Inactivation of Normal β-d-Galactosidase by Antibodies to Defective Forms of the Enzyme*

(Received for publication, April 3, 1975)

RICHARD A. ROTH and BORIS ROTMAN

From the Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912

A counterpart of the antibody-mediated activation of genetically defective enzymes is reported here. Antibodies elicited by certain mutant forms of β-d-galactosidase (EC 3.2.1.23) of Escherichia coli were found to inactivate the normal form of the enzyme. (Antibodies elicited by normal β-d-galactosidase do not affect the enzyme's catalytic activity.) We present evidence that the inactivating antibodies are directed against one or a few determinants of the enzyme.

The level of inactivation caused by the antibodies was independent of temperature below 25°C and increased with temperature above 25°C. The inactivation was proportional to the concentration of antiserum until a maximum level of 50% inactivation was reached. Antibodies capable of inactivating up to 87% of the activity were obtained after the antiserum was partially absorbed in an affinity column. This antibody preparation showed a 10-fold enrichment of inactivating antibodies over other antibodies directed against the enzyme. The antibody-mediated inactivation caused a reduction in the V_max of β-d-galactosidase without affecting the apparent K_m of the enzyme. In contrast to antibodies to normal β-d-galactosidase, inactivating antibodies changed the response of the enzyme to cations.

To explain these results, we present a model in which there is a temperature-dependent equilibrium between two active forms of β-d-galactosidase. Inactivation results from a conformational change induced by the binding of inactivating antibodies to only one of these two forms.

The catalytic activity of an enzyme may be unaffected, inhibited, increased, or protected from inactivation in the presence of specific antibodies (1). For Escherichia coli β-d-galactosidase, antibodies which mediate only two of the three effects have been reported: activating antibodies that increase the enzymatic activity of defective β-d-galactosidases (2, 3) and protecting antibodies that prevent heat denaturation of the normal and mutant enzymes (4). Inactivating antibodies, the third class, hitherto have not been found in antisera from different animal species immunized with normal β-d-galactosidase of either eukaryotic or prokaryotic origin (5).

The results reported here demonstrate that antibodies which inactivate normal β-d-galactosidase can be elicited in rabbits immunized with several defective β-d-galactosidases. The defective enzymes used as immunogens resulted from at least two mutational events and differ from the normal enzyme in specific activity and temperature sensitivity.

MATERIALS AND METHODS

Buffers—Buffer A contained 20 mM sodium phosphate (pH 7.2) and 0.2% bovine albumin (Cohn fraction V, Armour Pharmaceutical Co., Chicago, Ill.). Buffer B contained 10 mM tris(hydroxymethyl)amino-methane, 10 mM MgCl_2, and 100 mM NaCl and its pH was adjusted to 7.45 (23°C) with acetic acid. The complete buffer was prepared daily by adding 50 mM 2-mercaptoethanol to the stock salt solution. Buffer T contained 20 mM tris(hydroxymethyl)amino-methane (adjusted to pH 7.2 with acetic acid) and 0.2% bovine albumin.

Assays of β-d-Galactosidase—For routine experiments, the enzyme was assayed at 37°C in a 2-ml volume of Buffer B containing 3 mM chromogenic substrate. The enzymatic hydrolysis was allowed to proceed until color was visible and then the reaction was stopped by adding 3 ml of 200 mM Na_2CO_3. With o-nitrophenyl-β-d-galactopyranoside as the substrate, the absorbance of the reaction mixture was read at 420 nm and a molar extinction coefficient of 4700 was used to convert absorbance readings to o-nitrophenol concentration. With p-nitrophenyl-β-d-galactopyranoside as the substrate, absorbance was read at 400 nm and the value 18,400 was used for converting to p-nitrophenol concentration.

A kinetic assay was also used. For this, the enzymatic hydrolysis of o-nitrophenyl-β-d-galactopyranoside was followed spectrophotometrically at 22°C (room temperature) by reading the absorbance at 420 nm every 45 s. The volume, buffer, and substrate concentration were the same as for the routine assay.

An enzyme unit for β-d-galactosidase is defined as the amount of enzyme liberating 1 nmol of substrate in 1 min under the described conditions.

