HEAT-LABILE ISOZYMES OF ISOCITRATE LYASE FROM AGING TURBATRIX ACETI

Uzi Reiss and Morton Rothstein*

Division of Cell & Molecular Biology
State University of New York at Buffalo
Buffalo, New York 14214

Received October 25,1974

SUMMARY: The temperature stabilities of pure isocitrate lyase, isolated from young and old Turbatrix aceti, have been compared. The "old" enzyme shows the presence of a heat-labile component lacking in the enzyme derived from young organisms. This component has been shown to be associated with two of the five isozymes comprising the isocitrate lyase. A mechanism is proposed which might account for the presence of altered enzymes in aged organisms.

It is becoming clear that one of the biochemical manifestations of aging in an organism may be the increasing presence of altered enzyme. Thus, Gershon and Gershon (1), by means of antibody titrations, showed that the specific activity of isocitrate lyase decreased with the age of the free-living nematode, Turbatrix aceti. Subsequently, similar results were obtained with nematode aldolase (2) and mouse liver aldolase (3). Further evidence for age-related changes in enzymes was supplied by Holliday and Tarrant (4) who reported the presence of heat-labile forms of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in crude homogenates of late-passage fetal lung fibroblasts (MRC-5).

In our laboratory, we have shown that several enzymes lose specific activity with the age of T. aceti (5). Pure isocitrate lyase (6) from aged T. aceti has a sharply lowered specific activity, compared to that from young organisms (7). The enzyme consists of five isozymes, three of which constitute the major protein of the enzyme (6). All show the reduction in specific

*Reprint requests should be addressed to Morton Rothstein.

activity. Other chemical properties such as Km, inhibitor effects and binding during affinity chromatography are unchanged (7).

With the above results in mind, it was of interest to compare the heat stabilities of pure isocitrate lyase obtained from young and old T. aceti.

PROCEDURES

Heat Inactivation: Pure samples of enzyme from young (6-day) and old (27-day) cultures were prepared as previously described (6). The initial activity in both preparations was adjusted to approximately the same level (14.6 and 13.4 units, respectively, in 0.5 ml of Tris buffer (6)). The young and old preparations were incubated at 45°C and 0.05 ml aliquots withdrawn and assayed for activity at the indicated time intervals (Figure 1).

Gel Patterns after Heating: Samples of pure isocitrate lyase from young and old animals were heated at 45°. Aliquots were taken after 5, 10 and 15 minutes of incubation at 45° and were subjected to centrifugation (5000 xg, 10 minutes) to remove denatured protein. Samples (0.1 ml) were placed on top of 6% polyacrylamide gels. Electrophoresis and scanning for enzyme activity were performed as previously described (6).

RESULTS AND DISCUSSION

Figure 1 shows heat inactivation patterns for young and old preparations. Whereas the enzyme activity from young T. aceti declines in a straight line, purified "old" enzyme exhibits a biphasic heat inactivation plot, indicating the existence of a rapidly deteriorating component in the latter. After 15 minutes at 45°C, the slopes representing the rate of inactivation of both young and old preparations are essentially equal. The heat-labile portion of "old" enzyme amounts to about 53% of the total enzyme.

Though qualitative isozymic differences between young and old preparations were not found, there were substantial quantitative differences. Isozyme III is predominant in young organisms and isozyme I is predominant in old (7). In order to determine whether the heat-labile component of old preparations is associated with all the isozymes or is found only in specific isozymes, both "old" and "young" enzymes were heated at 45° for various times, and the remaining activities analyzed directly on acrylamide gels.

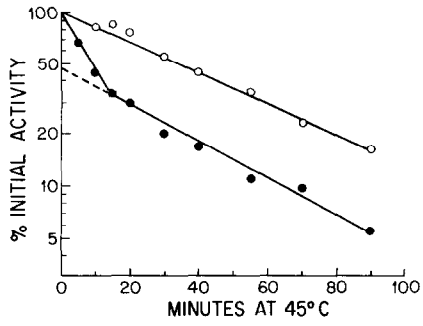

Figure 1: Heat inactivation of purified isocitrate lyase from young and old T. aceti. o——o, young (specific activity, 348 units/mg protein); ●——●, old (specific activity, 180 units/mg protein).

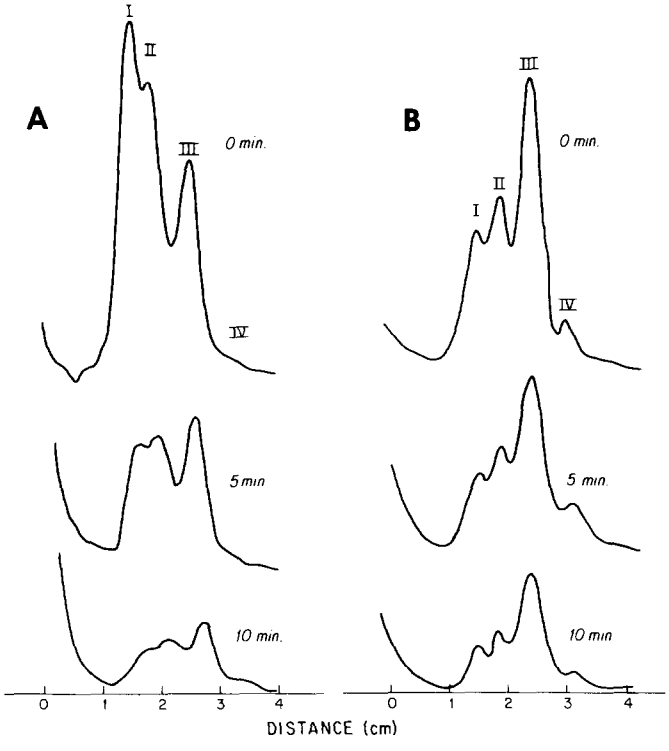

Figure 2: Activity of "young" and "old" isozymes as seen on polyacrylamide gels after heat inactivation at 45°C. A, scans of activities of "old" enzyme; B, scans of activities of "young" enzyme.

