J. Biosci., Vol. 4, Number 2, June 1982, pp. 191-195.

Kinetic characterization of rat liver nuclear lysozyme

V. SIDHAN and S. GURNANI

Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay 400 085

MS received 12 October 1981; revised 7 April 1982

Abstract. The kinetic properties of rat liver nuclear lysozyme, earlier purified to homogeneity in our laboratory, have been studied. The enzyme was found to be maximally active in the pH range 4.2 to 5.4 in 0.02 M buffer. Its K_m was found to be 333 mg/litre. It was heat sensitive even in the acidic pH range. The enzyme exhibited tissue specific differences when compared with the rat kidney nuclear lysozyme.

Keywords. Nuclear lysozyme; characterization; tissue specific differences; pH optimum; K_m value.

Introduction

Recently it was shown that multiple forms of rat liver lysozyme were located in the subcellular organelles (Sidhan and Gurnani, 1981). However, only the nuclear enzyme could be purified to electrophoretic homogeneity (Sidhan and Gurnani, 1981). Further, it was shown that rat liver nuclear lysozyme differed from rat kidney nuclear lysozyme not only with respect to its chemical properties but also with respect to its latency. In liver nuclei, lysozyme was found complexed with a protein inhibitor whereas this inhibitor was not present in the nuclear fraction of the kidney homogenate (Sidhan *et al*., 1976). Therefore, it was of interest to examine whether the liver nuclear enzyme was kinetically different from kidney nuclear lysozyme.

Materials and methods

Glucosamine was obtained from Sigma Chemical Company, St. Louis, Missouri, USA; N-acetyl Glucosamine was the product of Kochlight Laboratories, England; Analar grade urea and guanidine hydrochloride were from Fluka (Switzerland); and EDTA was from BDH England.

The enzyme was purified (Sidhan and Gurnani, 1981; 1978) and assayed for activity (Raghunathan and Gurnani, 1971). The heat stability of the enzyme was determined by heating it in 0.2 M acetic acid for 5 min at different temperatures in the range 37°C to 80°C.

The effect of inhibitors, denaturing agents, salts etc., was examined by incorporating these agents in the buffer used for the preparation of the substrate or cell suspension.

Results

The activity of the enzyme was linearly dependent on protein concentration (30-70 µg), buffer concentration (0.0-0.02 M), incubation time of the assay (0.0-30 min) and temperature (25°C-37°C). The K_m of the enzyme calculated from a Lineweaver-Burk plot was 333 mg/litre.

The pH optimum of the enzyme was checked using different buffer systems as shown in figure 1. The enzyme attained maximum activity at pH 5.4 in phosphate

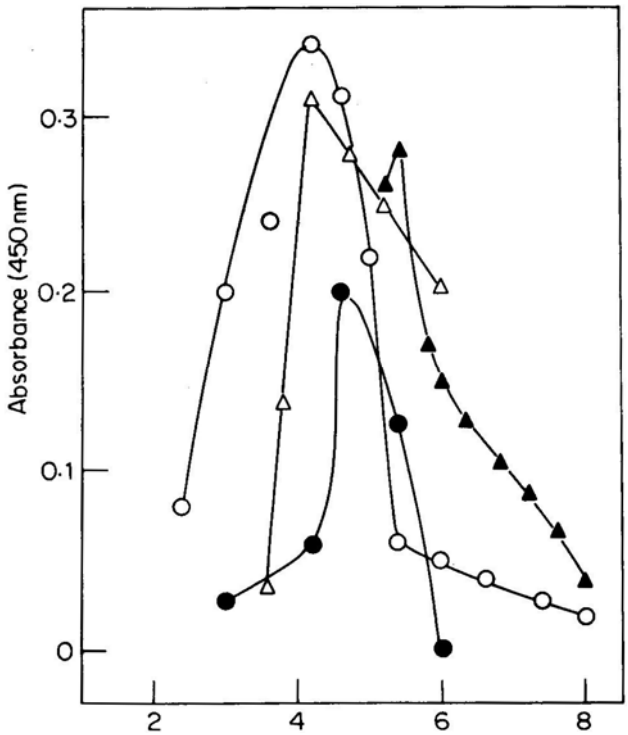

Figure 1. pH-activity profile of the rat liver nuclear lysozyme in different buffers. In each case the activity was measured at different pH values keeping the same buffer concentration (0.02 M) for 10 min at 37°C. The enzyme concentration used is indicated in parenthesis.

