ELECTROPHORETIC PROPERTIES AND TISSUE DISTRIBUTION OF MULTIPLE FORMS OF HEXOKINASE IN VARIOUS MAMMALIAN SPECIES

Lionel Grossbard, Marc Weksler and Robert T. Schimke

Laboratory of Biochemical Pharmacology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

Received May 26, 1966

In this communication we present evidence which indicates that the existence of multiple forms of hexokinase (ATP: D-hexose 6-phosphotransferase), with differing electrophoretic and kinetic properties, is a general phenomenon in mammalian species.

Previous studies, limited to rat tissues, have demonstrated four forms of hexokinase, separable by DEAE-cellulose chromatography (Gonzalez _et al._, 1964) and by starch gel electrophoresis (Katzen _et al._, 1965). These forms in rat include both the high K_m glucokinase, with a K_m for glucose of $1 - 2 \times 10^{-2}$M, limited to liver, as well as three hexokinases with K_m values for glucose ranging from 10^{-4} to 10^{-6}M. These enzymes can be distinguished from each other by certain kinetic and physical properties, both in crude (Katzen and Schimke, 1965) and in highly purified preparations (Grossbard and Schimke, 1966). The three hexokinases constitute a family of enzymes, each uniform in kinetic and physical properties from tissue to tissue, but present in characteristic proportions in the various rat tissues (Katzen and Schimke, 1965).

The present studies indicate that multiple forms of hexokinase exist in all species examined. The tissue distribution of the various forms is similar to that found in the rat. However, the electrophoretic mobilities of one or more of the hexokinase forms varies from species to species, and are sufficiently unique to allow for an identification of species on this basis.

Materials and Methods

All animals were well fed, healthy, young adult males obtained from NIH colonies, except for the cow. Cow tissues were obtained from a local slaughter house. Details of tissue preparation and starch gel electrophoresis are described in the legend to Fig. 1. Hexokinase activity was assayed by the method of Salas et al.(1963).

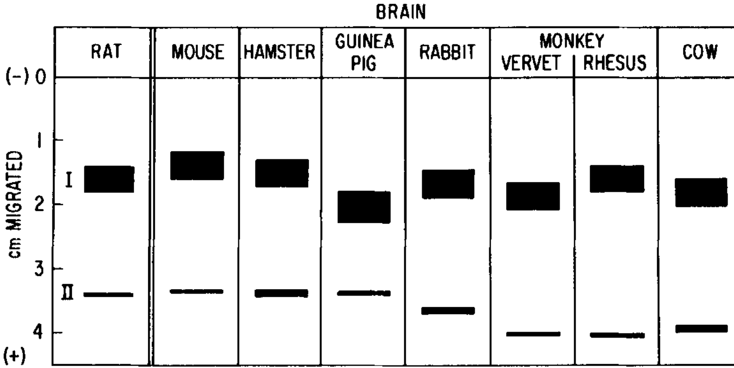

Fig. 1. Diagrammatic representation of the quantity and mobility of hexokinase forms, in extracts of brains, separated by starch gel electrophoresis. Extracts were prepared from the tissues of freshly killed animals by homogenization for 1 min in an equal volume of cold 0.01M potassium phosphate buffer, pH 7.0, containing 5mM mercaptoethanol, 5mM Na_2 EDTA and 10mM glucose. The homogenates were centrifuged for 45 minutes at 105,000 xg, and 30 µl of each of the supernatants were placed in each well. Vertical starch gel electrophoresis (Smithies, 1955) was performed using a 0.02M sodium barbital buffer, pH 8.4, containing 2.7mM Na_2 EDTA and 5mM 2-mercaptoethanol. Electrophoresis was carried out at 2° for 19 hours with a potential gradient of 6 volts/cm across the gel. Gels were sliced and stained for hexokinase activity as described previously (Katzen and Schimke, 1965), using two concentrations of glucose, 0.1M and 0.5mM. The electrophoretic forms present in brain (and in skeletal muscle - Fig. 2) stain at both concentrations of glucose, but slightly more intensely at the higher concentration. Under these conditions no bands of hexokinase activity with cathodal migration were present in any tissue.

The Roman numerals adjacent to the four forms of hexokinase in rat (Fig. 1-3) designate the forms which have been separated and characterized in rat tissues (Katzen and Schimke, 1965; Grossbard and Schimke, 1966).

Results

As shown in Figures 1 to 3 all species studied contained multiple forms of hexokinase with similar patterns of tissue distribution, but with differences in migratory properties. The hexokinase patterns are highly reproduc-

ible and represent results of electrophoreses of extracts from each tissue from each of 3 to 10 different animals of every species. The mobilities of the multiple forms are all presented relative to the forms in a rat liver extract, which was included as a reference in all electrophoretic runs.

