TISSUE-CHARACTERISTIC FORMS OF ADENOSINE DEAMINASE*

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SUMMARY

The starch gel electrophoresis of human tissue preparations results in multiple adenosine deaminase bands. The *in vitro* interconversion of the different bands or the formation of new ones, and the effects of the starch gel in the medium, suggest that the different bands represent different forms of the same enzyme, rather than different primary structures. The patterns obtained vary among different tissues, but the same type of tissue always exhibits the same characteristic pattern. This indicates that the different patterns are due to different chemical environments of the tissues, rather than artifacts introduced during the run. The results suggest the possibility of enzyme control by conformational changes.

INTRODUCTION

Adenosine deaminases are widely distributed in animal tissues[1]. Although relatively little is known about their metabolic role, it is believed that their primary function is detoxification[1,2]. Adenosine deaminase is not known to require any prosthetic groups, and little evidence has been available that normal physiological constituents are required for activation[1,3]. In terms of substrate specificity and pH optima, the adenosine deaminases from different organs within a given species seem to be identical. Adenosine deaminase is known, however, to exist in multiple molecular forms[1,2], and the apparent molecular weight and V_{max} of the enzyme from different organs of a given species may vary[2]. Evidence has also been reported that the multiple molecular forms of adenosine deaminase can be due to conformational differences[2].

The present communication presents the results of starch gel electrophoretic investigations of adenosine deaminase in human tissues. The results suggest that even though different molecular forms may exist within a given tissue, the particular form that is exhibited depends upon the type of tissue.

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METHODS

Horizontal starch gel electrophoresis was conducted with a phosphate–citrate or a tris–citrate buffer[4]. Both were at pH 7.0 and gave comparable results. Electrophoresis with 0.4% agarose, instead of a starch gel, was done as previously described[5]. Starch gels were used except when otherwise specified.

All studies were conducted with human tissues obtained from autopsy. The tissues were briefly washed to remove loose blood, weighed, and homogenized with a glass homogenizer. 1.5 ml of water per gram of tissue (wet weight) was used for the homogenization. Approximately 0.05 ml of the homogenate was applied to the gel by wetting a small square (6 mm^2) of Whatman No. 17 filter paper with it, and inserting the paper into a slot in the starch gel.

After electrophoresis, bands with adenosine deaminase activity were detected by a modification of the method of Tully and Walsh[1]. After slicing the gels, a solution containing 0.5 g of dissolved agar, 3.7 ml of a saturated aqueous solution of bromthymol blue, 5.0 ml of a saturated aqueous solution of phenol red, 0.02 *M* of adenosine, and water to 100 ml was poured over the gel to a depth of approximately 1 mm. (Demineralized water was used throughout.) When the agar had gelled, the surface was covered with Saran Wrap. The incubation was conducted at room temperature. The bands with adenosine deaminase activity could be seen by the marked change in color of the indicators, which were associated with the pH changes resulting from the ammonia liberation. When the substrate had been omitted, no bands were detected. It did not appear that the indicators affected the enzyme activity, since the addition of the indicators before or after the incubation resulted in comparable intensities (when the incubation periods were the same).

In order to further verify that the bands represented adenosine deaminase activity, the bands in the opposite half of a gel were detected by a modification of the Berthelot reaction[6]. The concentration of the reagents was twice as great as that used by Chaney and Marbach[6], and the reagents were stabilized by the addition of agar. After incubating the gel with a solution of adenosine, the reagents were poured over the gel surface, in the proper order. The bands which were detected had the same mobility as those detected with the indicator method. With either method, the bands tended to diffuse after a period of time. The indicator method has the advantage, however, that the formation of the bands can be observed during the incubation, before they are blurred by diffusion.

RESULTS AND DISCUSSION

Fig. 1 shows the results obtained after electrophoresis of a human spleen homogenate and of a solution of a commercial preparation of adenosine deaminase (Sigma). The latter solution was applied at two different concentrations. Fig. 1 shows that the bands detected with the indicators have the same mobility as those detected with the Berthelot reagents. It also shows that the mobility does not depend upon the amount of sample applied (within the limits of the concentrations used). The sample with the lower concentration exhibits multiple bands. The other samples also exhibited multiple bands at earlier stages of development.

The distribution of enzyme activity in some human tissues are presented in Fig.

2. The most intense bands in liver (not shown) have the same mobilities as in the heart, the most intense bands of kidney and lung have less mobility, while spleen has a greater mobility. Dozens of samples from different cadavers were analyzed, and the mobilities of these tissues were always the same. Although there was some overlapping of the subbands between different tissues, the consistency of the results suggests that molecular forms of adenosine deaminase *in vivo* are characteristic for different types of tissues.

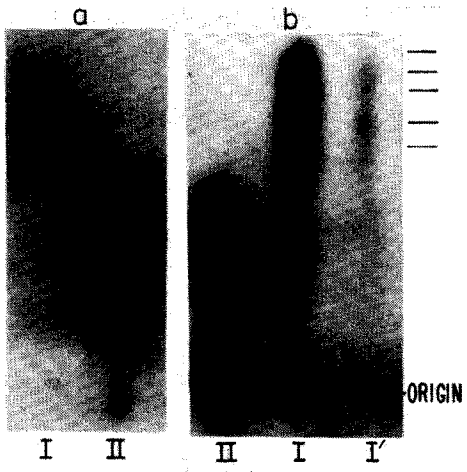

Fig. 1. Detection of adenosine deaminase activity by means of the Berthelot reaction (in a), and by indicators (in b). The samples applied were I: 0.5 mg/ml of a commercial preparation of the enzyme from calf intestinal mucosa, I': 0.2 mg/ml of the same preparation, and II: a homogenate of human spleen (1 g in 3 ml). Sub-bands in I' are indicated by dashes. Migration towards the anode was upward. (Cathodal band is hemoglobin, and not adenosine deaminase).

