BBA 66621

GLUCONO-δ-LACTONASE FROM *ESCHERICHIA COLI*

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(Received February 7th, 1972)

SUMMARY

Escherichia coli K12 has been shown to contain a glucono-δ-lactonase (D-glucono-δ-lactone hydrolase, EC 3.1.1.17). A convenient assay has been developed which is based on the spectral change of a pH indicator during the hydrolysis of the lactone. The enzyme has been purified about 105-fold. Its substrate specificity has been investigated. The enzyme is inactivated by 10 mM EDTA and is not inactivated by 0.1 mM *p*-chloromercuribenzoate (PCMB).

INTRODUCTION

In connection with our investigations of the *Escherichia coli* aldose mutarotase (aldose 1-epimerase, EC 5.1.3.3) we wanted to know if this enzyme splits as well as the sugar cyclosemiacetal the structurally similar ring of the sugar lactone[1]. Our enzyme preparations indeed hydrolyzed glucono-δ-lactone, but we were able to show that this reaction was not caused by the mutarotase itself but by an impurity, a lactonase (D-glucono-δ-lactone hydrolase, EC 3.1.1.17) not yet described in *E. coli*. We do not intend to deal with this enzyme comprehensively, but we investigated some of its properties sufficient for its characterization and for its separation and discrimination from mutarotase.

METHODS AND RESULTS

Reagents

D-Glucono-δ-lactone was obtained from K and K Laboratories Inc., D-ribono-γ-lactone and D-galactono-γ-lactone from Mann Res. Lab. (New York), L-gulono-γ-lactone and D-heptaglucono-δ-lactone from Fluka AG (Buchs) and D-glucosaccharic acid dilactone from Roth (Karlsruhe). All other reagents were usual commercial products.

Abbreviation: PCMB, *p*-chloromercuribenzoate.

Assay of glucono-δ-lactonase

There are two lactonase assays described in the literature. Brodie and Lipmann[2] determined, after incubation of the lactone with the enzyme, the residual substrate (according to the method of Hestrin[3]) as ironhydroxamic acid complex. Winkelman and Lehninger[4] determined the amount of CO_2 set free with a Warburg manometer from a bicarbonate buffer by the resulting aldonic acid. The latter method has the drawback of being limited to the narrow pH range of the buffer. The former is not very reliable, since in the alkaline assay mixture N_2 evolves from hydroxylamine which forms bubbles on the cuvette walls and makes the measurement of the extinction of the complex difficult. We developed a convenient optical assay which is based on the change in absorbance of a nitrophenol pH indicator caused by the formation of free acid from the lactone. The substrate solution is prepared immediately before the addition of the enzyme: 10 ml of a 0.25 mM *p*-nitrophenol solution in 10 mM phosphate buffer pH 6.8, are added to 18.5 mg D-glucono-δ-lactone. (The resulting final substrate concentration in the cuvette is 10 mM.)

After the addition of the *p*-nitrophenol to the lactone the stop watch is started. In a cuvette, 0.1 ml protein solution is mixed with 2.5 ml substrate solution. After 2 and 7 min the absorbance at 405 nm is measured (25 °C). The control, the non-enzymatic hydrolysis, causes during that time interval a change in absorbance of $\Delta A_{\text{spontaneous}} = 0.244$. The enzymatic activity is defined as the difference ΔA_E between the change of absorbance during the enzymatically catalyzed and the spontaneous lactone hydrolysis. The linearity of the assay is shown in Fig. 1. Activity is expressed as ΔA_E per mg protein in the assay.

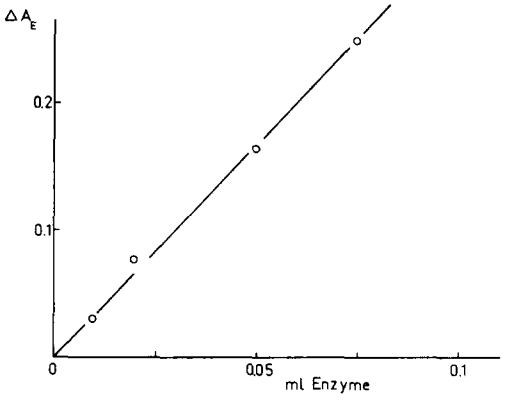

Fig. 1. Linearity of the lactonase assay. Assay conditions as described in the text.

This assay is commendable especially for probing the many protein fractions during the purification of the enzyme. For precise kinetic investigations the pH-stat yields better results, though this method is much more time consuming.