Enzyme Purification—Strain 7-1, a β-d-galactosidase constitutive mutant of Escherichia coli K12 grown in tryptone-lactate broth (6), was used for the production of normal β-d-galactosidase. The mutant enzymes were extracted from independent Lac' revertants of S30 (i^{+}_2y^{a-}) (isolated by Dr. Bruce Levin, University of Massachusetts) grown at 37°C in Davis' minimal medium (7) containing 0.4% sodium lactate (Fisher Scientific Co., Fairlawn, N.J.) as sole carbon source and 500 μM isopropyl-1-thio-β-d-galactopyranoside as inducer.

The β-d-galactosidases from the constitutive E. coli or the revertants

* This work was supported by Research Grant GB 41333 from the National Science Foundation.
† Supported by Training Grant AI 00418 from the National Institutes of Health. In partial fulfillment of the requirements for the degree of Doctor of Philosophy.
were purified as follows. Bacteria were harvested at stationary phase, washed three times with Buffer B, resuspended in the same buffer to a density of about 10^10 cells/ml, and disrupted in a French press cell. Cell debris was removed by centrifuging at 27,000 x g for 20 min and the supernatant was brought to 32% saturated ammonium sulfate by the addition of saturated ammonium sulfate (70.6 g of ammonium sulfate dissolved in 100 ml of water and adjusted to pH 7.2 with 5 M NaOH). The resulting precipitate was removed by centrifugation at 27,000 x g for 15 min and the supernatant was brought to 40% saturated ammonium sulfate. The precipitate formed was separated by centrifugation and stored at 4° under 50% saturated ammonium sulfate solution in Buffer B. Samples were taken from this stock suspension as needed for daily use. For this, a given volume of the suspension was spun at 27,000 x g for 15 min, and the pellet obtained was dissolved in the original volume of Buffer B. The solution was dialyzed against Buffer B for about 6 hours at 4° (two changes of 360 times the original volume).

**Immunizations**—New Zealand white rabbits, weighing between 2 and 5 kg, were injected subcutaneously in the scapular region. Each animal received 0.5 to 3 mg of β-D-galactosidase dissolved in 1 ml of Buffer B and emulsified with 1 ml of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The animals were then challenged using the same procedure 30 to 60 days after the first injection. At varying intervals beginning 1 week after the challenge the rabbits were bled from the marginal ear veins.

**Purification of Antibodies and Preparation of Fab Fragments**—Fractions containing γ-globulin were obtained by precipitating serum proteins with 40% saturated ammonium sulfate. Fab fragments, the univalent cleavage products of immunoglobulins, were prepared by digesting the γ-globulins with papain (9) and separating undigested antibodies by Sephadex chromatography.

**Removal of Antibodies from Antiserum**—Antiserum was precipitated at equilibrium with either β-D-galactosidase or sheep anti-rabbit immunoglobulin (a gift of Dr. Paul Knopf of this University). The reaction was allowed to proceed for 60 min at 37° and then overnight in 37°. The precipitate was then centrifuged in a Clay-Adams microchemistry centrifuge (20,000 x g) for 10 min.

**Specific Purification of Inactivating Antibodies**—Normal β-D-galactosidase was coupled to Sepharose 4B-200 (Sigma, St. Louis, Mo.) which had been activated by cyanogen bromide (9). A column consisting of Sepharose coupled to normal β-D-galactosidase. The reaction was allowed to proceed for 60 min at 37° and then overnight in 37°. The precipitate was then centrifuged in a Clay-Adams microchemistry centrifuge (20,000 x g) for 10 min.

**Measurement of Inactivation**—The kinetics of the antibody-mediated inactivation of β-D-galactosidase were studied in detail because the reaction is too fast to be analyzed by conventional methods. For all the experiments described below, inactivation was measured after incubating enzyme and antibody for 10 min, a time more than ample for completion of the reaction. As shown in Fig. 1, no detectable inactivation occurred during the enzyme assays since linear rates of hydrolysis were obtained with enzyme samples inactivated by several concentrations of Fab fragments.

