SUBSTRATE-INDUCED DISSOCIATION OF RABBIT MUSCLE ALDOLASE INTO ACTIVE SUBUNITS*

Beulah M. Woodfin**

Department of Biological Chemistry, Medical School
University of Michigan, Ann Arbor, Michigan 48104

Received September 11, 1967

For several years, data have been presented which suggested that the enzyme aldolase, from rabbit muscle, is composed of three subunits of identical, or nearly identical, size and structure (Drechsler *et al.*, 1959; Kowalsky and Boyer, 1960; Schachman, 1960; Stellwagen and Schachman, 1962; Deal *et al.*, 1963; Rutter *et al.*, 1963; and Winstead and Wold, 1964). More recent data, however, indicate the presence of four subunits of at least two types (Herskovits *et al.*, 1967; Kawahara and Tanford, 1966; Penhoet *et al.*, 1966 and Rajkumar *et al.*, 1966) including the possibility of two identical half-molecules (Chan and Morse, 1967; Lai *et al.*, 1965; Udenfriend and Velick, 1951). The data to be presented here support this latter model.

Materials and Methods

Aldolase was prepared from rabbit muscle by the method of Green and Taylor as modified by Drechsler, Boyer and Kowalsky (1959) and recrystallized at least four times to a specific activity of 15-17 units/mg protein. Fructose-1,6-diphosphate, sodium salt (FDP) was obtained from Calbiochem; glyceraldehyde-3-phosphate dehydrogenase from Boehringer; and NADH and glycylglycine were obtained from Sigma Chemical Co. Aldolase activity was

---

*This work was supported in part by a grant (NSF-GB 4237) from the National Science Foundation. During part of this work, the author was supported by a post-doctoral fellowship of the National Institute of General Medical Sciences, U.S. Public Health Service.

**Present address: Department of Biochemistry, School of Medicine, The University of New Mexico, Albuquerque, New Mexico 87106.

assayed as described by Rutter et al, 1963). A unit of enzyme activity is defined as the cleavage of one μmole of substrate per minute.

Formol Titrations: Reagent grade formaldehyde (30%) was adjusted to pH 9.0 (this was freshly prepared each day). The enzymes in 0.05 M KCl were diluted to 5 to 15 mg/ml protein and carefully titrated to pH 9.00-9.05, using standardized NaOH solutions of approximately 0.01 M and 0.05 M. A 5-ml aliquot of the pH 9.0 formaldehyde was added, and the pH again titrated to 9.00-9.05. Volumes and concentrations were adjusted to give a total volume of 15 ml at the end of the titration.

Molecular weight determinations were made by molecular sieve chromatography with Sephadex G-200 in a manner similar to that of Andrews (1965). A 1.0 cm x 40 cm column was used with 0.05 M tris-HCl, 0.1 M KCl, 0.005 M FDP, pH 7.5 buffer. Two mg of each protein, in a volume of 0.5 ml, was layered under the buffer head onto the top of the column bed. Pyruvate kinase was obtained from Mr. Paul Hollenberg and Dr. M. J. Coon of this department, and fumarase, L-amino acid oxidase and lipoyl dehydrogenase from the laboratory of Dr. Vincent Massey. The other proteins used were obtained from commerical sources.

Disc electrophoresis was performed by the technique of Davis (1964).

Results and Discussion

The ultraviolet absorbance of aldolase is slowly altered and the enzyme develops a yellow color upon incubation with the substrate, FDP. At room temperature (ca. 22°C), more than 60 hours are required to complete this reaction. Although the solution develops a strong yellow color, absorbance maximum is at approximately 330 mμ, trailing into the visible region, Figure 1. The spectral change occurs at protein concentrations ranging from 1 to 16 mg/ml, with a molar ratio of FDP/aldolase of 1000. Although glycylglycine, 0.05 M, pH 7.5, was used in most incubations, similar results were obtained with phosphate and Tris-HCl buffers. During the incubation the pH dropped from 7.5 to approximately 6.7.

