DISRUPTION OF ACTIVITY OF INDUCED PERMEASES BY TRIS(HYDROXYMETHYL)AMINOMETHANE IN COMBINATION WITH ETHYLENEDIAMINETETRAACETATE[1]

R. G. Eagon and Mary Ann Asbell

Department of Bacteriology
University of Georgia
Athens, Georgia 30601

Received May 31, 1966

In a previous publication (Asbell and Eagon, 1966), we reported that osmotically fragile rods (i.e., osmoplasts) resulted when Pseudomonas aeruginosa strain OSU 64 was incubated with ethylenediaminetetraacetate (EDTA) and tris(hydroxymethyl)aminomethane (Tris) buffer. Osmoplasts resulting from this treatment could be restored to osmotically stable cells by the addition of multivalent cations.

In a forthcoming publication (J. Bacteriol.), we showed (1) that the highest percentage of viable cells capable of multiplication resulted when osmoplasts were restored with the same divalent cations as those detected in the cell wall; and (2) that the percentage of survivors was increased seven-fold when phosphate buffer was substituted for Tris buffer. Thus, from these studies, and from studies by Goldschmidt and Wyss (1966) as well, it was concluded that Tris buffer compounded the lethal effects of EDTA.

We also observed that restored cells were incapable of forming an

[1]This investigation was supported in part by a Public Health Service Research Grant (AI-05156-04) from the National Institute of Allergy and Infectious Diseases awarded to the senior author and by a PHS Pre-Doctoral Fellowship (1-FM-GM-30,204-02) from the National Institute of General Medical Services awarded to the junior author.

induced permease to citrate if the osmoplasts had been prepared in Tris buffer. Osmoplasts prepared in phosphate buffer, however, and restored with Ca^{++}, Mg^{++} or Mn^{++}, but not with Zn^{++} or La^{+++}, were able to form an induced permease to citrate. Thus, the investigations described in this paper were undertaken to provide further information on the effect of Tris buffer and EDTA on induced permeases.

EXPERIMENTAL METHODS

P. aeruginosa was cultivated 14-16 hr at 37 C on a rotary shaker in the basal salts solution of Grelet (1951) to which was added 30 g of glucose or 10 g of either mannitol or sodium citrate per liter. Yeast extract (3 g/l) was added to the glucose medium but not to the mannitol or citrate media.

Osmoplasts were prepared in Tris buffer as follows: 1 μmole EDTA, pH 8, 33 μmoles Tris buffer, pH 8, and 3.6×10^9 cells per ml of 0.55 M sucrose. After incubation for 10 min at room temperature, osmoplasts were restored by adding cations to give a final concentration per ml as follows: Ca^{++}, 1 μmole; Mg^{++}, 1.3 μmole; and, Zn^{++}, 0.6 μmole.

Osmoplasts were prepared in phosphate buffer as follows: 10 μmoles EDTA, pH 8, 33 μmoles phosphate buffer, pH 8, and 3.6×10^9 cells per ml of 0.55 M sucrose. After incubation for 30 min at room temperature, osmoplasts were restored by adding cations to give a final concentration per ml as follows: Ca^{++}, 10 μmoles; Mg^{++}, 13 μmoles; and, Zn^{++}, 6 μmoles. The higher concentration of EDTA and the longer period of incubation were necessary to achieve results equivalent to the Tris buffer system as evidenced by the rate and extent of lysis in the absence of hypertonic sucrose solution.

Restored cells were collected by centrifugation at 3,500 x g for 12

min and resuspended in water.

The rate and extent of formation of induced permeases and of dissimilation of substrates were measured by the Warburg respirometer at 30 C. Each Warburg vessel contained 4 μmoles substrate, 800 μmoles phosphate buffer, pH 7, 2 ml normal or restored cell suspension (3.6×10^9 cells/ml) and 0.2 ml of 40% KOH in the center well to give a total volume of 3.2 ml. Endogenous activity was subtracted.