**Specificity of Antibody-mediated Inactivation**—The results presented in Table I provide evidence that the inactivation is caused by specific antibodies. While normal serum and Fab fragments against normal β-D-galactosidase did not cause significant inactivation, antisera from rabbits immunized with the temperature-sensitive enzymes inactivated up to 49% of the enzyme. The inactivating capacity of these antisera was shown to reside in the purified Fab fragments prepared from the γ-globulin fraction. The specific removal of either the bulk of the immunoglobulin or the anti-β-D-galactosidase antibodies resulted in the loss of the inactivating capacity. Below 0.2 mg of Fab fragments/ml, the extent of inactivation was proportional to the Fab fragments concentration. Above this concentration the inactivation remained constant at about 50% (inset, Fig. 1).

**Enrichment for Inactivating Antibodies**—Melchers and Messer obtained an enrichment for activating antibodies by partially absorbing an anti-β-D-galactosidase serum on an affinity column (11). We tried a similar approach to enrich for inactivating antibodies. The γ-globulin fraction of an inactivating antisera was passed through an affinity column consisting of Sepharose coupled to normal β-D-galactosidase. The material which did not absorb to the column was enriched for inactivating antibodies since it inactivated the enzyme to a higher maximum (87%) and had a reduced titer of binding antibodies (Fig. 1 and Table I). As with the whole antisera, we demonstrated that the inactivation caused by the enriched preparation was antibody-mediated since the specific removal of its immunoglobulin resulted in loss of 99% of its inactivating activity. Purified Fab fragments prepared from this material retained the ability to inactivate the enzyme at the maximal level of 87% (Table I). The specific activity of the antibody preparation showed that a 10-fold enrichment for inactivating antibodies had been achieved. Specific activity is defined here as the ratio between inactivating and binding antibodies (Table I).

**Effect of Temperature on Inactivation**—The extent of inactivation caused by Fab fragments at several temperatures is shown in Table II. Enzyme alone and enzyme in the presence of antibodies. One of the 14 was found to be indistinguishable from the normal enzyme. When the immunogenic properties of these mutant β-D-galactosidases were compared with those of the normal enzyme, major differences were observed. Normal β-D-galactosidase elicits antisera which precipitate the enzyme but do not affect the enzyme's catalytic activity (10). Furthermore, these antisera activate AMEF (2, 3). (The same immunogenic properties were found for the mutant enzyme resembling the normal β-D-galactosidase in heat stability.) In contrast, the temperature-sensitive enzymes elicited antibodies which did not activate AMEF and, more important for this study, these enzymes elicited a new class of antibodies which inactivated the normal enzyme. The properties of these inactivating antibodies are the subject of this report.

**RESULTS**

**Mutant β-D-Galactosidases as Immunogens**—Fourteen mutant β-D-galactosidases were obtained from the extracts of 14 independent Lac + revertants of S30, a lac 2 strain of Escherichia coli which produces AMEF, a defective β-D-galactosidase (Table I). Thirteen of these enzymes were obtained from the extracts of 14

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1 The abbreviation used is: AMEF, a defective β-D-galactosidase which is activatable by antibodies (2).
normal Fab fragments were not inactivated at temperatures below 51°C. In contrast, enzyme incubated with inactivating Fab fragments was 25% inactivated at 0°C. (The activity of β-D-galactosidase in cell-free extracts stored at 0°C has a half-life of 17 years.) Between 0 and 25°C only a slight increase in inactivation was observed. Above 25°C, the antibody-mediated inactivation increased more rapidly and reached 50% inactivation at 45°C.

