CARBOXYPEPTIDASE STUDIES ON β-GALACTOSIDASE: DETECTION OF ONE C-TERMINAL LYSINE PER MONOMER*

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Received December 7, 1964

The large and complex enzyme from Escherichia coli, β-galactosidase, has a molecular weight of about 520,000 (Sund and Weber, 1963). Based on studies of the number of catalytic sites (Cohn, 1957; Wallenfels and Malhotra, 1961), on measurements of sedimentation and diffusion in guanidine hydrochloride (Wallenfels, Sund, and Weber, 1963), on hybridization experiments involving heavy and light β-galactosidase molecules (Zipser, 1963), and on electron micrographs (Karlsson, et al, 1964), there seems little reason to doubt that the enzyme is composed of 4 identical monomers. It is the purpose of this communication to present evidence obtained from studies with carboxypeptidases A and B that the galactosidase monomer of molecular weight 130,000 contains one C-terminal lysine.

Galactosidase was prepared from the constitutive strain of E. coli ML 308 as previously described (Karlsson et al, 1964), except that final purification was achieved by an additional one or two passages through DEAE cellulose when necessary instead of crystallization. No contaminants or only traces could be detected in the ultracentrifuge or by starch gel electrophoresis. To denature the protein and yet keep it soluble for attack by carboxypeptidase, sodium dodecyl sulfate was added to a concentration of 0.5 per cent to galactosidase in 0.01 M Tris, 0.005 M mer-

*Supported in part by a grant, No. AI 04181 from the National Institutes of Health.

captoethanol, and 2 x 10^{-4} M $MnCl_2$, pH 7.7. The solution was then heated 10 minutes at 100°, yielding a clear solution with no galactosidase activity. Reaction with dinitrofluorobenzene showed that no new end groups were exposed, indicating that covalent bonds were not split by this treatment.

Incubation of the denatured protein with DFP-treated carboxypeptidase A or with DFP-treated carboxypeptidase B containing 0.001 M hydrocinnamic acid as inhibitor of A activity was carried out at 25°. Substrate to peptidase ratios were 10 or 20 to 1 by weight. After digestion, protein was precipitated with trichloroacetic acid to 5 per cent and the supernatant solution was lyophilized. The residue was taken up in water and lyophilized again several times to remove most of the trichloroacetic acid before analysis with an amino acid analyser.

Treatment of denatured galactosidase with carboxypeptidase A for 1 hour released no amino acids. To make certain of this, 60 mg of β-galactosidase were incubated for 3 hours with 3 mg of crystalline carboxypeptidase A. At the end of 1 hour, an aliquot corresponding to 20 mg of substrate was removed and 2 mg of carboxypeptidase A were added to the remainder. At the end of 2 hours, an additional aliquot of 20 mg was removed and 1 mg more of carboxypeptidase A was added. Just traces of amino acids were liberated during the 3 hour treatment, to an extent no greater than that obtained in a control experiment without substrate. The lack of degradation of galactosidase was not due to rapid denaturation of the peptidase since treatment of thiogalactoside transacetylase under similar conditions (to be reported elsewhere) caused rapid release of amino acids.

Carboxypeptidase B, on the other hand, did degrade galactosidase. In Experiment 1, Table I, results of a typical one hour incubation are shown. It may be seen that lysine was released in the greatest amount, followed in decreasing order by glutamine and tyrosine. Carboxypeptidase B releases basic amino acids from the free carboxyl terminus of a peptide

Table I

Amino Acids Released from β-Galactosidase by Treatment with Carboxypeptidase B

Experiment	Incubation Time, Minutes	Amino Acids micromoles/micromole of monomer of M.W. 130,000						
		Lys	$GluNH_2$	Tyr	Leu	His	Arg	Val
1	60	0.66	0.44	0.24	Trace			
2	10	0.73	0.36	0.10	Trace			Trace
	30	0.82	0.40	0.12	Trace			Trace
3	60	0.84	0.60	0.92	0.50	0.49	0.44	0.40
	120	0.83	1.64	1.43	0.88	0.78	0.59	0.58
	180	0.82	1.38	1.73	1.23	0.95	0.76	0.71

Legend to Table I.

β-galactosidase at a concentration of 15 to 20 mg per ml in buffer, pH 7.7, containing 0.5 per cent sodium dodecyl sulfate was heated 10 minutes at 100° before incubation at 25° with a highly purified sample of carboxypeptidase B. Reaction mixtures contained 0.001 M hydrocinnamic acid to partially inhibit A activity. For Experiment 1, 17 mg of substrate were treated with 1 mg of the peptidase, and in Experiment 2, 40 mg of galactosidase were incubated with 2 mg of the peptidase. In Experiment 3, 61 mg of protein were treated initially with 2.7 mg of enzyme. At 60 minutes, an aliquot of one third was removed and 1 mg more enzyme was added to the incubation mixture. At 120 minutes, one half of the remaining reaction mixture was removed and 0.7 mg more carboxypeptidase was added. Reaction was stopped with trichloro-

acetic acid; the supernatant solutions were dried and analysed with an amino acid analyser. Controls were of two kinds: 1) complete system at zero time, and 2) incubation for the same time periods without substrate. The values shown in the Table are those obtained after subtraction of the very low levels found in the control experiments. In addition to the amino acids shown above, others were found in lesser amounts.

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chain. Since the preparations used here contained low levels of A activity, this would account for the liberation of both basic and neutral amino acids. Similar results were obtained in another experiment (2, Table I) in which aliquots were removed from an incubation mixture after 10 and 30 minutes. Here also the sequence at the C-terminal end appears to be ....Tyr-GluNH_2-LysCOOH.

The absolute amount of amino acids released by peptidase action varied in the different experiments, but the amounts of lysine released were always less than 1 micromole per micromole of monomer (130,000). In order to determine the maximum amount of lysine that could be removed, and thereby the number of chains ending in lysine within the monomer, denatured protein was incubated with carboxypeptidase B for 3 hours. At the end of the first and the second hours, aliquots were removed for analysis and additional carboxypeptidase was added. It may be seen (Experiment 3) that release of lysine did not exceed approximately 0.84 micromoles, even though glutamine, tyrosine and leucine were obtained in quantities greater than 1 micromole per micromole of monomer in the longest incubation period.

Physical studies by Wallenfels and his collaborators indicate that β-galactosidase may be dissociated by performic acid oxidation (Wallenfels, Sund, and Weber, 1963) or by glacial acetic-formic acids (Weber, Sund, and Wallenfels, 1964) to yield polypeptide chains of approximately 30,000 or 40,000 in molecular weight. In order to determine whether such treatment would cause release of amino acids by carboxypeptidase different than that

shown in Table I, a sample of β-galactosidase was oxidized with performic acid (Hirs, 1956). Fifteen mg of lyophilized product were then dissolved in buffer containing 0.5 per cent detergent and treated with 1.5 mg of carboxypeptidase A as before for one hour. No hydrolysis was detected. Another aliquot of the oxidized protein was treated similarly with carboxypeptidase B for 1 hour to yield per micromole of monomer 0.63 micromoles of lysine, 0.43 micromoles of glutamine, and smaller amounts of other amino acids. Therefore, no additional lysine was uncovered by performic acid oxidation. It is evident that there can be only one polypeptide chain ending in lysine in the β-galactosidase monomer. It follows, if the measurements made by Wallenfels and his collaborators are correct, that different polypeptide chains are present in β-galactosidase, one which ends in ...Tyr-GluNH_2-LysCOOH, and another or others which are not susceptible to attack by carboxypeptidase.

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