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# Application of Conformation-Dependent Antibodies: Enhancement of Enzyme Activity of L-Glutamate Dehydrogenase [L-Glutamate NAD(P) Oxidoreductase (Deaminating) EC 1.4.1.3]

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**Abstract** □ By using a nonprecipitating system, it was found that the enzyme activity of L-glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3] (I) is enhanced by the addition of small amounts of anti-I-serum. It is suggested that this enhancement is due to the ability of conformation-dependent antibodies to maintain L-glutamate dehydrogenase in a more favorable conformation and thereby influence the rate of conversion of sodium α-ketoglutarate to sodium glutamate.

**Keyphrases** □ L-Glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3]—immunochemical properties □ Antibodies, L-glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3] produced—specificity □ Enzyme activity—enhancement of L-glutamate dehydrogenase

It has been shown that the enzymatic activity of L-glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3] (I) is dependent upon the conformation of the molecule (1–7). Immunochemical studies with this enzyme, using a precipitating system, showed that rabbit antibodies possess specificities not only for the primary structure but also for the conformation of the antigen (8, 9).

The effect of conformation-dependent antibodies on their homologous antigens is of interest. With respect to I, it was reported (10) that normal rabbit serum activates purified preparations of human and bovine I by about 40% in reactions using α-ketoglutarate and glutamate as substrates. It was also reported (10) that anti-I-serum enhanced the enzyme activity of I using α-ketoglutarate as substrate. It was, therefore, of interest to extend the reported investigations by studying the effect produced by these conformation-dependent antibodies over a period of time on the enzyme activity.

## EXPERIMENTAL

Nicotinamide adenine dinucleotide reduced (NADH), α-ketoglutarate, and bovine liver L-glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3] were purchased[1]; the latter material was obtained as a crystalline suspension in ammonium sulfate. Compound I was prepared for use by dialyzing against a standard buffer solution (0.03 *M* $Na_2HPO_4$–0.01 *M* $NaH_2PO_4$–$1 \times 10^{-3}$ *M* disodium edetate), pH 7.4.

**Immunochemistry**—Ten rabbits were treated at weekly intervals with 5 mg. of I. The first 3 weeks they were injected intradermally, using complete Freunds adjuvant as the suspension medium. The rabbits were bled the following week using the standard heart puncture technique. Thereafter, the rabbits were bled and immunized on alternate weeks. Incremental amounts of I were added to 1-ml. aliquots of the antiserum obtained from each rabbit. These mixtures were incubated at 37° for 1 hr. and then stored at 4° for 48 hr. Each rabbit antiserum gave a precipitin reaction, and the amount of protein precipitated was quantitated by analysis for nitrogen (Kjeldahl).

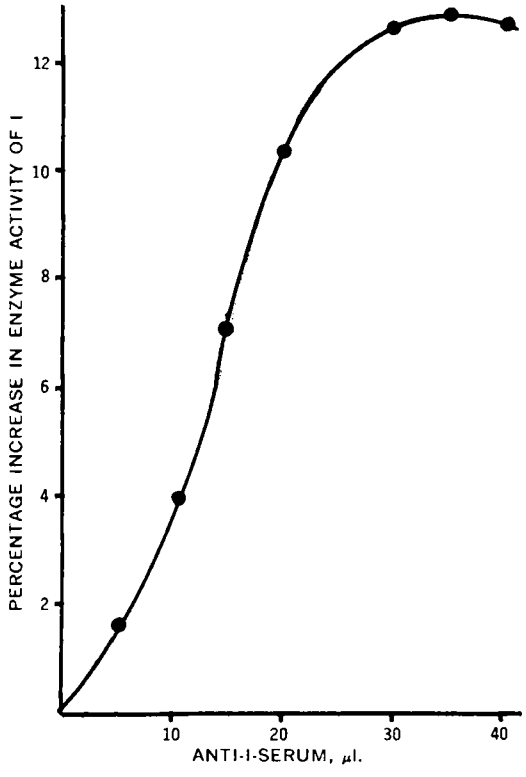


**Figure 1**—*Percentage increase in enzyme activity of I in the presence of anti-I-serum.*

[1] Sigma Chemical Co.

