ANTIGENIC STRUCTURE OF AMP-DEAMINASE: ISOZYME SPECIFICITY OF ANTIBODIES DIRECTED AGAINST PURIFIED ERYTHROCYTE ENZYME

Shelia LEMLEY and Oscar P. CHILSON*

Department of Biology, Division of Biological and Biomedical Science, Washington University, St. Louis, MO 63130, USA

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1. Introduction

AMP-deaminase is an allosteric enzyme that exists in several molecular forms [1–3]. Characterization of the antigenic structure of this enzyme is of interest because specific antibodies are useful probes for detecting developmental differences in isozyme distribution [4]. Interest in this protein as an antigen also arises from the observation that immunization of rabbits with skeletal muscle AMP-deaminase elicits production of circulating auto-antibody [5].

In an earlier report we compared the effect of rabbit antibody directed against homogeneous avian breast muscle AMP-deaminase on the enzymatic activity in relatively crude preparations from brain and erythrocytes [1]. We have now purified erythrocyte AMP-deaminase ~7000-fold and have carried out a reciprocal study of the interaction between rabbit antibody directed against highly-purified erythrocyte AMP-deaminase and purified isozymes from breast muscle and erythrocytes.

The degree to which these isozymes share similar sequence and/or conformational antigenic determinants is not known. However, it is now clear that these isozymes are very similar with respect to the molecular weights of the native enzymes (i.e., 276 000), that each enzyme is a tetramer of subunits of identical size (i.e., 69 000; [6]), and that their amino acid compositions are similar, but not identical (Kruckeberg, S. L. and O. P. C., unpublished observations).

Antibodies obtained from rabbits following immunization with highly purified AMP-deaminase from chicken erythrocytes can inhibit > 90% of the enzymatic activity of highly purified AMP-deaminases from both avian erythrocytes and breast muscle, depending upon the substrate concentration used for assay of enzymatic activity. However, a much greater amount (~ 5-fold) of antibody was required to inhibit the muscle isozyme than was needed to obtain an equivalent degree of inhibition of a comparable amount of erythrocyte AMP-deaminase.

It is concluded that the AMP-deaminase isozymes from avian erythrocytes and breast muscle, though not identical, share similar antigenic determinants.

2. Materials and methods

All chemicals were of reagent grade and were obtained from the sources reported in [1].

AMP-deaminase was isolated from lysates of adult chicken (*Gallus domesticus*) erythrocytes by a procedure to be described in detail elsewhere (Kruckeberg, S. L. and O. P. C., unpublished results). Breast muscle AMP-deaminase was purified as in [5].

Rabbit antisera directed against purified erythrocyte AMP-deaminase (spec. act. 950 U/mg) were obtained as follows: 0.5 mg lyophilized enzyme in 0.5 ml phosphate buffered saline (PBS; 8.8 g NaCl + 7.0 g $NaH_2PO_4 \cdot H_2O$ per liter) was emulsified with 0.5 ml complete Freund's adjuvant and injected at multiple sites in the neck region. A secondary response was elicited by injection of 1 mg lyophilized enzyme in 1 ml PBS in the ear vein on days 32, 34, 37 and 39 after the primary injection. The antisera used in the

* To whom correspondence should be addressed

studies to be described here were obtained from blood samples collected from the marginal ear vein on days 41 and 60, following the primary injection. Rabbit antisera directed against homogeneous AMP-deaminase from chicken breast muscle were obtained as in [5]. Purified immunoglobulin fractions were isolated from pre-immune (control) sera and anti-sera by sodium sulfate fractionation as in [7]. Sera and immunoglobulin fractions were stored at −20°C. Double diffusion (Ouchterlony) tests were carried out as in [5]. Immunoinhibition of enzymatic activity was assayed as in [1,5]. The buffer used for assays of enzymatic activity contained 150 mM KCl + 50 mM Tris–(hydroxymethyl)-amino methane 2-[*N*-morpholino] ethane sulfonic acid (pH 6.5, Tris–MES) instead of Tris–succinate. Details regarding individual experiments are provided in figure legends.

3. Results

Rabbit antibody directed against purified erythrocyte AMP-deaminase inhibits the enzymatic activity of this isozyme; the extent of this inhibition varies with the concentration of 5′-AMP that is used in the assay of activity after preincubation with antiserum. The data summarized in fig.1 show that at antibody to enzyme ratios which showed > 90% inhibition, when assayed at 100 μM 5′-AMP, showed ~ 50% inhibition when these mixtures were assayed at 30 mM 5′-AMP. Approximately 90% inhibition was observed, even when assayed at 30 mM 5′-AMP, when the ratio of antiserum to enzymatic activity was increased to 50×10^{-3} ml/U (data not shown).

Double diffusion analysis showed a single, sharp precipitin band when these antisera were tested against erythrocyte AMP-deaminase, but similar experiments, using the muscle isozyme, were uniformly negative, suggesting strict antibody specificity for the erythrocyte enzyme (Kruckeberg, S. L. and O. P. C., unpublished observations). However, positive evidence that rabbit anti-erythrocyte AMP-deaminase interacts with muscle AMP-deaminase was obtained from enzyme inhibition experiments.

