PRELIMINARY NOTES

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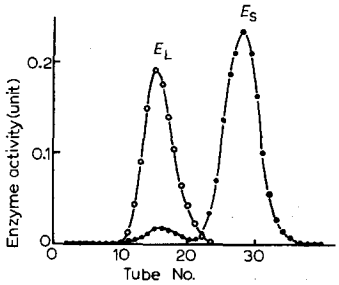
Adenosine deaminases of two different molecular sizes in human tissues

Although multiple forms of adenosine deaminase have been demonstrated on the basis of its behavior on electrophoresis[1-3] and ion-exchange column chromatography[4,5], the molecular properties of this particular enzyme in relation to its physiological function have not yet been clarified. We recently found in human tissues two species of the deaminase which differ from each other in molecular size when tested by gel filtration. During the course of this study MA AND FISHER[6-8] (personal communication) reported similar findings on the deaminase in the livers of a wide variety of amphibians and mammals.

Human tissue specimens obtained by either operation or autopsy were homogenized in 20 mM phosphate buffer (pH 7.0) containing 2 mM 2-mercaptoethanol, using a Waring Blendor. The homogenate was centrifuged at 105 000 $\times$ g for 90 min and the supernatant applied to a Sephadex column which had previously been equilibrated and was thereafter eluted with the same buffer as described above. The enzymic activity was measured either colorimetrically according to the method of MARTINEK[9] or spectrophotometrically by the method of KAPLAN[10]. Analytical and preparative electrophoresis was carried out for 2–6 h at a constant current of 7 mA/cm^2 at 4° with a 5% polyacrylamide gel plate in Tris–borate buffer, I = 0.05, pH 8.6. In preparative electrophoresis, the enzyme was eluted with the same phosphate buffer after slicing the gel into segments.

Fig. 1 shows a typical gel filtration pattern of adenosine deaminase of larger molecular size (E_L) and of smaller molecular size (E_S) in normal human lung and stomach tissue. The distribution of E_L and E_S in other human tissues was investigated and the results so far obtained indicated that normal lung, liver and serum preferentially contained E_L, whereas normal and leukemic leucocytes contained only E_S. In contrast, stomach tissue, either normal or cancerous, and lung cancer tissue demonstrated both types of the deaminase.

In order to compare physicochemical properties of the two deaminases, E_L from normal lung and E_S from normal stomach were purified by procedures including $(NH_4)_2SO_4$ fractionation, DEAE-cellulose and Sephadex G-200 column chromatography, and preparative electrophoresis on polyacrylamide gel. E_L and E_S were purified about 2000- and 1000-fold, respectively, by these procedures with yields of 1–3%. The final preparations exhibited a single protein band corresponding to the enzymic activity upon analytical electrophoresis. The molecular sizes of the purified enzymes estimated using a calibrated Sephadex G-200 column were approx. $2 \cdot 10^5$ for E_L and $5 \cdot 10^4$ for E_S, respectively. Studies on substrate specificity with respect to adenosine and deoxyadenosine revealed no apparent difference between the two enzymes, suggesting that E_L and E_S are very similar to the Type A and Type C of adenosine deaminase, respectively, reported by MA AND FISHER. Other catalytic properties in terms of K_m values, pH activity profiles and effects of various inhibitors such as substrate analogues and p-chloromercuriphenylsulfonic acid also indicated no remarkable difference between the two enzymes. The antiserum obtained from



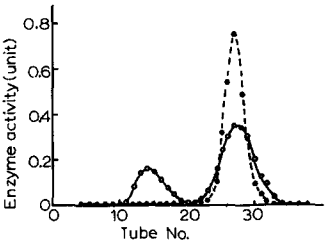


Fig. 1. Fractionation of adenosine deaminase in human lung and stomach on Sephadex G-200. 1 ml of soluble fractions of the tissue homogenates was applied to a Sephadex G-200 column (1.6 cm × 24 cm). 1-ml fractions were collected. ○—○, normal lung; ●—●, normal stomach. An unit of enzyme activity was defined as the activity catalyzing the liberation of 1 μmole NH_3–N from adenosine per min.

Fig. 2. Elution profile of E_S previously incubated with the conversion factor from normal lung. ○—○, partially purified E_S (0.05 ml, 77 μg protein) was incubated at room temperature for 1 h with the conversion factor (0.95 ml, 24 mg protein) partially purified by $(NH_4)_2SO_4$ and acetone fractionation. ●— — —●, as a control E_S was incubated with bovine serum albumin (1 mg/ml). The mixture (0.5 ml) was applied to a Sephadex G-200 column (1.6 cm × 24 cm). 1-ml fractions were collected.

rabbit or guinea pig immunized with either E_L or E_S neutralized the enzymic activity of the other.

Incubation of the purified E_L preparation with 2.5 M guanidine sulfate resulted in the disappearance of its activity in the fraction corresponding to E_L with a concomitant appearance of the activity in the elution position of E_S upon Sephadex G-200 gel fractionation. In addition, E_L thus treated with guanidine recovered its activity in the E_L fraction upon dialysis against phosphate buffer, followed by concentration. These results indicate that E_L and E_S are interconvertible. Two possible interpretations of the structural relationship between E_L and E_S may be considered: (a) E_L is a polymeric form of the active subunit, E_S, and (b) E_L is a complex of E_S and some other protein molecule(s). In the latter, E_L in the soluble fraction of tissues could be a kind of functional unit in the form of an enzyme complex.

Although the molecular size of E_S did not significantly change during the course of purification from stomach, we found that incubation of purified E_S with the soluble fraction of normal lung homogenate resulted in the enlargement of the molecular size of E_S to that of E_L. The factor, presumably present in normal lung, which promotes an apparent conversion of E_S to E_L, was inactivated by heating at 80° for 10 min and was nondialyzable. The factor was purified free from adenosine deaminase activity due to E_L in the lung by procedures of ammonium sulfate and acetone fractionation and Sephadex G-200 column chromatography. Fig. 2 shows the elution profile of E_S previously incubated with the factor. The specific converting activity (defined as percent ability to convert E_S to E_L per mg of protein) of the final preparation was increased more than 50-fold by the procedures. The physicochemical nature and the physiological role of the factor is at present under study.

Department of Biochemistry,
The Center for Adult Diseases, Osaka,
Osaka (Japan)

HITOSHI AKEDO
HIROMU NISHIHARA
KIYOKO SHINKAI
KEIKO KOMATSU

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