ISOELECTRIC PROPERTIES OF NEMATODE ALDOLASE AND RAT LIVER SUPEROXIDE DISMUTASE FROM YOUNG AND OLD ANIMALS

Phyllis GOREN, Abraham Z. REZNICK, Uzi REISS and David GERSHON
Department of Biology, Joseph and Arlene Taub Institute of Biological Research, Technion, Israel Institute of Technology, Haifa, Israel

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1. Introduction

The occurrence and accumulation of faulty enzyme molecules with no or with reduced catalytic activity have been demonstrated in cells of ageing animals. This phenomenon has been described for a considerable number of enzymes from a variety of cell types, various animal species and was suggested to be of key importance in the physiological deterioration of ageing animals (review [1]). Among these enzymes we have recently focused our studies on fructose-1,6-diphosphate aldolase from the nematode *Turbatrix aceti* (NA) [2] and cytoplasmic superoxide dismutase (SOD) from liver, brain and heart of rats and mice [3,4].

The nature of the mechanisms which lead to the age-related modifications of enzymes has been under active investigations in the last several years (cf. [1,5]). These age-related modifications must be of a subtle nature since no differences could be discerned in the following properties of enzymes derived from old animals: antigenic identity, K_m, K_i and electrophoretic mobility in polyacrylamide and SDS–polyacrylamide gels. However, noticeable decrements have been observed in the specific activity per unit of enzyme antigen or per milligram of purified enzyme protein. Also, temperature sensitivity of many enzymes changes as a function of age [1,5]. The changes that occur in 'old' enzymes can conceivably be attributed to subtle chemical alterations leading to charge differences which may not be discerned in regular polyacrylamide gel electrophoresis. Such modifications may be acetylation, phosphorylation, adenylylation, deamidation, carbamylation, glycosylation, oxidation of sulfhydryl bonds, or amino acid substitutions. It is obviously important to determine the nature of such putative alterations which lead to the formation and accumulation of faulty enzyme molecules in ageing animals.

In this communication we report that no charge differences could be found between 'young' and 'old' nematode aldolase and rat liver SOD when the high resolution isoelectric focusing technique was used.

Abbreviations: NA, Nematode fructose-1,6-diphosphatase aldolase; SOD, superoxide dismutase; RMA, rabbit muscle aldolase

2. Materials and methods

2.1. *Enzymes*

Fructose-1,6-diphosphate aldolase (NA) was purified from young (7 days) and old (35 days) nematodes by a method described [6]. SOD was purified from livers of young (8 months) and old (32 months) rats as described [3]. The purified aldolase from young nematodes had spec. act. 8.0 units/mg compared to 4.2 units/mg for the enzyme purified from old nematodes. Purified SOD of young rats had a specific activity of 2460 units/mg as compared to 1008 units/mg for the enzyme from liver of old rats. Rabbit muscle aldolase (RMA) was purified as described [7].

2.2. *Isoelectric focusing*

Focusing was conducted according to the method

of Henrick and Margolis [8] with some modifications. Runs were performed in glass tubes of 2.7 mm inner diameter. Gel solution, 8%, was prepared by adding 0.8 g acrylamide, 42 mg *N,N'*-methyl-bis-acrylamide, 0.1 ml ampholine solution (LKB-Produkter) and 0.05 mg riboflavin to final vol. 10 ml in distilled water. Enzyme samples were added to the gel solutions before polymerization in concentration of 10–20 μg/tube and the gels were photopolymerized for 30 min. The upper (+) electrode solution was made of 0.2% sulfuric acid while the lower electrode (–) solution contained 0.4% triethanolamine solution. Aldolase was focused with ampholines of the pH range 7–9, SOD with ampholines of the pH range 5–7 and RMA with ampholines of the pH range 7–10. Determination of the pH gradient was done by cutting the gels into 5 mm slices, eluting the slices separately overnight in distilled water and measuring the pH in each test tube.

3. Results

Figure 1 shows the isoelectric pattern of aldolase from young nematodes (A), from old nematodes (B) and from mixed 'young' and 'old' samples (C). All gels were stained with Coomassie blue for proteins. Gel A contained about 20 μg 'young' aldolase while gel B contained about 10 μg 'old' aldolase. In gel C a mixture of 10 μg each of A and B were run. It can be seen that only a single protein band was observed in both 'young' and 'old' aldolases with an isoelectric point of 7.10. No additional bands were observed in gel C.