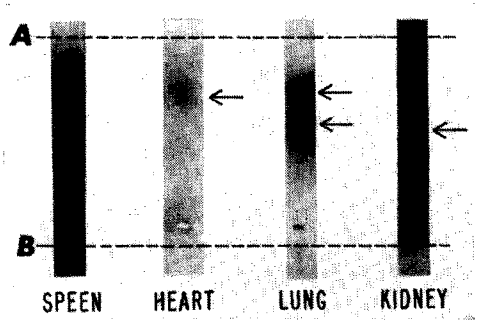

Fig. 2. Electrophoretic patterns of human tissue homogenates. The relative position of human serum albumin is indicated by "A", and of hemoglobin by "B".

Figs. 3 and 4 present evidence that different molecular forms or conformations are represented by the different bands. Fig. 3 indicates that heating the spleen homogenate at 60° for 30 min converts a large proportion of the rear band to the forward band. This conversion has previously been demonstrated, in the case of the purified calf serum enzyme, by Cory *et al.*[2].

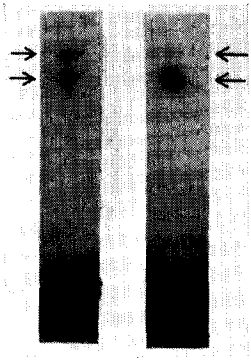

Fig. 3. Effect of heating at 60° for 30 min upon spleen subbands. Sample on the left was heated, sample on the right was not.

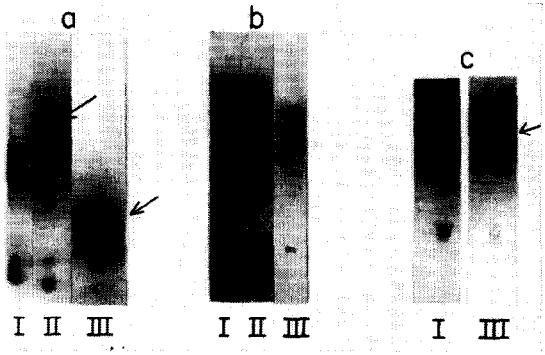

Fig. 4. Effect of the medium upon adenosine deaminase patterns. 15 g/100 ml of starch was used in a, and 9.6 g/100 ml (the concentration generally utilized) was used in b, 0.4 g/100 ml of agarose was used in c. The samples applied were: I spleen, without any treatment; II spleen after dialysis and heating, and III lung.

Fig. 4-a and -b shows that if the spleen homogenate is dialyzed at 3° for 16 h against a 0.05 *M* phosphate buffer pH 7, containing 10^{-3} mole of EDTA, and then heated for 30 min at 60°, a third, more rapidly migrating band is exhibited at a high starch gel concentration (15%, 9.6% was generally used). This band is not observed with the lower concentration of starch. Fig. 4 also demonstrates that the difference in mobility between the major bands of spleen and lung is greatly accentuated at the higher starch gel concentration. These observations are consistent with multiple molecular forms which differ in molecular size[7]. A difference in molecular size is also consistent with the results presented in Fig. 4-c. When the electrophoresis was conducted in agarose, which does not have the molecular sieving effect of starch gels, the lung and spleen both exhibit a single, broad band, with similar mobilities. It is also possible that the above effects of variation in concentration of starch gels are due to differences between the multiple molecular forms in asymmetry, rather than in molecular size. In either case, the effects of the electrophoretic media, the interconvertability of the bands, and the *in vitro* development of new bands suggest that differences in primary structure are not necessary in order to account for the multiple forms of adenosine deaminase.

The dependence of the results upon the type of methodology employed should be considered. It is well known that a single, pure protein can exhibit multiple bands, or a greatly altered mobility. This can be due to interactions or binding with components of the electrophoretic medium, or to dissociation or aggregation of the protein during the migration. In the present case, if adenosine deaminase were the same in all of the samples, and different bands were due to artifacts introduced during the run, then the characteristic patterns of different tissues could not be accounted for. Any artificial influences present during the migration would be the same for all of the samples. Consequently, the different patterns must be due to differences in the tissue samples themselves.

Since the samples were crude homogenates, different patterns might be ascribed to impurities in the preparations. Except for a uniform dilution, the samples were applied directly to the gels. It is possible that the mobility differences do involve binding of other cellular constituents. The consistent type of pattern exhibited by each type of tissue would suggest that such binding is related to the chemical environment of adenosine deaminase in the cell and is not haphazard. Conformational changes can do, of course, involve binding of substances which can act as allosteric effectors.

Since the consistency of the patterns of a given type of tissue suggests that differences in the molecular form or conformation is an *in vivo* characteristic of tissue type, rather than artifactual, and since there are also gross differences in the V_{max} of the enzyme from different tissues of the same species[2], it is possible that these characteristic differences are related to the control of enzyme activity. The involvement of the enzyme in detoxification would also suggest a relationship between the molecular form of adenosine deaminase and pathological processes. These possibilities can, of course, only be verified by further investigations.

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