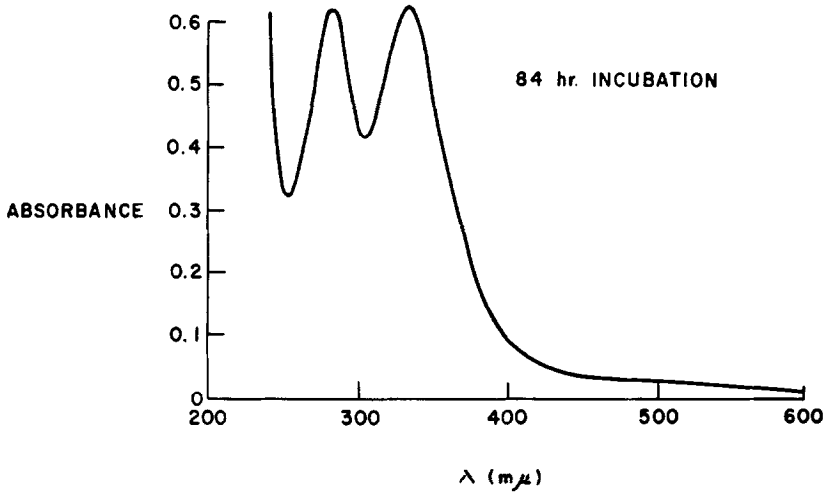

Fig. 1. Spectrum of Aldolase-Substrate Mixture after 84 hours at ca. 22° C. The enzyme (150 mg) at 10 mg/ml protein in 0.05 M glycylglycine, pH 7.5, was placed in a dialysis bag and dialyzed vs. 30 ml of 0.10 M fructose-1,6-diphosphate in 0.05 M glycylglycine, pH 7.5, at ca. 22°C. After 84 hours, 50 μl of the bag contents were diluted to 1.0 ml with dialysis medium and the spectrum measured in a Cary Model-14 spectrophotometer vs. the same dialysis medium as blank.

Sedimentation velocity ($S_{20,w} \doteq 5.9$ S) and diffusion ($D_{20,w} = 6.7 \times 10^{-7}$ cm^2 sec^{-1}) measurements on the product gave molecular weights of approximately 85,000, indicating a half molecule of aldolase. On dialysis to remove excess substrate, the product is stable and retains approximately 15% of the specific activity of the native enzyme, but the intensity of the yellow color is decreased. Formol titration yields 110-113 amino groups per 158,000 molecular weight units of native enzyme, and 29-34 for the modified protein, thus indicating blockage of 65-70% of the lysine ε-amino groups suggesting a Schiff s base formation with the carbohydrate which apparently stabilizes the half-molecule. Amino acid analyses on the product, after reduction with sodium borohydride, revealed less than half the expected lysine, and as as-yet-unidentified compound.

The molecular weight determination by molecular sieve chromatography with eleven proteins of known molecular weight and Blue Dextran 2000 (mol. wt. 2,000,000) as standards is shown in Figure 2. Based on 158,000 as mole-

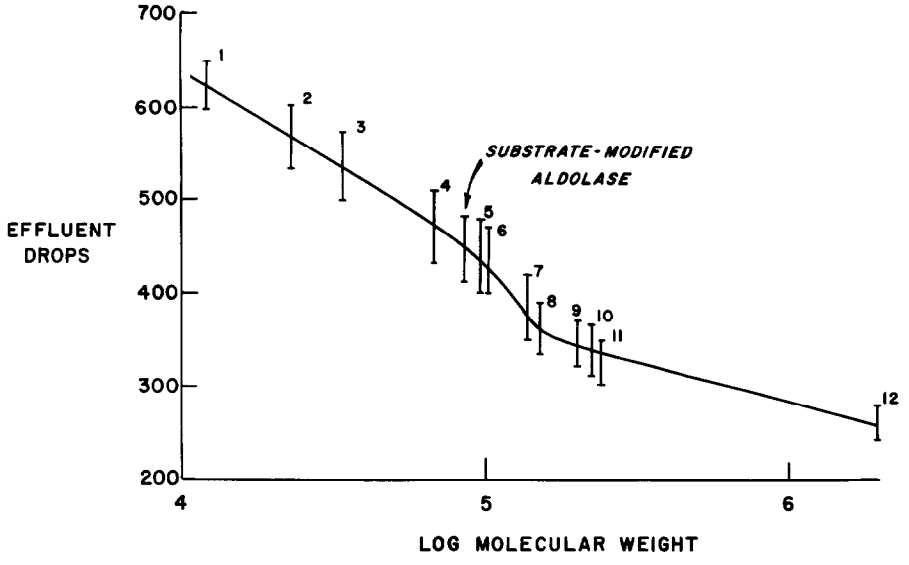

Fig. 2. Molecular Weight Determination on Sephadex G-200. Conditions are described in the text. Proteins to standardize the column are: 1. cytochrome c, 2. trypsin, 3. carboxypeptidase, 4. bovine plasma albumin, 5. yeast hexokinase, 6. pig heart lipoyl dehydrogenase, 7. venom L-amino acid oxidase, 8. native muscle aldolase, 9. fumarase, 10. liver catalase, 11. pyruvate kinase. 12. is Blue Dextran 2000.