Partial purification of the enzyme

We accomplished about a 105-fold purification as compared to the cell free extract, following the purification procedure for the mutarotase[1] up to column

chromatography with Sephadex G-75 and separating the mutarotase and the lactonase on a Sephadex G-75 column with 10 mM phosphate buffer, pH 6.8, as elution buffer. The elution volume for the lactonase is slightly higher than for the mutarotase, indicating a lower molecular weight (mutarotase = 30 000[5]). ΔA_E increases from 0.015/mg in the crude extract to 1.57/mg in the preparations with the highest puritiy.

The highly purified enzyme rapidly loses activity and further purification was difficult.

Substrate specificity

Table I shows glucono-δ-lactone to be the best substrate. Among the other investigated aldono lactones only galactono-γ-lactone is hydrolyzed in significant amounts. Neither the γ-lactones of the ribonic, L-gulonic and heptagluconic acid nor D-glucosaccharic acid dilactone and glucuronolactone are substrates of *E. coli* lactonase.

TABLE I

SUBSTRATE SPECIFICITY

Relative rate of hydrolysis of various γ- and δ-lactones (D-glucono-δ-lactone = 100). Assay conditions as described in the text. Substrate concentrations 10 mM.

Substrate	*Rel. hydrolysis rate*
D-Glucono-δ-lactone	100
D-Galactono-γ-lactone	15
L-Gulono-γ-lactone	0
D-Ribono-γ-lactone	0
Heptagluconic acid γ-lactone	2
D-Glucosaccharic acid dilactone	2
D-Glucuronolactone	0

It should be mentioned that *p*-nitrophenyl acetate is readily hydrolyzed. This could mean that we are dealing with an esterase rather than a lactonase. But we have shown that esterases like papain and lipase (wheat) do not hydrolyze galactono-γ-lactone significantly.

Requirement for bivalent ions

Addition of bivalent ions to purified lactonase does not increase the enzymatic activity. 10 mM EDTA on the other hand inactivates the enzyme (which represents a significant difference to mutarotase). Addition of equal concentrations of Mg^{2+} or Ca^{2+} protects against this inactivation.

SH-groups

SH-groups do not seem to be essential for the lactonase activity. Unlike mutarotase *E. coli* lactonase is not inactivated by 25 min incubation with 10^{-4} M PCMB at 25 °C.

DISCUSSION

Cohen and Scott[6,7] have shown that part of the glucose in *E. coli* is converted to

pentose by oxidation *via* gluconolactone, gluconic acid, and 2-oxogluconic acid. Between 14 and 37% of the available glucose can be used this way, and the major portion of the ribose is formed by this pathway[8]. During their investigations of the respective metabolic pathways in other organisms Cori and Lipmann[9] postulated an enzyme capable of hydrolyzing the formed aldono lactone to the open chain acid. Such an enzyme was found in baker's yeast, rat liver, and *Azotobacter vinelandii*[2]. We found this enzyme to occur also in *E. coli*.

TABLE II

COMPARISON OF LACTONASES FROM DIFFERENT SOURCES

I, glucono-6-phospholactonase; II, glucono-δ-lactonase; III, uronolactonase. + and − indicate whether or not a substance is substrate or effector, respectively.

Substrate or effector	*Source of lactonase*					
	Yeast[10]		*Ps. fluorescens*[11]	*Rat liver*[4]		*E. coli*
	I	*II*		*II*	*III*	
Glucono-δ-lactone	−	+	+	+	−	+
Galactono-γ-lactone	−	+	−	+	−	+
Glucurono-γ-lactone	−	−	−	−	+	−
Gulono-γ-lactone	−	+	−	+	−	−
p-Nitrophenyl acetate	+	+			+	+
Bivalent ions	−	+		+	+	+
PCMB				+	+	−
PO_4	−	−	+	−	−	−

Comparing *E. coli* lactonase with lactonases from other organisms (Table II) we can state some differences in its substrate specificities. In contrast to the yeast enzyme[10] it does not hydrolyze L-gulono-γ-lactone, also, unlike the enzyme from *Pseudomonas fluorescens*[11] it does hydrolyze galactono-γ-lactone. The data available on the rat liver glucono-δ-lactonase indicate that this enzyme in contrast to the *E. coli* lactonase is sensitive to PCMB, requires Mn^{2+} and hydrolyzes gulono-γ-lactone[4].

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