A NOVEL METHOD FOR DETERMINATION OF DEOXYRIBONUCLEASE AND DEOXYRIBONUCLEASE INHIBITOR

TOMOYOSHI YANAGIDA, HIROSHI KANEKO, KAZUHIKO KAWAUCHI and HIROSHI OGAWARA
Second Department of Biochemistry, Meiji College of Pharmacy, Nozawa-1, Setagaya-ku, Tokyo 154, Japan
(Received for publication October 27, 1981)

Deoxyribonucleases (DNase) are known to have crucial roles in recombination, replication and repair systems. However, the detailed mechanism of their function has not been elucidated as yet. One strategy to gain further insight into the mechanism they display is to use specific DNase inhibitors.

Recently, ROSENTHAL and LACKS1) described a method for the detection of nucleases separated in acrylamide gels containing nucleic acid and sodium dodecyl sulfate. On the basis of this principle, we devised an agar plate method for the determination of DNase and DNase inhibitors. Screening DNase inhibitors by this method, we found that a Micromonospora strain produced an inhibitor in broth filtrate. The following paper describes the properties of the inhibitor.

Fig. 1 shows the effect of DNase I concentration on the hydrolysis of calf thymus DNA (Worthington Biochemical Co.). When the diameter of the zone size was plotted against the logarithm of the concentration of DNase, a straight line was obtained over a wide range of DNase concentration (about 25 to 3,200 µg/ml). The size of the zone depended on the incubation time; thus, the longer the incubation period, the greater the zone size.

A similar relationship was observed with endonucleases such as porcine spleen DNase II and DNases from various Streptomyces strains2), and exonucleases such as bovine spleen phosphodiesterase II (PL-Biochemicals, Inc.). However, in these cases the amount of enzyme needed to give a zone of similar size was much greater. Unexpectedly, although the optimum pH for DNase II has been reported to be 4.6, zone size was larger at pH 7.6 than at pH 4.6. The same finding was obtained using DNase I. The diameter is larger at pH 7.6 than at pH 7.0 (optimum pH). This may be due to differences in diffusion rate of DNases with pH.

Up to the present time, there has been no report describing a specific DNase inhibitor of low molecular weight that interacts strongly with the enzyme itself. On the other hand, specific inhibitors that were proteins in nature have been isolated from serum3), liver4), Actinomyces5), Aspergillus6) and other sources. Thus, we probed the assay method for a DNase inhibitor using rat serum as a standard. Agar plate method for the

Fig. 1. The effect of DNase concentrations on the hydrolysis of DNA by DNase I.

To 100 ml of 2 mM MgCl₂ and molten agar (2%) in 0.04 M tris-HCl of pH 7.6, 0.8 ml of DNA solution (0.125%) was added at 40°C, mixed well and 10 ml were poured into Petri dishes (80 mm diameter). Paper discs (8 mm diameter) containing 20 µl of DNase solutions of various concentrations were placed on the agar surface, maintained at 5°C for 1 hour and incubated overnight at 27°C. Then, 1 to 2 ml of an ethidium bromide solution (1 mg/ml) was poured onto the surface and the clear area formed as a result of hydrolysis of DNA by DNase was detected under ultraviolet light.

The concentrations of DNase solutions were, turning clockwise from the upper top, 500, 250, 100, 50, 25 and 12.5 µg/ml, respectively.
Fig. 2. Inhibition of DNase I activity by rat serum.

A: Inhibition of DNase was determined using paper discs containing serum diluted serially indicated in the figure. The discs were put on the upper dots and the bar indicated the position of the DNase strip.

B: DNase inhibition by paper strip method. The dots indicated the edges of the strips containing test solutions and the bar indicated the DNase strip.

Recently, 2-nitro-5-thiocyanobenzoic acid was reported to inhibit DNase I specifically by cleaving the peptide bonds on the amino-terminal side of serine and threonine residues. However, this reagent exhibited only weak activity as measured by a spectrophotometric method, since prolonged treatment of DNase I with high concentrations of the reagent was necessary. This also affected the agar plate method. No inhibitory zone could be detected by the method described. However, the zone formed by hydrolysis of the DNA by DNase I decreased with increasing reaction time of DNase I with 2-nitro-5-thiocyanobenzoic acid (data not shown). Actinomycin D, on the other hand, which is known to inhibit DNase activity by forming complex with DNA, did not show inhibition of DNase I on the agar plate.

Thus, although this method is not completely quantitative, it can be used to determine the inhibitory activity of a test sample semi-quantitatively by means of serial dilution. It may be applicable for screening inhibitors against not only endonucleases but also exonucleases. In addition, it may also be useful for detection of substances that interact with RNase and histone, because these proteins can be detected in a similar way on an agar plate as well as on an acrylamide gel.

Acknowledgments

We express our great thanks to Prof. H. Umezawa for his hearty encouragement throughout the present work. This work is supported by the Institute of Microbial Chemistry and a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

References