MUSCLE-TYPE ALDOLASE ISOLATED FROM A LIVER TUMOR[1]

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SUMMARY

Fructose diphosphate aldolase has been crystallized from a Novikoff ascites hepatoma and its properties compared with those of aldolases of normal rat liver and muscle. The identity of the tumor enzyme with the normal muscle enzyme was established on the basis of amino acid composition and tryptic fingerprints, as well as the sensitivity to inactivation by L-glyceraldehyde 3-phosphate.

Studies carried out with crude tissue extracts have suggested that in the development of poorly differentiated liver tumors the normal liver aldolase is replaced by an enzyme with substrate specificity (1,2) and immunological properties (3) resembling those of muscle aldolase. We have now isolated for the first time a crystalline fructose diphosphate aldolase from the ascitic form of Novikoff hepatoma.[6] The enzymes from normal rat liver and normal rat

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[6] Aldolase from Rhodamine sarcoma has recently been crystallized by Matsushima et al. (personal communication).

muscle have also been obtained in pure form, thus permitting a comparison of the three enzymes at the molecular level.

METHODS

Aldolases were assayed as described previously (4). Erythrose 4-phosphate and L-glyceraldehyde 3-phosphate were prepared and the concentrations determined enzymatically as described by Lai et al. (5). Samples were prepared for amino acid analysis as previously described (4) and analyzed according to Spackman et al. (6). Samples of aldolases for tryptic fingerprints were first labeled with ^{14}C-dihydroxyacetone phosphate and S-carboxymethylated (7), and then digested at pH 8.0 at an aldolase/trypsin ratio of 100:1. Peptides were lyophilized, dissolved in water, and chromatographed for 24 hours on Whatman 3 MM paper in butanol:acetic acid:water (3:1:1). Subsequent electrophoresis was carried out in pyridine:acetic acid:water (1:10:89), pH 3.5, at 4000 volts for 1.5 hours. Peptides were visualized after spraying with ninhydrin; peptides containing arginine were located with the Sakaguchi reagent (8).

RESULTS AND DISCUSSION

The three aldolases were purified by chromatography on phosphocellulose columns utilizing both substrate (9) and salt gradient elutions (4). The specific activities of the liver, muscle, and tumor enzymes, were, respectively, 1.64, 13.8 and 12.7 μmoles FDP cleaved per min per mg of protein at 25°. After purification, the enzymes were shown to be homogeneous by sedimentation velocity and sedimentation equilibrium ultracentrifugation, as well as by electrophoresis on polyacrylamide gel and cellulose acetate.

Although the muscle and liver aldolases were similar in amino acid composition, they differed significantly with respect to their content of several amino acids, including arginine, lysine, glutamic acid, proline, methionine, and tyrosine. The liver enzyme contained only half as much proline (49 residues as compared with 86 for the muscle aldolase) and three times as much

methionine (24 residues as compared with 8 for the muscle enzyme). The total amino acid composition of Novikoff hepatoma aldolase was identical to that of the muscle enzyme.

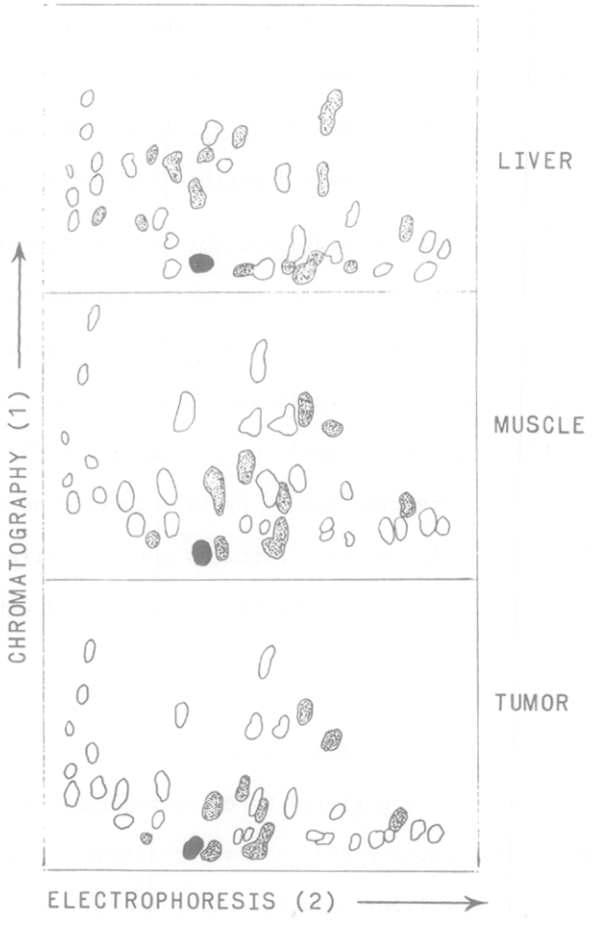

Figure 1. Tryptic fingerprints of rat liver, rat muscle, and Novikoff tumor aldolases. Initial chromatography in butanol:acetic acid:water was followed by electrophoresis at right angles in pyridine:acetic acid:water. The labeled active-site lysine peptides are shown by solid shading, and the arginine-containing peptides by the stippled shading.

