A NEW PROCEDURE FOR THE SOLUBILIZATION OF BEEF SPLEEN DIPHOSPHOPYRIDINE NUCLEOTIDASE

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Received November 21, 1960

Diphosphopyridine nucleotidases (DPNases) of mammalian origin have been found, with one exception, (Leone and Bonaduce, 1959) to be tightly bound to the cell structure and resistant to the usual methods of solubilization. In 1956 Alivisatos and Woolley published a method for the solubilization of beef spleen DPNase. The method involves homogenization, repeated washing, and extraction of the suspended precipitate with an equal volume of isoamyl alcohol in presence of DNA. The procedure resulted in a 13 % yield of soluble enzyme, calculated on the basis of activity present in the washed suspension. The specific activity of the soluble enzyme is 3.9 (Folin method).

In a recent publication Alivisatos et al. (1960) described a modification of the above method in which the yield was increased to 28 % by repeated extractions.

We would like to report a new procedure which has been developed in this laboratory and which is simple, time-saving and highly reproducible and yields more than 50 % soluble enzyme. The following details apply to model experiments carried out with 50 g of beef spleen pulp.

The beef spleen is freed from connective tissue and macerated. The pulp is stirred at 37° C with ca. 80 ml 0.25 % trypsin (DIFCO TRYPSIN 1:250) dissolved in 0.055 M phosphate buffer, pH 7.25, containing 0.077 M NaCl. The tryptic digestion was carried out in the Dostal modification (1957) of the apparatus described by Rappaport (1956) for tryptic degradation of monkey kidney. The modified apparatus is not the continuous flow-type described by Rappaport, but one which permits discontinuous removal of digest. The diameter of the inner beaker is 6.7 cm whereas the diameter of the outer beaker is 7.6 cm. The digest is sucked off every hour from the outer beaker and sufficient trypsin solution is added to keep the pulp covered. After 2.5 hours total digestion time, the combined digests (250 ml) are centrifuged[x)] for 1 hour. The sedimented material is suspended in 200 ml 0.055 M phosphate buffer, containing 0.077 M NaCl, pH 7.25, stirred for 1 hour and finally centrifuged for 30 minutes. To obtain cytolysis, the residue is suspended in 130 ml of 0.022 M phosphate buffer, pH 7.25, and stirred for 60 minutes. After centrifugation for 45 minutes no activity is found in the supernatant. The precipitate, in which 80 - 85 % of the activity of the pulp is found, is suspended in approximately 100 ml of 0.11 M phosphate buffer, pH 7.25 (preparation P). The mixture is stirred for 2 hours with 1 gram DNA (DNA ex herring, Light and Co.) per gram protein and then 22 ml pre-cooled isoamyl alcohol (MERCK, p.A., redistilled) per 100 ml suspension, containing about 10,000 units (1 unit splits 1 µmole DPN^+ in 1 hour at 37° C) are added dropwise. After stirring vigorously for 60 minutes at

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x) All centrifugations were carried out at 17,000 x g and 0° C unless otherwise indicated.

5° C and centrifuging 90 minutes, the upper (isoamyl alcohol) and middle (debris) layers are carefully separated and discarded. The watery lower phase is dialysed in a rocking apparatus against flowing 0.013 M phosphate buffer, pH 7.25, (1 liter/hour) for 24 hours. The dialysed solution is centrifuged for 2 hours. The clear supernatant (with a specific activity of 9.4) contains 55 % of the activity of the washed precipitate (preparation P).

The preparation is fairly stable at 0° C and for at least two weeks at -15° C. After centrifugation at 37,500 rpm (average 92,660 x g) for 60 minutes (Spinco L) all of the activity remained in the supernatant. The soluble enzyme transfers the adenosinediphosphate ribose moiety of DPN^+ to acetylpyridine forming the acetylpyridine analogue. Almost no activity was found after incubating the soluble preparation for 2 hours at 35° C with a 0.25 % trypsin solution in 0.067 M phosphate buffer, pH 7.25. The fact that inactivation of the enzyme by tryptic digestion was observed only after the cytolysis step has led us to investigate which cell type in the spleen carries DPNase activity. The additional work on this enzyme will be published in the near future.

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