ENHANCED STABILITY AGAINST HEAT DENATURATION OF E. COLI WILD TYPE AND MUTANT ß-GALACTOSIDASE IN THE PRESENCE OF SPECIFIC ANTIBODIES.

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SUMMARY

The enzyme ß-galactosidase from E. coli K12 3300 and ten mutant proteins, activatable to ß-galactosidase activity by specific antibodies, exhibit an increased stability against heat denaturation when complexed with specific antibodies. One mutant protein, activated by specific antibodies to enzyme activity, does not show this antibody-mediated increase in stability against heat denaturation, suggesting that activation of the mutant proteins to enzyme activity and stabilization against heat denaturation are mediated by two different populations of anti ß-galactosidase antibodies binding to different antigenic determinants on the enzyme molecule.

Antibodies against ß-galactosidase precipitate, but do not inactivate the enzyme (1). Binding of antibody molecules thus does not interfere with the native state of the active site. Mutant proteins of ß-galactosidase have been found, which can be activated to enzyme activity by specific antibodies (2, 3). Reconstitution of the active site in these mutant proteins by specific antibody suggested to us that some of the antibodies directed against ß-galactosidase could also have stabilizing effects on the native state of the wild type enzyme. Such a stabilization may be detectable, when wild type enzyme as well as the antibody activatable mutant proteins are denatured by heat in the presence and in the absence of specific antibodies.

MATERIALS AND METHODS

Extracts of wild type E. coli K12 3300 and of eleven mutants activatable to enzyme activity by specific antibodies were obtained as described (3). These extracts were incubated for 30 minutes at 37° C either with (a) specific antibody, e. g. in the presence of excess rabbit anti wild type ß-galactosidase antibody (3), or (b) unspecific antibody, e. g. in the presence of rabbit anti ovalbumin antiserum. For heat inactivation antibody-extract mixtures were then incubated for 20 minutes at various temperatures between 36° and 72° C (see Figure 1). Thereafter anti ß-galactosidase antibody was added to (b) and anti ovalbumin serum to (a) for another 30 minutes of incubation at 37° C. Finally wild type or antibody-activated mutant ß-galactosidase enzyme activity was determined with p-nitrophenyl-ß-D-galactopyranoside (Serva Entwicklungslabor, Heidelberg, Germany) as described (3).

RESULTS

Wild type ß-galactosidase and all antibody-activatable mutant proteins, with the exception of mutant protein 959, showed an increase in their stability against heat denaturation in the presence of specific antibodies (Figure 1). Wild type enzyme exhibits the highest stability against heat denaturation of its active site. This finding may be used to distinguish possible mixtures of wild type enzyme - and of antibody-activatable mutant protein - antibody complexes (2), i. e. by heat denaturation at 62° C.

Within the antibody-activatable mutant proteins two main groups can be distinguished by the temperature of denaturation (T_d) of their active site in the presence (+Ab) and the absence

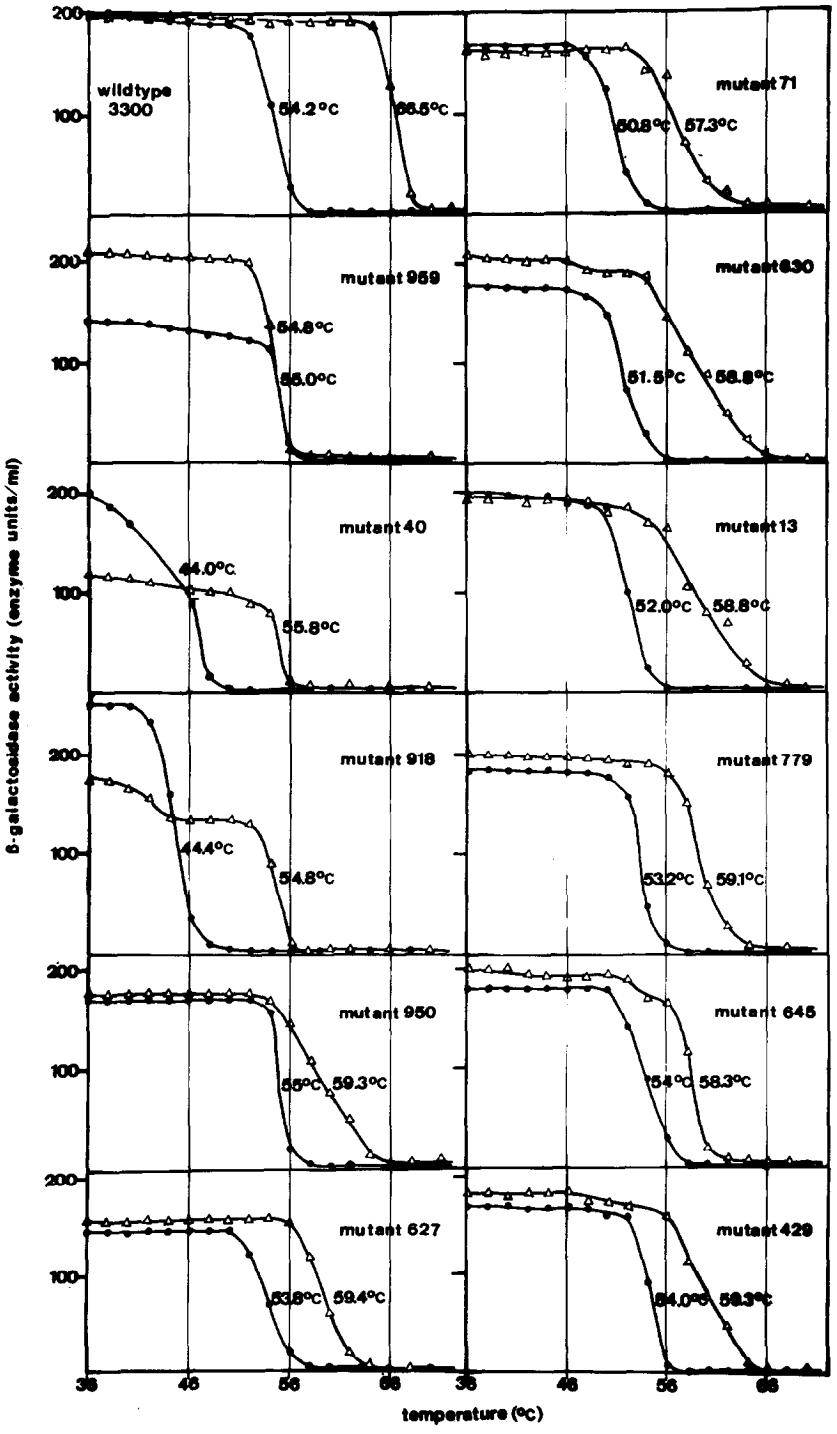
wildtype 3300
54.2°C
56.5°C
mutant 71
50.8°C
57.3°C
mutant 959
54.8°C
55.0°C
mutant 630
51.5°C
56.8°C
mutant 40
44.0°C
55.8°C
mutant 13
52.0°C
58.8°C
mutant 918
44.4°C
54.8°C
mutant 779
53.2°C
59.1°C
mutant 950
58°C
59.3°C
mutant 645
54°C
58.3°C
mutant 627
53.8°C
59.4°C
mutant 429
54.0°C
58.3°C
200
100
36
46
56
66
β-galactosidase activity (enzyme units/ml)
temperature (°C)

