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ADENOSINE DEAMINASE INHIBITORS: DIFFERENTIAL EFFECTS ON MULTIPLE FORMS OF ADENOSINE DEAMINASE

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SUMMARY

Three forms of calf intestine adenosine deaminase were resolved by gel filtrat in Sephacryl C-200 into species of 38,000 (E_S), 57,000 (E_I) and greater than 200,00 (E_L) daltons. The sensitivities of the three forms of adenosine deaminase to the adenosine deaminase inhibitors, 2'-deoxycoformycin (dCF) and erythro-9-(2-hydroxy-3 nonyl)adenine (EHNA) were measured. The concentration of inhibitor producing 50% i hibition of E_L, E_I, and E_S was 2×10^{-8} M, 8×10^{-11} M and 2×10^{-11} M, respectivel for dCF and 1×10^{-7} M, 3×10^{-10} M and 2×10^{-11} M, respectively, for EHNA. Thus the E_S and E_I forms of adenosine deaminase could be distinguished from the E_L form the basis of their respective sensitivities to dCF and EHNA.

INTRODUCTION

Adenosine deaminase (E.C. 3.5.4.4) catalyzes the hydrolytic deamination of ade sine to inosine and ammonia. This enzyme is responsible for the inactivation of se antineoplastic agents which are adenosine analogs (1-4). In addition, the absence adenosine deaminase in lymphocytes and other tissues is associated with one form o the clinical syndrome, severe combined immunodeficiency disease (5).

Multiple forms of adenosine deaminase have been reported in amphibian, avian mammalian tissues as demonstrated by ion exchange chromatography, starch gel electr phoresis, gel filtration, and antibody affinity chromatography (6-12). Three majo isoenzymes each containing additional variants have been described in human tissue with molecular weights of 33,000-47,000, 114,000 and 230,000-440,000 (6, 8, 13-16) The major form of adenosine deaminase found in spleen, stomach, intestine, and eryt cytes is that of the small molecular weight variant, while lung, liver and kidney tain the large form (6, 16-21). In addition, there is some evidence that the smal

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nd large molecular weight forms of adenosine deaminase are interconvertible via the ction of a "conversion factor" (16, 17) although the two forms of adenosine deaminase ave similar kinetic properties (6, 16, 18-21).

The recently developed and highly potent adenosine deaminase inhibitors, 2'-deoxy-oformycin (dCF) (22) and erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (23) have become f interest because of their utility in potentiating some anticancer agents which are denosine analogs (1, 24-27) and because of their value in mimicking the pathologic defect f adenosine deaminase deficiency in severe immunodeficiency disease (28-32). Therefore, t was of interest to ascertain if multiple forms of adenosine deaminase displayed ifferences in their relative sensitivities to dCF and EHNA as a possible means of dis-riminating between two or more forms of the enzyme.

ATERIALS AND METHODS

Materials. Calf intestine adenosine deaminase was purchased from Boehringer annheim Co. Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) and 2'-deoxycoformycin (dCF) ere kindly provided by Dr. Harry B. Wood Jr., Drug Synthesis and Development Branch, ational Cancer Institute.

Chromatography. Adenosine deaminase (6,000 units) was applied to a column (2.5 x 0 cm) of Sephacryl C-200 equilibrated with 10 mM potassium phosphate (pH 7.4) - 25% lycerol. Adenosine deaminase activities were eluted with the same buffer and collected n 5 ml fractions. Molecular weight standards were: bovine serum albumin (67,000), valbumin (43,000), chymotrypsinogen (25,000), ribonuclease A (13,700) and cytochrome (12,500).

Assay. Adenosine deaminase activity was measured spectrophotometrically at 265 nm n an assay mixture (final volume 1.0 ml) containing: 0.1 mM adenosine, 15 mM potassium osphate (pH 7.4), 1.25% glycerol and 0.05 ml of enzyme. One unit of activity repre-ents the deamination of one μmole of adenosine per min at 37°.

Protein was measured by the method of Bradford (33) using bovine serum albumin as standard.

ESULTS AND DISCUSSION

Gel filtration chromatography of calf intestine adenosine deaminase resulted in e resolution of three molecular species (Fig. 1). Their molecular weights rresponded to 38,000 (E_S), 57,000 (E_I) and greater than 200,000 (E_L) daltons. e specific activities for E_L, E_I and E_S were 133, 456, 985 units/mg protein, spectively.