**DISCUSSION**

The results presented here show that sera from rabbits

![Graph showing activity of Fab fragments](image-url)

**Figure 1.** Inactivation of β-D-galactosidase by different preparations of Fab fragments. Prior to assay, 0.4 ml of enzyme (550 enzyme units/ml in Buffer A), equilibrated at 45°C, was mixed with 0.1 ml of the indicated preparation of Fab fragments and then incubated for 10 min at 45°C. The mixture was cooled at 0°C for 5 min and then 0.1-ml samples were removed for kinetic assays of enzymatic activity as described under "Materials and Methods." The activity is expressed in nanomoles of o-nitrophenyl-β-D-galactopyranoside hydrolyzed per ml of assay solution. A: Buffer A (A), Fab fragments prepared from immunoglobulin of normal rabbits (0.2 mg of protein/ml) ( ), Fab fragments prepared from antibodies against normal β-D-galactosidase (0.2 mg of protein per ml) (○); B, C, and D: Fab fragments from antibodies against temperature-sensitive enzyme containing 0.1, 0.2, 0.4 mg of protein/ml, respectively; E: Fab fragments from purified inactivating antibodies. For the experiment with purified inactivating Fab fragments (Curve E), we used a preparation with 70% the titer in binding units of the crude Fab fragments (Curve D). Each point is the mean of nine determinations; the vertical bars show standard errors. The inset is a plot of relative concentration of Fab fragments versus percentage of β-D-galactosidase inactivated. The enzyme was inactivated and assayed as described for the other experiments shown in this figure. ○, Fab fragments from antibodies against a temperature-sensitive enzyme; ●, Fab fragments from purified inactivating antibodies.

β-D-galactosidase without affecting the apparent $K_m$ of the enzyme. The purified inactivating Fab fragments caused a greater reduction of the $V_{max}$ (in proportion to their greater inactivating capacity), also without affecting the apparent $K_m$.

**Effect of Different Cations and Substrates on Activity of Antibody-enzyme Complex**—The activity of β-D-galactosidase is influenced by the monovalent cations present in the assay and the extent of the effect depends upon the substrate and temperature-sensitive enzyme containing 0.1, 0.2, 0.4 mg of protein/ml, respectively; E: Fab fragments from purified inactivating antibodies. For the experiment with purified inactivating Fab fragments (Curve E), we used a preparation with 70% the titer in binding units of the crude Fab fragments (Curve D). Each point is the mean of nine determinations; the vertical bars show standard errors. The inset is a plot of relative concentration of Fab fragments versus percentage of β-D-galactosidase inactivated. The enzyme was inactivated and assayed as described for the other experiments shown in this figure. ○, Fab fragments from antibodies against a temperature-sensitive enzyme; ●, Fab fragments from purified inactivating antibodies.

The effect of substrate concentration on the enzymatic reaction catalyzed by the enzyme-Fab fragment complex is shown in Fig. 2 as a Lineweaver-Burk plot. From this graph we conclude that inactivating Fab fragments caused a reduction in the $V_{max}$ of β-D-galactosidase without affecting the apparent $K_m$ of the enzyme. The purified inactivating Fab fragments caused a greater reduction of the $V_{max}$ (in proportion to their greater inactivating capacity), also without affecting the apparent $K_m$.