RESULTS

The experimental results shown in Fig. 1 indicate that induced permeases to citrate, fructose and mannitol were formed by normal cells of

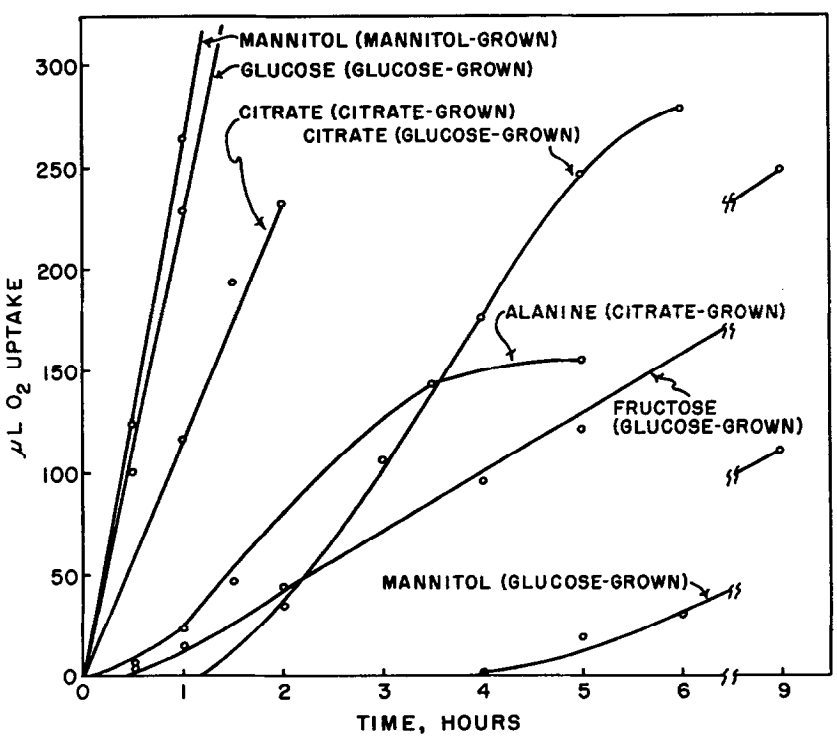

Fig. 1. Comparison of rates of dissimilation of various substrates and of formation of induced permeases by resting normal cells of Pseudomonas aeruginosa. Normal cells are defined as those cells that were handled in the same manner as restored osmoplasts prepared in Tris buffer but were not exposed to EDTA.

glucose-grown P. aeruginosa. This microorganism has been reported to be cryptic to citrate (Kogut and Podoski, 1953; Barrett, Larsen and Kallio, 1953) and to fructose (Eagon and Williams, 1960). In the case of mannitol, it is probable that an induced dehydrogenase and/or kinase must be formed in addition to the permease. P. aeruginosa is constitutively permeable to glucose (Eagon and Williams, 1960) and all bacteria are believed to be permeable to amino acids (Kepes and Cohen, 1962). The latter is borne out by results shown in Fig. 1. Although not shown here, the response to glutamate was nearly identical with that to alanine.

The data in Fig. 1 also show that cells cultivated on citrate or mannitol oxidized these substances without a lag period as evidenced by an immediate uptake of oxygen.