---

cular weight for the native enzyme (Kawahara and Tanford, 1966), the molecular weight of the half-molecule would be 79,000. If 41 lysines per half-molecule reacted with the dihydroxyacetone-phosphate moiety, the molecular weight for the substrate-modified enzyme would be about 84,000. From the molecular sieve column, see Figure 2, one arrives at a molecular weight of approximately 86,000.

If this enzyme-substrate reaction blocks the ϵ-amino groups of the lysine residues as suggested by the formol titration data, one would expect the electrophoretic behavior of the protein to be changed markedly. The broad band for the substrate-modified material on disc electrophoresis, Figure 3, suggests a considerable variation in the number of lysine ϵ-amino groups reacted for any molecule. Thus the number determined by formol titration must be an average. There does not appear, however, to be native enzyme present to account for the residual activity observed.

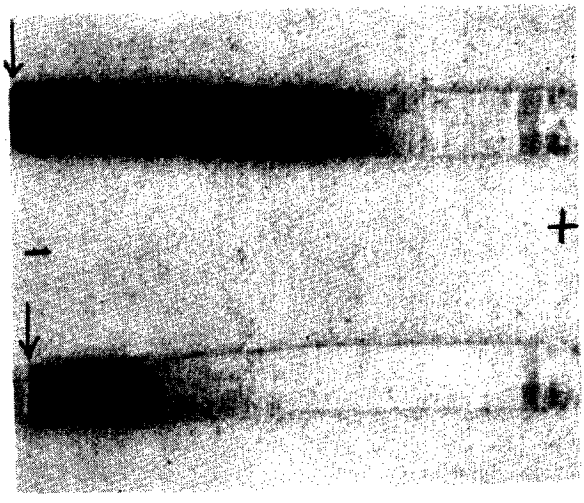

Fig. 3. Disc electrophoresis of Native Aldolase (left) and Substrate-modified Aldolase (right). Arrows indicate the top of the separation gel. Sample approximately 15 ug.

Acknowledgements

The author wishes to express gratitude to Dr. Vincent Massey, in whose laboratory this work was performed, and his associates for valuable discussion and criticism.

References

Andrews, P., Biochem. J., 96, 595 (1965).
Chan, W., and Morse, D., Federation Proc., 26, Abst No. 1931. (1967).
Davis, B. J., Ann. N.Y. Acad. Sci., 121, Art. 2, 404 (1964).
Deal, W. C., Rutter, W. J., and VanHolde, K. E., Biochemistry 2, 246 (1963).
Drechsler, E. R., Boyer, P. D., and Kowalsky, A. G., J. Biol. Chem., 234, 2627 (1959
Herskovits, J. J., Masters, C. J., Wassarman, P. M. and Kaplan, N. O., Biochem. Biophys, Res. Comm. 26, 24 (1967).
Kawahara, K., and C. Tanford, Biochemistry, 5, 1578 (1966).
Kowalsky, A., and Boyer, P. D., J. Biol. Chem., 235, 604 (1960).
Lai, C. Y., Tchola, O., Cheng, T., and Horecker, B. L., J. Biol. Chem. 240, 1347 (1965).

Penhoet, E., Rajkumar, T. V., and Rutter, W. J., Proc. Natl. Acad. Sci., U.S., 56, 1275 (1966).
Rajkumar, T. V., Penhoet, E., and Rutter, W. J., Federation Proc. 25, abst. No. 1878 (1966).
Rutter, W. J., Woodfin, B. M., and Blostein, R. E., Acta Chim. Scand., 17, 226 (1963).
Schachman, H. K., in Protein Structure and Function, Brookhaven Symposium, 1960, p. 49.
Stellwagen, E., and Schachman, H. K., Biochemistry, 1, 1056 (1962).
Udenfriend, S., and Velick, S. F., J. Biol. Chem., 190, 733 (1951).
Winstead, J. A., and Wold, F., J. Biol. Chem., 239, 4212 (1964).