**DISCUSSION**

The results presented here show that sera from rabbits

*Z. B. Rotman, unpublished results.*
TABLE I
Specificity of inactivation

To 0.45 ml of β-D-galactosidase in Buffer A, previously equilibrated at 45°, 0.05 ml of the preparation to be tested was added. After 10 min of incubation at 45°, the mixture was cooled at 0° for 5 min; 0.1-ml samples were removed from the mixture and assayed for enzymatic activity by the routine procedure described under "Materials and Methods." The rate of hydrolysis is given as enzyme units per ml of mix. Standard errors were calculated from nine determinations. Inactivating units correspond to the number of enzyme units inactivated by 1 μl of the tested preparation. The ability to bind β-D-galactosidase was assayed as described under "Materials and Methods." Binding units correspond to the number of enzyme units bound by 1 μl of the preparation.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Rate of hydrolysis</th>
<th>Inactivation*</th>
<th>Inactivating units</th>
<th>Binding units</th>
<th>Specific activitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal serum (diluted 1:4)</td>
<td>880 ± 16</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fab fragments to normal β-D-galactosidase</td>
<td>856 ± 10</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivating serum (diluted 1:4)</td>
<td>444 ± 16</td>
<td>49</td>
<td>4.4 ± 0.4</td>
<td>370 ± 20</td>
<td>1.2</td>
</tr>
<tr>
<td>γ-globulin fraction of inactivating serum</td>
<td>394 ± 10</td>
<td>55</td>
<td>4.9 ± 0.4</td>
<td>370 ± 20</td>
<td>1.3</td>
</tr>
<tr>
<td>Inactivating Fab fragments</td>
<td>476 ± 20</td>
<td>46</td>
<td>4.0 ± 0.4</td>
<td>370 ± 20</td>
<td>1.1</td>
</tr>
<tr>
<td>Purified inactivating immunoglobulin</td>
<td>189 ± 12</td>
<td>79</td>
<td>1.0 ± 0.1</td>
<td>70 ± 4</td>
<td>19</td>
</tr>
<tr>
<td>Purified inactivating Fab fragments</td>
<td>118 ± 9</td>
<td>87</td>
<td>7.6 ± 0.5</td>
<td>30 ± 3</td>
<td>25</td>
</tr>
<tr>
<td>Inactivating serum after removal of anti-β-D-galactosidase immunoglobulin (diluted 1:4)</td>
<td>882 ± 43</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivating serum after removal of immunoglobulin (diluted 1:4)</td>
<td>877 ± 21</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All additions were in Buffer A.

a The value of 880 enzyme units/ml was taken as 100% activity.

b The specific activity is (inactivating units/binding units) x 100.

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TABLE II
Inactivation as function of temperature

Inactivations were performed as in the legend to Table I except that the temperature of inactivation was varied as indicated in the table. Assays were performed by the kinetic method described under "Materials and Methods." Linear rates of hydrolysis were observed as in the experiments shown in Fig. 1. Standard errors were calculated from nine determinations.

Although linear rates of hydrolysis were found, it was still possible that the inactivation observed at 0° really occurred after the sample from the antibody-enzyme mix was added to the assay solution. This possibility was ruled out since only 5% inactivation was observed when antibody was added to the assay solution prior to the addition of enzyme.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Temperature of inactivation</th>
<th>Rate of hydrolysis</th>
<th>Inactivation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>enzyme units/ml ± S.E.</td>
<td></td>
</tr>
<tr>
<td>A. Buffer A</td>
<td></td>
<td>560 ± 12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>562 ± 7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>528 ± 7</td>
<td>6</td>
</tr>
<tr>
<td>B. Fab fragments from γ-globulin of normal rabbits (0.2 mg of protein/ml)</td>
<td>0</td>
<td>573 ± 17</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>562 ± 15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>532 ± 8</td>
<td>5</td>
</tr>
<tr>
<td>C. Inactivating Fab fragments (0.2 mg of protein/ml)</td>
<td>0</td>
<td>421 ± 1</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>397 ± 11</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>349 ± 7</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>280 ± 8</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>198 ± 4</td>
<td>65</td>
</tr>
</tbody>
</table>

* The value of 560 enzyme units/ml was taken as 100% activity.

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immunized with several different temperature-sensitive β-D-galactosidases differ from antisera against the normal enzyme in two respects: they do not activate AMEF and they contain antibodies which inactivate the normal enzyme. That the inactivating ability of the antisera resides in specific antibodies is shown by four criteria: (a) absorption of the antisera by precipitation with β-D-galactosidase removed their inactivating effect; (b) absorption of the antisera by precipitation with sheep anti-rabbit immunoglobulin also removed the inactivating effect; (c) both the purified γ-globulin and the Fab fragments prepared from it retained the original inactivating capacity of the antisera; (d) fractionation of an antiserum by affinity chromatography resulted in enrichment for inactivating antibodies; that is, we obtained a preparation relatively high in inactivating antibodies but low in other antibodies to β-D-galactosidase. From this last result, we concluded that inactivating antibodies are a specific class of antibodies directed against one or a few determinants of β-D-galactosidase.

Antibody-mediated enzyme inactivation may occur by several mechanisms (1). We have ruled out that inactivation results from aggregation of the enzyme-antibody complex since monovalent Fab fragments inactivate the enzyme.

Inactivation could also stem from a steric blockage of the
The catalytic sites of the enzyme. The following results argue against this mechanism for the antibody-mediated inactivation described here for the unfractionated Fab fragments.