To assess the degree of sensitivity of the unchromatographed enzyme mixture and e three forms of adenosine deaminase to dCF and EHNA, equivalent enzyme activity

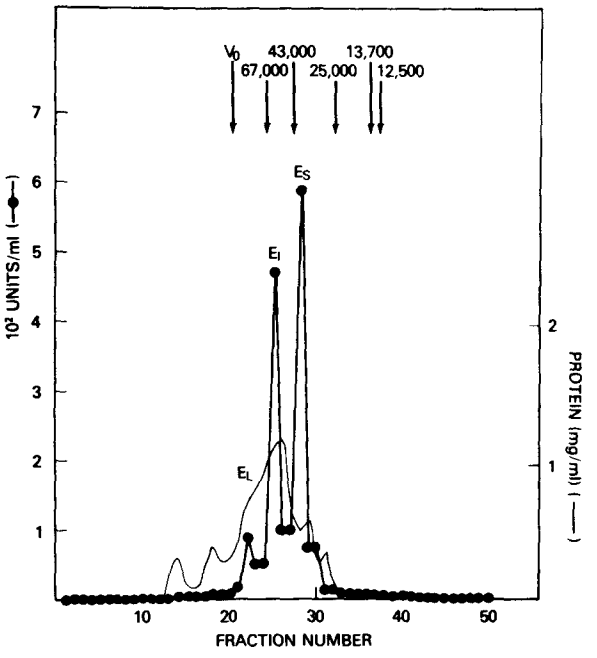
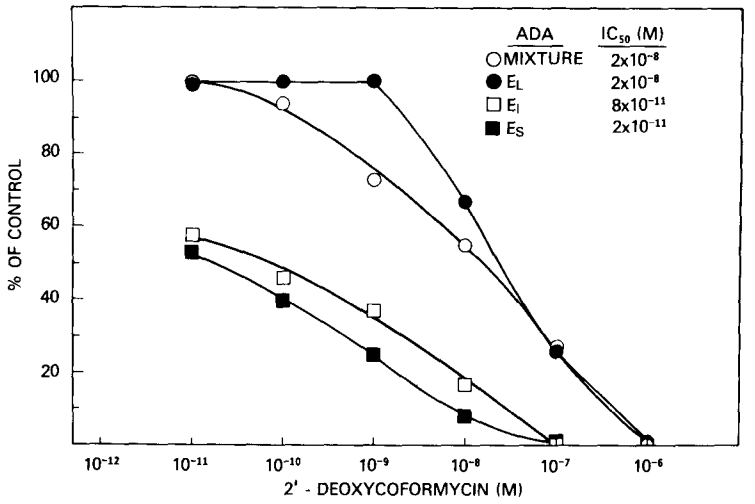


Figure 1. Molecular heterogeneity of calf intestine adenosine deaminase.

units of the enzyme mixture and E_L, E_I and E_S were preincubated for 10 min at 37° with varying concentrations of each inhibitor (Fig. 2). The median inhibitory concentration (IC_{50}) for dCF for the enzyme mixture and E_L, E_I and E_S was 2×10^{-8} M, 2×10^{-8} M, 8×10^{-11} M and 2×10^{-11} M, respectively, while the IC_{50} for EHNA for the same forms of adenosine deaminase was 6×10^{-7} M, 1×10^{-7} M, 3×10^{-10} M and 2×10^{-11} M, respectively. Thus, the sensitivity of E_L was similar to that of the unchromatographed mixture of adenosine deaminase, whereas E_I and E_S were 150-250 a 1,000-2,000 times more sensitive, respectively, than E_L to the two drugs.

The binding characteristics of dCF and EHNA for adenosine deaminase are marke different (22-27). dCF is a tight binding inhibitor whereas the effects of EHNA a easily reversible. However, the similarities in the IC_{50} between dCF and EHNA for E_I and E_S suggest that the greater sensitivities of these species of adenosine deaminase in comparison to E_L are due to intrinsic differences in their binding si from the binding site in E_L rather than differences in the binding kinetics of the inhibitors for the three forms of the enzyme.



gure 2. Inhibition of adenosine deaminase by dCF and EHNA. Assays were carried out by preincubation of enzyme with varying concentrations of each inhibitor for 10 min in a reaction mixture containing in 0.9 ml: 10 mM potassium phosphate (pH 7.4), 1.4% glycerol, inhibitor and 0.03 units of enzyme. Adenosine deaminase activity was measured after the addition of 0.1 mM adenosine (in a final assay volume of 1.0 ml). Results are expressed as a percent of control values without added inhibitor. Each value is the mean of triplicate determinations.

The findings of this study of a differential effect between two adenosine deaminase inhibitors on the E_L and E_S or E_I forms of adenosine deaminase provides a sensitive pharmacological tool for discriminating between the small and large molecualr weight variants of this enzyme in normal and pathological tissues, and may also explain the differential toxicity to lymphoid tissues by dCF (34) where the E_S form of the enzyme predominates.

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