Sodium phosphate buffer, (76 µg, ▲);
Sodium phosphate-citric acid buffer, (78 µg, O);
Sodium acetate buffer, (80 µg, Δ); and
Sodium citrate-citric acid buffer, (78 µg, ●)

buffer. With acetate buffer and phosphate-citrate buffer, it showed a pH optimum of 4.2. Using sodium citrate-citric acid buffer, its maximum activity was observed at pH 4.4. When the effect of buffer concentration was examined at pH 5.4, it gave maximum activity at a buffer concentration of 0.02 M.

The optimum temperature required for the maximum activity was 37°C. With further increase in temperature, the activity of the enzyme decreased. At 80°C

only 25% of its original activity remained. The enzyme was found to be heat sensitive under acidic as well as alkaline conditions. In acidic conditions (see experimental section) at 80°C, it showed only 20% activity as compared to that observed at 37° whereas under alkaline conditions, it was inactivated completely within 2 min (Sidhan and Gurnani, 1978).

Glucosamine was a more potent inhibitor of the liver nuclear enzyme than N-acetyl-glucosamine (figure 2). At a concentration of 0.05 M, inhibition due to N-acetyl-glucosamine was 25 per cent, whereas that with Glucosamine was 80 per cent. Since Glucosamine was used in its hydrochloride form, the effect of

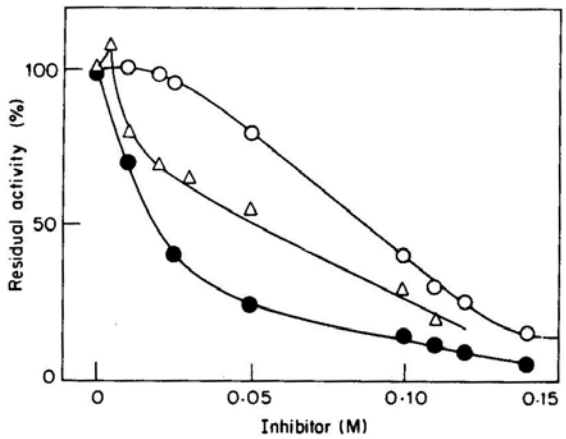

Figure 2. The effect of inhibitors on the activity of rat liver nuclear lysozyme. The assay was carried out at 37°C for 10 min in 0.02 M sodium phosphate buffer, pH 5.4 using 75 µg enzyme. NAGA (O); GA (●); NaCl, (Δ).

different concentrations of sodium chloride, on the activity of the enzyme was checked to eliminate the possibility of inhibition due to the salt. It is quite clear from the results shown in figure 2 that apart from inhibition due to the salt, there was additional inhibition (25%) caused by glucosamine, at a corresponding molar concentration.

Protein denaturing agents like urea and guanidine-HCl were also studied for their denaturing action on liver nuclear lysozyme. Guanidine-HCl, at lower concentration (0.01 M) produced a slight activation. With further increase in concentration, the activity decreased progressively. Complete inactivation was observed at 0.4 M guanidine-HCl (figure 3a). With urea, 70 per cent inhibition was observed at a concentration of 3 M (figure 3b).

Discussion

The kinetic properties of the homogeneous rat liver nuclear lysozyme showed some similarities as well as some differences when compared to the kidney nuclear enzyme. The K_m of the liver nuclear lysozyme was high having a value of 333 mg/ litre as compared to that of kidney nuclear enzyme which was 200 mg/litre (Raghunathan and Gurnani, 1975).

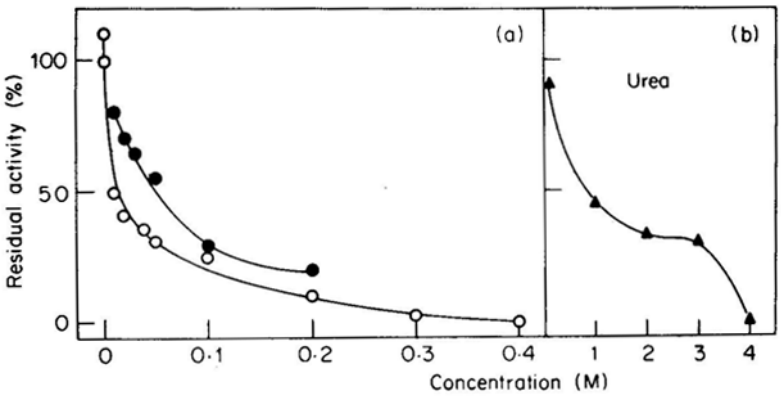

Figure 3a. Effect of guanidine-HCl and NaCl on the activity of rat liver nuclear lysozyme. Guanidine-HCl and NaCl were incorporated along with the substrate in the assay system containing 0.02 M sodium phosphate buffer pH 5.4, enzyme 75 µg, incubated at 37^{0}C for 10 min.
Guanidine-HCl, (O); NaCl (●).

