ISOLATION AND PURIFICATION OF ACID PROTEASE FROM MOUSE FIBROBLASTS L-929

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Purification of a kinin-forming acid protease identified in mouse fibroblasts grown in stationary cell culture (Back, N., & Steger, R., Proc. Soc. Exp. Biol. & Med. 143: 769, 1973) was undertaken. The fibroblast cell line L-929 was cultured in Spinner flasks with Minimal Essential Media containing 10% fetal calf serum and 0.4% lactalbumin. The cells (10^9) were homogenized for 8 minutes, dialyzed for 18 hours against 0.01M Na_2PO_4 buffer (pH 6.8), 0.1M NaCl and 1mMEDTA, and centrifuged at 12,000 rpm for 45 minutes. The supernatant digested denatured hemoglobin when incubated for 1 hour at pH 4.0. The supernatant (57mg) was fractionated on a G-200 Sephadex column (2.5x90cm) and kinin-forming acid protease activity identified in fractions 25-40 when incubated with rat plasma for 24 hours at pH 4.0 and assayed on the isolated rat uterus. The active fractions were purified 3 fold on a hydroxylapatite column (2.0x30cm), and after treatment with cysteine the activity increased 5 fold. A final purification was carried out on a DEAE-A50 Sephadex ion exchange column (0.9x20cm). The eluant was 0.01M K_2PO4 buffer (pH 6.8), 0.1M KCl and 2.5mM mercaptoethanol. The purification factor was 9.35 with a 13.8% yield and a specific activity of 2063ng kinin per mg protein.
The substrate from rat plasma also was purified on a DEAE-A50 Sephadex ion exchange column (2.5x35cm). The substrate was assayed in a mixture of 0.1 ml (1.2 mg/ml) G-200 Sephadex-purified acid protease, 0.1 ml SBTI (10mg/ml), 0.2 ml 0.56M acetate buffer (pH 4.0) incubated for 18 hours. Kininogen was found in fractions 25-45 (peak I) and 55-65 (peak II). The two peaks were combined and eluted from a G-100 Sephadex column (2.5x90cm). Two peaks emerged with the major peak having been purified 3.9 fold. The DEAE-A50 Sephadex purified enzyme and substrate were used to establish the pH optimum of enzyme activity at 3.8. The molecular weights of the purified enzyme and substrate estimated on a G-200 Sephadex column (1x57cm) were 39,000 and 110,000 respectively. The acid protease activity was localized primarily in the 10,000 g supernatant cell fraction. While the 1500 g precipated fraction also exhibited activity, the 10,000 g precipitate did not contain any enzyme activity. (Supported by USPHS grant HE-11492-07).