The total number of tryptic peptides in each case was consistent with the four-subunit model proposed for aldolase (10-13). The peptide patterns (Fig. 1) demonstrate certain regions of homology in the liver and muscle enzymes, particularly with respect to the active site lysine peptide which occupies the same position in all three fingerprints. Differences between

the muscle and liver aldolases are clearly shown by the overall distribution of peptides, as well as by the number and location of the arginine-containing peptides. The identity of the Novikoff hepatoma enzyme with muscle aldolase is confirmed by the tryptic fingerprints.

Finally, the susceptibility to inactivation by L-glyceraldehyde 3-phosphate further characterizes the muscle and Novikoff hepatoma aldolases. Exposure to L-glyceraldehyde 3-phosphate (Fig. 2) results in progressive inactivation of the muscle and tumor enzymes, while the liver aldolase remains unaffected by this treatment. The sensitivity of aldolases from skeletal muscle to inactivation by triose and tetrose phosphates (5,14) is in marked contrast to the resistance of rat liver aldolase reported here and observed also for the enzyme from rabbit liver;[7] this difference serves to characterize the two types of aldolases.

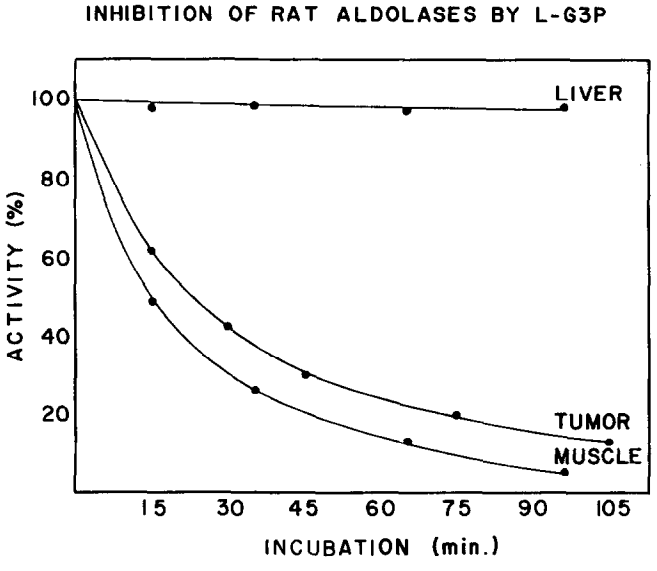

Figure 2. Inactivation of liver, muscle, and Novikoff hepatoma aldolases by L-glyceraldehyde 3-phosphate. The reaction mixtures contained 0.012 mM aldolase, 40 mM triethanolamine (pH 7.5), 1 mM EDTA, and 0.10 mM glyceraldehyde 3-phosphate at 25°. Aliquots were withdrawn and assayed as indicated.

[7] Unpublished results from this laboratory.

The results reported here provide conclusive evidence for the identity of Novikoff hepatoma aldolase with the rat muscle enzyme. The studies are now being extended to hepatomas in tissue culture, in an effort to elucidate the factors which control the expression of the aldolase genes.

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REFERENCES

1. Adelman, R. C., Morris, H. P., and Weinhouse, S., Cancer Res., 27, 2408 (1967).
2. Nordmann, Y., and Schapira, F., Eur. J. Cancer 3, 247 (1967).
3. Matsushima, T., Kawabe, S., Shibuya, M., and Sugimura, T., Biochem. Biophys. Res. Commun. 30, 565 (1968).
4. Gracy, R. W., Lacko, A. G., and Horecker, B. L., J. Biol. Chem. 244, 3931 (1969).
5. Lai, C. Y., Martinez-de Dretz, G., Bacila, M., Marinello, E., and Horecker, B. L., Biochem. Biophys. Res. Commun. 30, 665 (1968).
6. Spackman, D. H., Moore, S., and Stein, W. H., Anal. Chem. 30, 1190 (1958).
7. Lai, C. Y., Hoffee, P., and Horecker, B. L., Arch. Biochem. Biophys. 112, 567 (1965).
8. Smith, I., and Horsewell, H. G., in "Chromatographic and Electrophoretic Techniques", Vol. I (I. Smith, ed.), Interscience Publishers, Inc., New York 1960, p. 222.
9. Penhoet, E., Kochman, M., and Rutter, W. J., Biochemistry (in press).
10. Kawahara, K., and Tanford, C., J. Biol. Chem. 241, 3228 (1966).
11. Penhoet, E., Rajkumar, T., and Rutter, W. J., Proc. Natl. Acad. Sci. 56, 1275 (1966).
12. Sia, C. L., and Horecker, B. L., Arch. Biochem. Biophys. 123, 186 (1968).
13. Lai, C. Y., and Chen, C., Arch. Biochem. Biophys. 128, 212 (1968).
14. Rose, I. A., and O'Connell, E. L., J. Biol. Chem. 244, 126 (1969).