 4: Five minutes incubation with staining mixture. 1′ to 4′: 0, 10, 20, 30 min incubation, respectively. Fig. 3b is a diagram of 3a, 1′ to 4′.

e) An attempt to hybridize F and S in fibroblast extracts has been made, using 8 M urea and 10 mM mercaptoethanol followed by dialysis. Patterns were unchanged: reactivation was complete but no hybridization occurred. This would have been unlikely since no in vivo hybrid is produced.

The hypothesis arises that F could be an hybrid between S and VF. In order to check this hypothesis we are attempting to isolate VF, which is present in small amounts.

In conclusion, the human cultured fibroblast displays a unique isozymic pattern of TPI which is independent of the tissue from which the culture has been started, and of the age of the donor, whether embryonic or adult. The relationship between the slow and fast types of enzyme, the biological role of the fast enzyme, and the conditions which allow the depression of the fast isozymes in vitro, remain to be elucidated.

References

[1] J.C. Kaplan, L. Teeple, N. Shore and E. Beutler, Biochem. Biophys. Res. Commun. 31 (1968) 768.
[2] W.K.G. Krietsch, P.G. Pentchev, H. Klingenbürg, T. Hofstätter and T. Bücher, European J. Biochem. 14 (1970) 289.
[3] R.K. Scopes, Biochem. J. 107 (1968) 139.
[4] W.K.G. Krietsch, P.G. Pentchev, W. Machleidt and H. Klingenbürg, FEBS Letters 11 (1970) 137.
[5] M.C. Meienhofer, D. Delain, A. Hanzlikova-Leroux, A. Boue and J.C. Dreyfus, Protides of Biological Fluids, Bruges 18 (1970) 103.