Figure 2 shows the results. Isozymes I and II in old preparations are clearly more sensitive to heat than their counterparts from young organisms. Isozymes I, II and III from young worms degrade at about equal rates so that their activities remain in the same relationship. The phenomenon can be

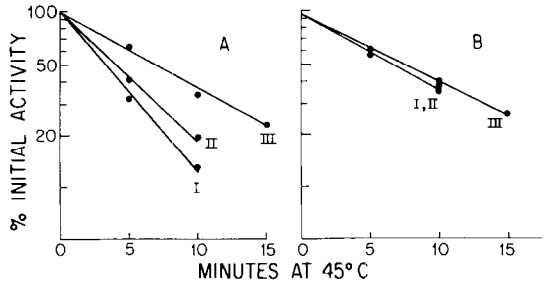

Figure 3: Rates of inactivation of "young" and "old" isozymes at 45°C. The plots were derived from the isozyme patterns of polyacrylamide gels in Figure 2. A, "old" isozymes; B, "young" isozymes.

seen clearly in Figure 3 in which the rate of inactivation of each isozyme is plotted. Isozyme III from both young and old preparations has a similar rate of inactivation.

The mechanism by which enzymes from old organisms are altered is not known. Accumulation of errors in amino acid sequence due to faulty protein synthesis, as originally postulated by Orgel (8), seems unlikely. Orgel himself has recently withdrawn support for the idea of "error catastrophe" (9). In the course of our work on the purification of isocitrate lyase, no changes in isolation patterns were observed which could be related to sequence (charge) changes in the "old" preparations.

Conformational changes in some of the molecules representing "old" enzyme are a likely possibility and could account for the existence of the heat-labile fraction observed in purified "old" enzyme. Thus, the following sequence of reactions could occur in aging organisms: original enzyme $\longrightarrow$ conformationally altered, meta-stable enzyme $\longrightarrow$ totally inactive enzyme $\longrightarrow$ eventual proteolysis.

In old T. aceti, isozymes I and II would exist, in large part, in the meta-stable form, readily destroyed by heating. Other enzymes might exist only briefly in this form, passing quickly or directly to an inactive form. This idea would explain the lack of heat-sensitive isozyme III in preparations from old T. aceti (Figure 3) even though the specific activity is somewhat reduced (7).

If protein turnover (both synthesis and breakdown) is slowed with age, the average dwelling time of each enzyme in cells would be lengthened. As a result, there would be a greater tendency to accumulate meta-stable and inactive forms of enzymes through chemical, kinetic or enzymic action. Initial results obtained by Dr. Roger Lane* show that in T. aceti, both synthesis and degradation of soluble proteins are slowed sharply with increasing age. In 9-day old organisms, the half-life is approximately 45 hours, whereas at 28 days, it is over 200 hours. Zeelon et al. have also indicated a greatly increased half-life for aldolase in old T. aceti (2).

Gershon and Gershon (1) postulated that old isocitrate lyase consists of a mixture or normally active and completely inactive molecules. However, the existence of heat-labile forms of isocitrate lyase (Figures 1, 2, 3), clearly supports the idea that the lowered specific activity of isocitrate lyase is the result, as least in part, of partially active molecules in the enzyme preparation. Further evidence for this idea is found in the fact that pure preparations of the "old" enzyme adhered to an affinity column (oxalate, bound to AH-Sepharose 4B) exactly like "young" enzyme, being removed only at the same concentration of substrate (7). No "inactive" protein came directly through the column.

*Biochemistry Department, State University of New York at Buffalo.

ACKNOWLEDGMENT: This work was supported in part by Program-Project HD06644 from the National Institute of Child Health and Human Development.

REFERENCES

1. Gershon, H. and Gershon, D. (1970) Nature, 227, 1214-1217.
2. Zeelon, P., Gershon, H. and Gershon, D. (1973) Biochemistry, 12, 1743-1750.
3. Gershon, H. and Gershon, D. (1973) Proc. U. S. Nat. Acad. Sci., 70, 909-913.
4. Holliday, R. and Tarrant, G. M. (1972) Nature, 238, 26-30.
5. Rothstein, M., Gupta, S. and Hieb, W. F. (1974) Gerontologist, 14, 32.
6. Reiss, U. and Rothstein, M. (1974) Biochemistry, 13, 1796-1800.
7. Reiss, U. and Rothstein, M. (1975) J. Biol. Chem., in press.
8. Orgel, L. E. (1963) Proc. U. S. Nat. Acad. Sci., 49, 517-521.
9. Orgel, L. E. (1973) Nature, 243, 441-445.