1. The same antibody-enzyme complex which showed inactivation when assayed with p-nitrophenyl-β-D-galactopyranoside in the presence of K⁺ was fully active when assayed with the same substrate in the presence of Na⁺. When o-nitrophenyl-β-D-galactopyranoside was the substrate, inactivation was observed in the presence of either cation.

2. The antibody-enzyme complex has the same apparent $K_m$ but a fraction of the $V_{max}$ of the normal enzyme. In order to explain this difference under a simple steric hindrance hypothesis, it is necessary to assume that some of the enzyme's binding sites are completely blocked, while others are completely free. This is untenable considering that all four sites appear unaffected when the complex is assayed with the para-substrate in the presence of Na⁺.

3. Inactivation is blocked by antibodies to normal β-D-galactosidase which do not affect the active sites of the enzyme as measured by the $K_m$ and $V_{max}$.

An alternate model for inactivation, similar to that postulated for antibody-mediated activation (11, 13), requires an equilibrium between (at least) two forms of β-D-galactosidase which differ in antigenic properties. One form, $Z^*$, would be the predominant molecular species at temperatures below 30° and would not bind inactivating antibodies. The other form, $Z^*_d$, would become more prevalent at temperatures above 30° and would have the antigenic determinant(s) for binding inactivating antibodies. According to this model, loss of enzymatic activity occurs as a result of a conformational change of $Z^*_d$ induced by the binding of inactivating antibodies to a determinant(s) $d$.

Several independent studies substantiate our model by demonstrating that different conformational forms of β-D-galactosidase exist in a temperature-dependent equilibrium: (a) a marked increase in the susceptibility of β-D-galactosidase

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**Table III**

*Effect of Na⁺ and K⁺ on activity of enzyme-antibody complexes*

A mixture of 0.35 ml of β-D-galactosidase in Buffer T and 0.35 ml of Fab fragments or Buffer T was incubated at 30° for 10 min and then was cooled at 0° for 5 min. For enzymatic assay, a sample of 0.1 ml was removed from the mix and added to 1.9 ml of $3 \times 10^{-3}$ M o-nitrophenyl-β-D-galactopyranoside (ONPG) or $3 \times 10^{-3}$ M p-nitrophenyl-β-D-galactopyranoside (PNPG) at 37° in either Na-PO₄ or K-PO₄, 0.1 M, pH 7.0. The reaction was stopped with 3 ml of 0.2 M Na₂CO₃. The amount of substrate hydrolyzed was determined spectrophotometrically as described under “Materials and Methods.” Standard errors were calculated from nine determinations. All preparations were dialyzed against Buffer T.

This experiment required a lower temperature for the inactivation since the enzyme by itself was not stable at temperatures higher than 41° in the Buffer T.

<table>
<thead>
<tr>
<th>Addition</th>
<th>ONPG as substrate</th>
<th>PNPG as substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate of hydrolysis</td>
<td>Rate of hydrolysis</td>
</tr>
<tr>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td></td>
<td>enzyme units/ml ± S.E.</td>
<td>%</td>
</tr>
<tr>
<td>Buffer T</td>
<td>328 ± 18</td>
<td>0</td>
</tr>
<tr>
<td>Fab fragments to normal enzyme</td>
<td>320 ± 19</td>
<td>2</td>
</tr>
<tr>
<td>(880 binding units)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivating Fab fragments</td>
<td>195 ± 8</td>
<td>41</td>
</tr>
<tr>
<td>(440 binding units)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The values obtained in the presence of Buffer T were taken as 100% activity.*
A mixture of 0.1 ml of β-β-galactosidase in Buffer A and 0.04 ml of the first addition was incubated at 45° for 10 min and then it received 0.04 ml of the second addition. All the solutions were prewarmed to 45°. After the second addition the mixture was incubated at 45° for 5 min and then cooled at 0° for 5 min. The enzymatic assay was performed by the routine procedure described under “Materials and Methods” using 0.05-ml samples. Standard errors were calculated on the basis of nine determinations.