Brain: The electrophoretic pattern of hexokinase activity from brain extracts of all species was similar (Fig. 1). All showed only two different forms with the preponderant form being that with the slower mobility. K_m determinations for glucose for the preponderant hexokinase type in mouse brain gave a value of 3.1×10^{-5}M, very similar to those reported for the preponderant form in rat brain (type I) (Katzen and Schimke, 1965) and in calf brain (Fromm and Zewe, 1962).

Skeletal muscle: Skeletal muscle extracts of all species contained the same two hexokinase types as brain, but with a relatively greater proportion of the more rapidly migrating form (Fig. 2). Mouse, hamster and cow were similar to rat in that the more rapidly migrating form was predominant. Guinea pig, rabbit and monkey had approximately equal amounts of both types. K_m values for glucose for the more rapidly migrating and predominant form in mouse and cow muscle, each isolated by use of DEAE-cellulose chromatography (Grossbard and Schimke, 1966), were 3.3×10^{-4}M and 2.8×10^{-4}M respectively. These were essentially identical to the value reported for the predominant form in rat muscle (type II) (Grossbard and Schimke, 1966).

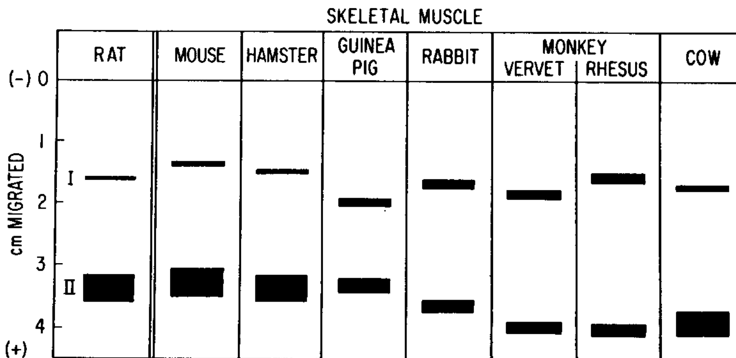

Fig. 2. Diagrammatic representation of the quantity and mobility of hexokinase forms in extracts of skeletal muscle.

Liver: The livers of all animals contained several forms of hexokinase, varying from three in mouse and rabbit to five in hamster (Fig. 3). Previous studies (Gonzalez et al., 1964; Katzen and Schimke, 1965) have indicated that rat liver contains, among its four forms, both a substrate inhibited hexokinase (type III) with a K_m of 5 - 7 X 10^{-6}M for glucose, as well as glucokinase (type IV) with a K_m of 1.6 - 2.0 X 10^{-2}M for glucose. As described previously for rat liver (Katzen and Schimke, 1965), these two enzymes may be identified on starch gel by their staining characteristics using two different concentrations of glucose. Figure 3 shows that all livers examined contained both a substrate inhibited form (which stains intensely with 0.5mM glucose, but which is virtually undetectable with 0.1M glucose in the developing solution), and an enzyme with a high K_m for glucose (evident only when the gels were incubated at 0.1M glucose, but not seen with 0.5mM glucose in the developing solution).

In all species the most rapidly migrating form was that form which appeared to have a high K_m for glucose. In order to determine a K_m for this form from a previously unstudied species, the most rapidly migrating enzyme from mouse liver was isolated by DEAE-cellulose chromatography. The K_m value for glucose was 2.0 X 10^{-2}M, a value similar to those reported for rat liver (Vinuela et al., 1963; Gonzalez et al., 1964; Katzen and Schimke, 1965), guinea pig liver (Walker, 1963), and rabbit liver (Salas et al., 1965) glucokinases.

Among the species studied only the mouse appeared to present an exception to the findings in rat that the hexokinase type with identical electrophoretic mobilities in different tissues had similar kinetic properties (Grossbard and Schimke, 1966). Thus, on starch gels of mouse tissues the substrate inhibited form in liver had a mobility identical to the predominant muscle form which was not substrate inhibited. The identical mobility of the two forms of hexokinase would appear to be fortuitous, in view of the marked species variation in mobility of the substrate inhibited