Figure 2 shows two identical SOD isozymes for 'young' enzyme (A,B), 'old' enzyme (C,D) and a mixture of both (E). Gels A, C and E contained 40 μg enzyme and were stained for protein with Coomassie blue. Gels B and D contained 0.2 μg enzyme and were stained for SOD activity [9]. The isoelectric point of the upper band is 5.37 and that of the lower band is 5.78 (fig.3). Nematode aldolase is focused at the acidic end of the pH 7–9 gradient used in fig.1. In order to see whether there are more additional acidic proteins present in the aldolase preparation, the enzyme was focused on gel using the 5–7 pH range. Figure 3 shows the presence of only a single aldolase band in the lower pH range also. Electrofocusing of rabbit muscle aldolase revealed the presence

Fig.1. Isoelectric focusing patterns of aldolase from young and old nematodes. (A) 'young' aldolase. (B) 'old' aldolase. (C) mixed 'young' and 'old' aldolases. pH range 7 (upper end) –9 (lower end).

of five differently charged isozymes, as shown in fig.4. We have used RMA as a control to indicate the high resolution power of this technique. Nematode aldolase has a much lower isoelectric point than rabbit muscle aldolase.

4. Discussion

The isoelectric focusing technique employed by us is very sensitive. This is shown by the ability to separate the five isozymic forms of RMA which differ from each other by one charge due to deamidation of one asparigine residue per subunit [10]. This technique has failed to reveal any differences between purified aldolase and SOD from young and old animals. Our

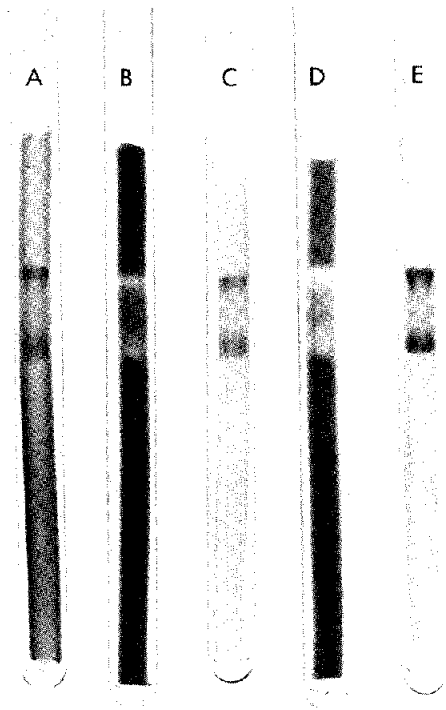

Fig.2. Isoelectric focusing pattern of rat liver SOD from young and old animals. (A) 'young' SOD stained for protein. (B) 'young' SOD stained for activity. (C) 'old' SOD stained for protein. (D) 'old' SOD stained for activity. (E) mixture of 'young' and 'old' SOD stained for protein. pH range 5 (upper end) – 7 (lower end).

findings provide firm evidence that age-related alterations, which cause the reduction in specific activity (48% reduction for aldolase and 60% reduction for SOD) and changes in temperature sensitivity of these enzymes, are not due to post-translational modifications which involve charge differences such as phosphorylation, deamidation, carbamylation, adenylylation, acetylation and glycosylation. Even more significantly