<table>
<thead>
<tr>
<th>First addition*</th>
<th>Second addition*</th>
<th>Rate of hydrolysis</th>
<th>Inactivation*</th>
<th>Competition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer A</td>
<td>Inactivating Fab fragments*</td>
<td>568 ± 21</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>135 binding units of Fab fragments to normal enzyme</td>
<td>Inactivating Fab fragments*</td>
<td>719 ± 20</td>
<td>44</td>
<td>21</td>
</tr>
<tr>
<td>540 binding units of Fab fragments to normal enzyme</td>
<td>Inactivating Fab fragments*</td>
<td>976 ± 31</td>
<td>24</td>
<td>57</td>
</tr>
<tr>
<td>2200 binding units of Fab fragments to normal enzyme</td>
<td>Inactivating Fab fragments*</td>
<td>1124 ± 31</td>
<td>13</td>
<td>77</td>
</tr>
<tr>
<td>2200 binding units of Fab fragments to normal enzyme</td>
<td>Buffer A</td>
<td>1185 ± 44</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Buffer A</td>
<td>Buffer A</td>
<td>1200 ± 38</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Inactivating Fab fragments*</td>
<td>2200 binding units of Fab fragments to normal enzyme</td>
<td>527 ± 13</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>

*All additions were in Buffer A.
*The value of 1290 enzyme units/ml was taken as 100% activity.
*Competition is defined here as the percentage of inactivation which has been blocked. The value of 56% inactivation was taken as 0% competition.
*810 binding units of inactivating Fab fragments were added.

to proteolytic attack by chymotrypsin was observed when the temperature was raised from 25 to 40° (14); (b) a temperature shift from 25 to 40° exposed new titrable sulfhydryl groups (15); (c) an Arrhenius plot of enzymatic activity has two slopes with a break at 30° (16); (d) an inhibitory effect of fructose 1,6-diphosphate on the enzyme was demonstrable at 45° but not at 30° (17).

Our data are consistent with a temperature dependent equilibrium between two forms of the enzyme. We found that inactivating antibodies bind more to normal enzyme at 37 than at 25°. Also, inactivation is a temperature-independent process from 0 to 25° while it increases with temperature above 25°.

Other results given here support the postulated antibody-mediated conformational change of Z°, as a cause of inactivation. We observed that in contrast to antibodies to normal β-β-galactosidase, inactivating antibodies affect the response of the enzyme to cations. This different response to cations of the Z°,-antibody complex is easily interpretable in terms of a change in its three dimensional structure.

Since inactivating antibodies are elicited only by mutant forms of β-β-galactosidase, we propose that the alteration in these mutant enzymes resides in the determinant d. Thus, antibodies against the altered d determinant would be present in the antisera produced against mutant enzymes. These antibodies would be capable of binding the normal d determinant, causing inactivation. This scheme also explains how antisera to normal enzyme can block inactivation since these sera contain antibodies against the normal d determinant which bind without inactivating.

The inability to achieve more than 50% inactivation when using a crude inactivating antiserum may be accounted for by the presence of competing anti-d antibodies with low or no inactivating ability. At 25° these competing antibodies appear to be bound preferentially to the enzyme since we were able to obtain an antibody fraction with higher levels of inactivation by partial absorption of the crude serum on an affinity column.

Although the model proposed here accounts for all our findings and is supported by independent evidence, it is not complete since several questions remain unanswered. For instance, does 50% inactivation of the enzyme represent a population of molecules in which 50% of them are fully active and 50% are completely inactive, or are all the molecules 50% active? How many antibody molecules are necessary for complete inactivation of one enzyme molecule? Do inactivating antibodies differ in their inactivating capacity? The above questions may be answered by experiments with purified inactivating antibodies and by analyses at the level of single antibody molecules (18).

Acknowledgments—We thank Dr. Paul Knopf for a gift of sheep anti-rabbit immunoglobulin and Dr. Bruce Levin for a set of revertant strains of S30.

REFERENCES
Inactivation of normal beta-D-galactosidase by antibodies to defective forms of the enzyme.
R A Roth and B Rotman


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