**Table I**—Percentage Increases in Enzyme Activity of I for Each Animal Tabulated against Length of Time from Initial Immunization

| Animal Number | Time from Initial Immunization, Weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| 1 | 0 | 0 | 3 | 11 | 8.5 | 10 | 14 |
| 2 | 0 | 6.8 | 5.2 | 8 | 4.5 | 6.5 | 10.5 |
| 3 | 7 | 4 | 2.6 | 4.2 | 9 | 6.5 | 9.8 |
| 4 | — | 6 | 14 | 7.5 | 11.7 | 14.5 | 17.5 |
| 5 | 4.5 | 7 | 7.5 | 7 | 13 | 14.5 | 31 |
| 6 | 2.5 | 18 | 23 | 17.5 | 7 | 6 | 0 |
| 7 | 7 | 6 | 14 | 7 | 15 | 6 | 5 |
| 8 | 6.5 | 12 | 23 | 12 | 15 | 13 | 3 |
| 9 | 5 | 10 | 21 | 11 | 7 | 14.5 | 10 |
| 10 | 3.5 | 9 | 9.5 | 12 | 10 | 2 | 7 |

Preimmunized serum was treated with I and run under identical conditions to those described for the antiserum. No precipitin reactions were observed.

**Enzyme Assays**—In a typical assay the concentration of I in a solution was determined by measurement of the absorbance at 280 nm., where $E_{max} = 1.0 \times 10^6\ M^{-1}\ cm.^{-1}$ for a molecular weight of $1.0 \times 10^6$. To 3 ml. of phosphate buffer (0.03 *M* $Na_2HPO_4$–0.01 *M* $NaH_2PO_4$–$1 \times 10^{-3}$ disodium edetate), pH 7.4, $1.0 \times 10^{-4}$ *M* NADH, $2 \times 10^{-3}$ *M* sodium α-ketoglutarate, and $1 \times 10^{-1}$ *M* $NH_4Cl$ was added 5 μl. of a solution of I (0.5 mg./ml.). The enzyme activity at 23° was obtained by measuring the decrease in the absorbance of the solution at 340 nm. The determination of the enzyme activity of I in the presence of antibody obtained from each rabbit and for each bleeding was performed in exactly the same manner as that already described. However, the solution containing I and 20 μl. of the specified antiserum were incubated at 37° for 1 hr. and then cooled to 23° before proceeding with the assay. Controls using I with and without preimmunized serum were run simultaneously with the conditions identical to those described for the antiserum enzyme assays.

## RESULTS

Antiserum from each rabbit and from each bleeding gave a positive precipitin reaction; however, controls using nonimmunized serum produced no precipitation. Initially, it was necessary to ascertain the optimum amount of antibody required to produce the largest change in enzyme activity without precipitation occurring. For this purpose, incremental amounts of antiserum were added to the standard aliquot of I and the increase in activity was ascertained. Typically, it was found that a maximum increase in enzyme activity occurred in the presence of 35 μl. of antiserum (Fig. 1). However, a small amount of precipitate was obtained under these conditions. An investigation to ascertain the largest amount of antiserum permissible before precipitation occurred was conducted. From these results (Fig. 2), it was evident that the largest amount of antiserum that could be used and still maintain solution was 20 μl. By using these conditions, it was found that practically every antiserum tested produced an enhancement of the activity of the enzyme I as measured by the increase in the rate of conversion of sodium α-ketoglutarate to sodium glutamate (Table I). No enhancement of enzyme activity was obtained with nonimmunized serum.

## DISCUSSION

Unlike the previous report (10), no enhancement of the enzyme activity of I was found in the presence of nonimmunized serum. However, by using the nonprecipitating system described, the reported enhancement of the enzyme activity of I by use of small amounts of rabbit anti-I-serum was corroborated. The rationale was that within the heterogeneous mixture of antibodies formed to the antigenic challenge of I, a definite population is conformation dependent. It is suggested that these antibodies are capable of maintaining I in a more favorable conformation to influence the rate of conversion of sodium α-ketoglutarate to sodium glutamate. However, it is evident from Table I that the concentration of the conformation-dependent antibodies varies with the length of time of immunization. A similar observation has been made of the concentrations of various antibody populations produced by rabbits upon continuous immunization with type III and type VIII pneumococci (11). It also has to be concluded that the antigenic determinants of I must be clearly different from those that constitute the enzyme active site.

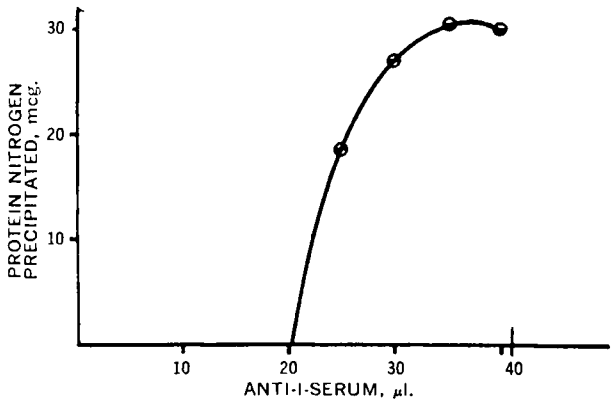


**Figure 2**—*Amount of protein nitrogen precipitated in the presence of anti-I-serum per aliquot of the standard enzyme assay system.*

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