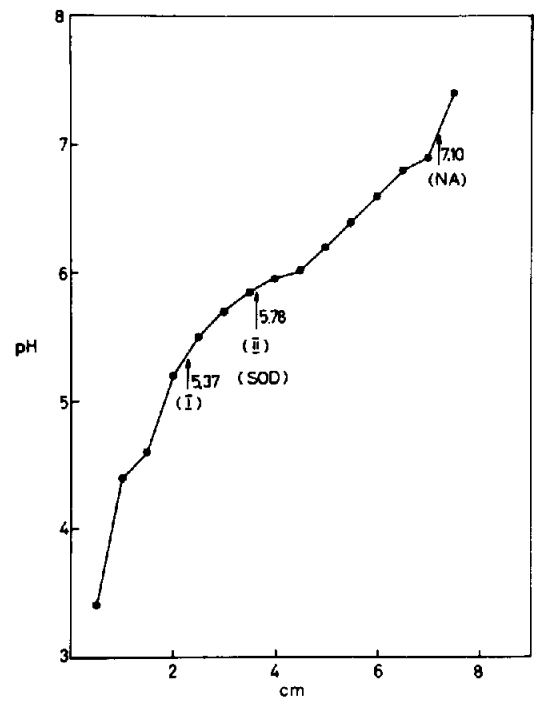

Fig.3. Isoelectric points of SOD isozymes and nematode aldolase. Duplicate gels were focused using pH range 5–7, one gel was stained for protein; the other was sliced to determine pH.

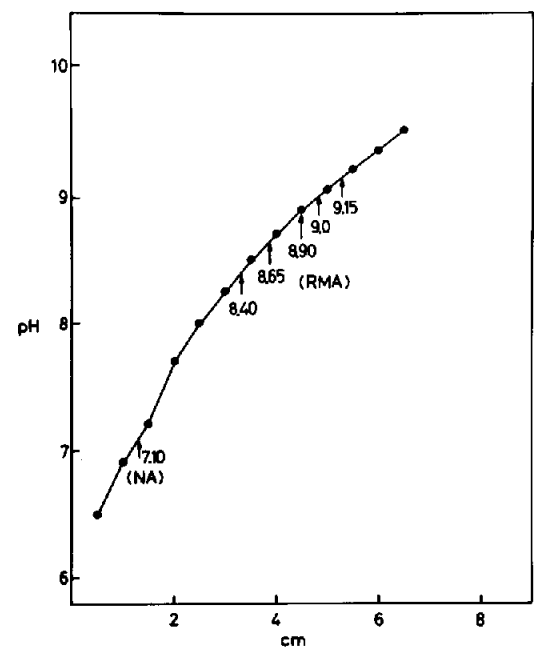

Fig.4. Isoelectric points of nematode aldolase and of rabbit muscle aldolase isozymes. Duplicate gels were focused using pH range 7–10, one gel was stained for protein; the other was sliced to determine pH.

our findings make it unlikely that random amino acid substitutions resulting from errors in synthesis are of major importance in the age-related changes of enzyme properties. This latter mechanism was postulated as a major cause of ageing of organisms [11,12] and has been widely acclaimed by gerontologists. Such random substitution would inevitably cause charge differences when amino acid residues differing in charge are substituted one for another. It is thus suggested by us that age-related alterations in enzymes are probably of a post-translational origin which do not involve charge differences.

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References

[1] Gershon, D., Gershon, H., Jacobus, S., Reiss, U. and Reznick, A. (1976) in: Metabolic Interconversion of Enzymes (Shaltiel, S. ed) pp. 227–232, Springer Verlag, Berlin.
[2] Reznick, A. Z. and Gershon, D. (1977) Mech. Age. Dev. 6, 345–353.
[3] Reiss, U. and Gershon, D. (1976) Eur. J. Biochem. 63, 617–623.
[4] Reiss, U. and Gershon, D. (1976) Biochem. Biophys. Res. Commun. 73, 255–262.
[5] Rothstein, M. (1975) Mech. Age. Dev. 4, 325–338.
[6] Reznick, A. Z. and Gershon, D. (1977) Int. J. Biochem. 8. 53–59.
[7] Gershon, H. and Gershon, D. (1973) Proc. Natl. Acad. Sci. USA 70, 909–913.
[8] Henrick, K. G. and Margolis, J. (1970) Anal. Biochem. 33, 204–207.
[9] Beauchamp, C. D. and Fridovich, I. (1971) Anal. Biochem. 44, 276–278.
[10] Gürtler, B. and Leuthardt, F. (1970) Helvetia Chim. Acta 53, 654–658.
[11] Orgel, L. E. (1963) Proc. Natl. Acad. Sci. USA 49, 517–521.
[12] Orgel, L. E. (1970) Proc. Natl. Acad. Sci. USA 67, 1476.