Typical data are summarized in table 1. In exp. 1, equal amounts of purified AMP-deaminase from muscle and erythrocytes were incubated with fixed amounts of antiserum (directed against the RBC

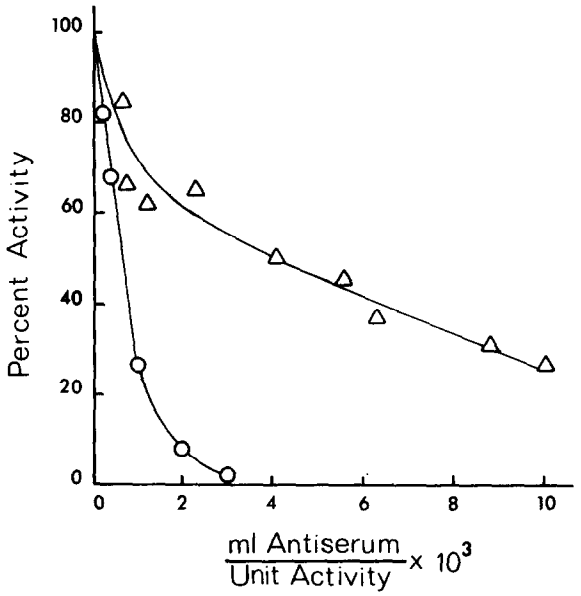

Fig.1. Inhibition of AMP-deaminase from chicken erythrocytes by rabbit antibody directed against the erythrocyte enzyme. (○) Aliquots (~ 10 μg each) of purified erythrocyte deaminase were incubated for 30 min at room temperature with increasing amounts (4–30 μl) antiserum, obtained from rabbits that were immunized with purified erythrocyte AMP-deaminase, or control serum obtained prior to immunization. Incubation mixtures were brought to total vol. 100 μl with phosphate-buffered saline (8.8 g NaCl + 7.0 g $NaH_2PO_4 \cdot H_2O$). Aliquots of these incubation mixtures were then assayed by the continuous spectrophotometric assay referenced under section 2, 100 μM 5′-AMP in 150 mM KCl + 50 mM Tris–MES, pH 6.5. (△) Same as (○), except 4.5 μg enzyme was incubated with antiserum (or control serum) 5–50 μl, in total vol. 875 μl and assayed by the discontinuous assay of ammonia production at 30 mM 5′-AMP.

enzyme) and assayed at 30 mM 5′-AMP. Under conditions where 61% activity of the RBC isozyme was inhibited, low, but significant inhibition (i.e., 11%) of the muscle enzyme was observed. In exp. 2, using an antiserum obtained at a different stage in the course of immunization, inhibition of the muscle isozyme was increased to 32%. Inhibition of muscle AMP-deaminase by rabbit antierythrocyte AMP-deaminase was also dependent on substrate concentration; when assayed at 100 μM 5′-AMP the degree of inhibition was 87% (expt 2, table 1).

Parallel double diffusion and immunoinhibition experiments, using rabbit antibodies directed against

Table 1
Inhibition of avian muscle and erythrocyte AMP-deaminases by rabbit anti-erythrocyte AMP-deaminase

	Enzyme source	Assay [AMP]	% Inhibition
Expt. 1[a]	Muscle	30 mM	11
	Erythrocytes	30 mM	61
Expt. 2[b]	Muscle	30 mM	32
	Muscle	100 μM	87

[a] The antiserum used for this experiment is the same one that was used to obtain the data that are summarized in fig.1. Muscle and erythrocyte enzymes were incubated with antiserum or control serum at a ratio of 7 μl/unit enzymatic activity. Following incubation for 30 min at room temperature enzymatic activity was determined by the discontinuous assay of ammonia production

[b] The antiserum used for this experiment was from a different bleeding, obtained at a different stage of immunization than for expt. 1. The ratio of antiserum (or control serum) to enzymatic activity was 2.1 μl/unit enzymatic activity. Enzymatic activity was determined either by the discontinuous assay of ammonia production (at 30 mM 5′-AMP) or by the continuous spectrophotometric assay (at 100 μM 5′-AMP)

breast muscle AMP-deaminase, yielded reciprocal results. Precipitin bands were obtained with muscle enzyme in the antigen wells but no bands were observed when this antiserum was tested against erythrocyte enzyme. Inhibition of the enzymatic activity of muscle AMP-deaminase was virtually complete over a 5-fold range of antigen: antibody ratios, when assayed at 100 μM 5′-AMP. Greater than 95% inhibition of erythrocyte AMP-deaminase by rabbit anti-muscle AMP-deaminase was also observed; however, ~ 5-fold greater amounts of antibody were required (data not shown).

4. Discussion

The immunoinhibition data we have presented show that muscle and erythrocyte AMP-deaminases share similar antigenic determinants. The mechanism by which immunoinhibition is diminished at elevated substrate concentration is not known. The AMP-deaminase isozymes are both allosteric proteins [1,6]. Thus it is possible that an allosteric transition occurs as the level of 5′-AMP is raised, producing a more active conformation or exposing one or more active sites that were not accessible to antibody, during preincubation of the antigen–antibody mixture. It is also possible that in the presence of 30 mM 5′-AMP the antigen–antibody complex dissociates (at least partially). Another explanation that is consistent with the data is that antibody does not interact with substrate binding or catalytic sites directly, but favors an enzyme conformation having low affinity for substrate. By this mechanism elevated levels of substrate would increase enzymatic rates via saturating these low affinity sites.

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References

[1] Henry, H. and Chilson, O. P. (1973) Comp. Biochem. Physiol. 44B, 121–136.
[2] Ogasawara, N., Goto, H. and Watanabe, T. (1975) FEBS Lett. 58, 245–248.
[3] Raggi, A., Benjamin, C. and Ronca, G. (1975) FEBS Lett. 58, 19–23.
[4] Sammons, D. W. and Chilson, O. P. (1977) Fed. Proc. Fed. Am. Soc. Exp. Biol. 36, 2387.
[5] Selig, D. and Chilson, O. P. (1969) J. Immunol. 103, 725–735.
[6] Kruckeberg, W. C. and Chilson, O. P. (1976) Fed. Proc. Fed. Am. Soc. Exp. Biol. 35, 519.
[7] Keckwick, R. A. (1940) Biochem. J. 34, 1248–1257.