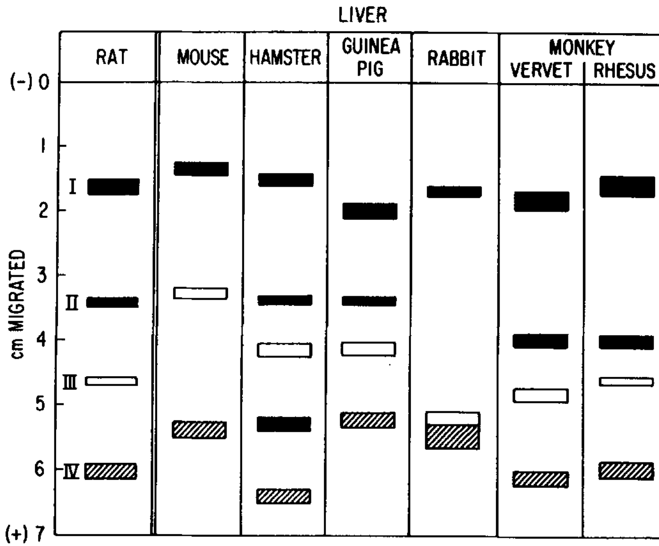

Fig. 3. Diagrammatic representation of the quantity and mobility of hexokinase forms in extracts of liver. ▬ -hexokinase forms staining at both concentrations of glucose (0.1M, 0.5mM) but slightly more intensely at the higher concentration. ▭ -substrate inhibited form of hexokinase; staining intensely in 0.5mM glucose and virtually undetectable in 0.1M glucose in the developing solution. ▨ -hexokinase form staining only when gels were incubated at 0.1M glucose; no staining with 0.5mM glucose.

Five different inbred strains of mice used (C57BL/6, C3H/HEN, CAF1, CdF1, ALN) gave identical results as did the two strains of rats used (Osborne-Mendel, and Sprague Dawley).

---

form (Fig. 3). Thus, the predominant mouse muscle enzyme, isolated by DEAE-cellulose chromatography, was not substrate inhibited and had a K_m for glucose of 3.3×10^{-4}M, whereas the enzyme with the same electrophoretic mobility isolated from mouse liver was substrate inhibited and had a K_m of 9.0×10^{-6}M for glucose. This latter value is similar to that reported for the substrate inhibited enzyme in rat liver (type III).

Other tissues: The distributions of the hexokinase types in kidney and epididymal fat pad of all animals were essentially the same as those described for rat (Katzen and Schimke, 1965). As with the rat, epididymal fat pad contained only the two slowly migrating forms. Kidney extracts all contained predominantly the slowest migratory type, but did contain detect-

able amounts of all forms present in liver except for the most rapidly migrating form.

Discussion

There is a striking similarity between the various mammalian species studied with respect to multiple forms of hexokinase, including both the number and tissue distribution of these forms. In addition, available evidence based on K_m values for glucose would suggest that each type in rat, whose properties have been studied in detail (Grossbard and Schimke, 1966), has a counterpart form in most other species, regardless of the differences in electrophoretic mobilities. Thus, in all species examined (rat, mouse and calf) the predominant hexokinase of brain (type I) has a K_m value for glucose of $3 - 6 \times 10^{-5}$M. The predominant hexokinase of skeletal muscle (type II) has a K_m of $1 - 3 \times 10^{-4}$M in the species studied (rat, mouse and cow). The substrate inhibited enzyme (type III), examined in rat and mouse, has a K_m of $5 - 9 \times 10^{-6}$M. The high K_m glucokinase (type IV), in rat, rabbit, guinea pig, and mouse has a K_m of $1 - 2 \times 10^{-2}$M.

The number of similarities among the species listed above would appear to be particularly important in view of the known nutritional and hormonal effects in controlling the level of glucokinase (type IV) in the rat (reviewed by Sharma *et al.*, 1964 and Sols *et al.*, 1964), and the level of type II hexokinase in rat epididymal fat pad (Moore *et al.*, 1964; Katzen and Schimke, 1965; McLean *et al.*, 1966).

REFERENCES

Fromm, H. J., and Zewe, W., J. Biol. Chem., 237, 1661 (1962).
Gonzalez, C., Ureta, T., Sanchez, H., and Niemeyer, H., Biochem. Biophys. Res. Commun., 16, 347 (1964).
Grossbard, L., and Schimke, R. T., J. Biol. Chem., in press, (1966).
Katzen, H. M., Soderman, D. D., and Nitowsky, H. M., Biochem. Biophys. Res. Commun., 19, 377 (1965).
Katzen, H. M., and Schimke, R. T., Proc. Natl. Acad. Sci. U.S., 54, 1218 (1965).
McLean, P., Brown, J., Greensalde, K., and Brew, K., Biochem. Biophys. Res. 23, 117 (1966).

Moore, R. O., Chandler, A. M., and Tettenhorst, N., Biochem. Biophys. Res. Commun., 17, 527 (1964).
Salas, J., Salas, M., Vinuela, E., and Sols, A., J. Biol. Chem., 240, 1014 (1965).
Salas, M., Vinuela, E., and Sols, A., J. Biol. Chem., 238, 3535 (1963).
Sharma, C., Manjeshwar, R., and Weinhouse, S., in G. Weber (Editor), Advances in Enzyme Regulation, Vol. 2, Pergamon Press, N.Y., 1964, p. 189.
Smithies, O., Biochem. J., 61, 629 (1955).
Sols, A., Salas, M., and Vinuela, E., in G. Weber (Editor), Advances in Enzyme Regulation, Vol. 2, Pergamon Press, N.Y., 1964, p. 177.
Vinuela, E., Salas, M., and Sols, A., J. Biol. Chem., 238, PC1175 (1963).
Walker, D. G., Biochim. Biophys. Acta, 77, 209 (1963).