Figure 3b. Effect of urea on the activity of rat liver nuclear lysozyme.

The pH optima and the effect of buffer molarity on the activity of the liver nuclear lysozyme also differed from those of the kidney nuclear lysozyme (pH 6.2 and 9.2 in 0.06 M), the former was maximally active at low buffer molarity (0.02 M), was active in the acidic pHrange4.2 and 5.4 and did not show a second pH optimum in the alkaline region (Raghunathan and Gurnani, 1971; 1972). There was a slight shift in the pH optimum with different buffer systems suggesting the influence of ionic species in the buffer on the activity. The liver nuclear lysozyme also shared some of these features with fish, T_4 and papaya lysozymes with respect to the requirement of low buffer concentration and low pH for its optimum activity (Sankaran and Gurnani, 1972; Jenson and Kleppe, 1972; Haward and Glazer, 1969).

Like lysozymes from other sources (Imoto *et al.,* 1972), liver nuclear lysozyme was maximally active at 37°C and was rapidly inactivated on heating and differed from the kidney nuclear lysozyme in being heat sensitive under acidic conditions.

The response of the liver nuclear lysozyme to the inhibitors, glucosamine and N-acetyl glucosamine, the known competitive inhibitors of lysozyme, was comparable to their effect on kidney nuclear lysozyme (Raghunathan and Gurnani, 1975). However, in the case of liver nuclear lysozyme, the former was more inhibitory than the latter; the opposite was true with the kidney nuclear lysozyme. At low concentrations, sodium chloride had an activating effect on the liver nuclear enzyme, but with increase in concentration there was progressive inhibition, similar to that seen in the case of the kidney nuclear lysozyme.

The effect of denaturing agents on the activity of the liver nuclear lysozyme appeared to suggest that guanidine hydrochloride was a more powerful denaturing agent than urea, as was earlier observed in the case of the kidney nuclear lysozyme (Raghunathan and Gurnani, 1975). The loss of activity of hen egg white lysozyme in a low concentration of guanidine-hydrochloride was shown to be due to the effect of ionic strength (Altekar and Gurnani, 1972). In this respect, the liver nuclear lysozyme responded like the kidney nuclear enzyme and hen egg white lysozyme.

Thus the investigation of the kinetics of rat liver nuclear lysozyme further confirms the tissue specific differences between rat liver nuclear lysozyme and rat kidney nuclear lysozyme. The differences in their physiochemical properties have been reported elsewhere (Sidhan and Gurnani, 1978).

References

Altekar, W., and Gurnani, S. (1972) *Indian J. Biochem. Biophys.,* **9,** 293.
Haward, J. B., and Glazer, A. N. (1969).*J. Biol. Chem.,* **244**,1399.
Imoto, T., Johnson, L. N., North, A. C. T., Philips, D. C, and Rupley, J. A. (1972) *The Enzymes* ed. P. D. Boyer, **7,** 665.
Jensen, H. B., and Kleppe, K. (1972) *Eur. J. Biochem.,* **28,** 116.
Raghunathan, R., and Gurnani, S. (1971) *Arch. Biochem. Biophys.,* **147,** 527.
Raghunathan, R., and Gurnani, S. (1972) *Indian J. Biochem. Biophys.,* **9**,166.
Raghunathan, R. and Gurnani, S. (1975). *Indian J. Biochem., Biophys.* **12**,13.
Sankaran, K. and Gurnani, S. (1972). *Indian J. Biochem., Biophys.,* **9,** 162.
Sidhan, V., Raghunathan, R., and Gurnani, S. (1976) *Indian J. Biochem. Biophys.,* **13,** 96.
Sidhan, V., and Gurnani, S. (1978) *Indian J. Biochem., Biophys.,* **15,** 273.
Sidhan, V., and Gurnani, S. (1981) *Agri. Biol. Chem.,* **45**,1817.