Fig. 1

Figure 1 Heat denaturation of wild type and mutant ß-galactosidase in the presence (—Δ—) and absence (—●—) of specific antibodies. Temperatures, at which half of the enzyme activity is inactivated (T_d), are indicated in the graphs.

(-Ab) of specific antibodies (Figure 1: Group 1 - mutant proteins 13, 71, 429, 627, 630, 645, 779, and 950 - $T_{d,\ -Ab}$ 50.5° - 55° C -, $T_{d,\ +Ab}$ 57.3° - 59.3° C, Group 2 - mutant proteins 40 and 918 - $T_{d,\ -Ab}$ 44° - 46° C; $T_{d,\ +Ab}$ 55° - 56° C). Smaller differences can be observed within one group of mutant proteins. These differences may reflect the structural and genetic differences of the mutant proteins (3). With the exception of mutant protein 959 the two groups 1 and 2 are identical with the two groups of mutant proteins, which are activated by two different populations of antibodies bound at two different antigenic sites (3).

Mutant activation (2, 3) and heat denaturation appear to be effected by different populations of anti ß-galactosidase antibodies bound at different sites on the enzyme molecule, since mutant protein 959 is activated to enzyme activity, but shows no increased stability against heat denaturation (Figure 1). By mutation protein 959 seems to have lost the antigenic site binding antibodies which stabilize the active site against heat denaturation.

Mutant proteins 40, 918, and 959 were activated to a different extent depending on whether they were preincubated and heated at temperatures below T_d in the presence of specific or of unspecific antibodies. While mutant protein 959 showed an increase, mutant protein 40 and 918 showed a decrease of activity with specific antibody. Antibodies inhibiting the activation of the mutants and differences in the incubation time needed to reach full activation of the different mutant proteins may offer explanations for these effects (4).

DISCUSSION

The majority of enzymes is inhibited in their activity by specific antibodies (5). A possible stabilization of the native conformation of the active site by specific antibodies can only be studied with enzymes, which are not inactivated by antibodies. Recently stabilization against heat denaturation of acetylcholinesterase by specific antibodies has been reported (5).

Within the population of anti wild type ß-galactosidase antibodies different functions can now be distinguished. Activation of two groups of mutant proteins (3), inhibition of mutant activation (4) and stabilization of the native conformation of the active site may well be mediated by different populations of antibodies binding to different antigenic sites on the enzyme molecule. Genetic loci within the structural gene for ß-galactosidase contributing to the establishment of the antigenic site binding heat-stabilizing antibody may be found, by searching for missense mutants of the wild type enzyme or of the antibody-activatable mutant proteins, which have lost the increased stability against heat denaturation by specific antibodies. Mutant protein 959, which is activated to enzyme activity, but not stabilized against heat denaturation, seems to be the interesting example of a single mutation (3), affecting two different functions of anti ß-galactosidase antibodies and thus probably two different antigenic sites on the enzyme.

Wild type ß-galactosidase has been used as antigen in the detection of lymphoid cells containing antibody directed against the enzyme (7). It is expected that the increased stability against heat denaturation in the presence of specific antibody may be used to improve the conditions for the quantitative detection of such antibodies on the cellular and subcellular level in eliminating

enzyme activity endogenous to or unspecifically adsorbed to lymphoid cells.

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