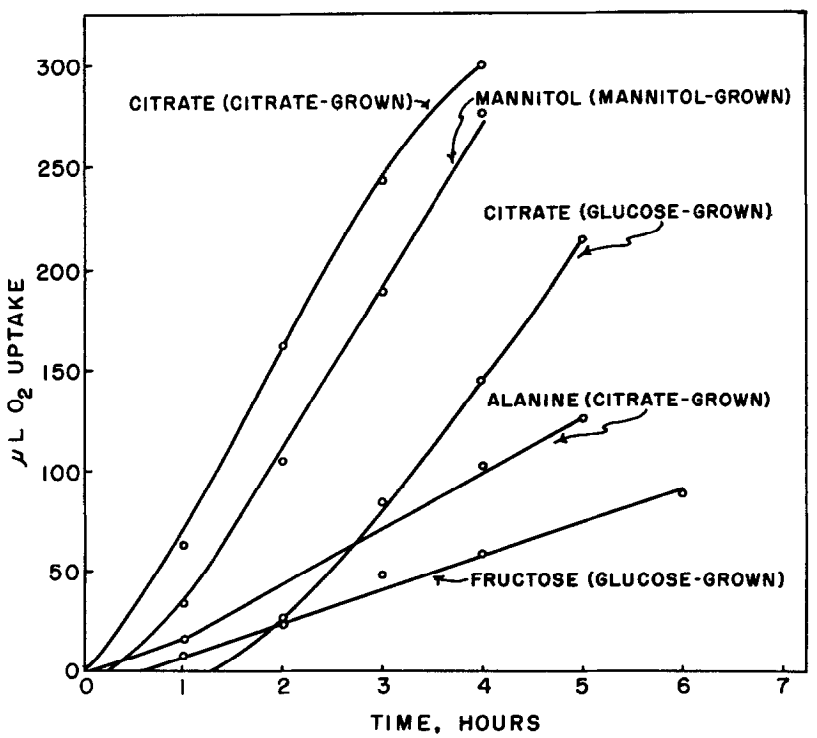

Fig. 2. Comparison of rates of dissimilation of various substrates and of formation of induced permeases by restored osmoplasts of Pseudomonas aeruginosa prepared in phosphate buffer.

The data depicted in Fig. 2 show results using cells restored after treatment in EDTA and phosphate buffer. Restored cells which had been previously cultivated on glucose were capable of forming induced permeases to citrate and to fructose. Mannitol was not used as substrate in this experiment. Restored cells which had been grown on citrate or mannitol, furthermore, retained the induced systems and oxidized these substances without a period of induction. Similarly, alanine and glutamate were oxidized without induction.

The results shown in Fig. 3 indicate data derived from cells restored after treatment in EDTA and Tris buffer. Here it is seen that glucose-grown restored cells were incapable of forming induced permeases to fructose and

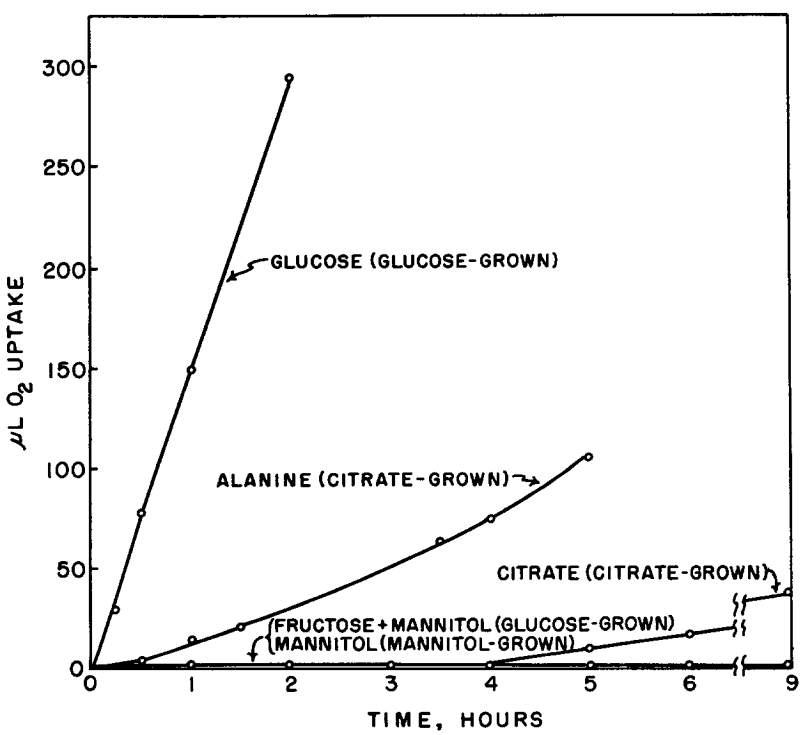

Fig. 3. Evidence that the activity of induced permeases are lost by restored osmoplasts of Pseudomonas aeruginosa prepared in Tris buffer but that penetration of substrates to which the cells are constitutively permeable (i.e., glucose and alanine) is unaltered.

mannitol. Moreover, restored cells that had been cultivated on citrate or mannitol lost their ability to transport these substances. The low rate of activity detected for citrate after 4 hr incubation may have been due to intact cells which escaped the effects of EDTA and Tris buffer. A low concentration of survivors from this treatment is always observed (Asbell and Eagon, 1966).

Penetration and dissimilation of substrate to which cells are constitutively permeable, on the other hand, were uneffected by EDTA and Tris buffer as indicated by the oxidation of glucose and alanine (Fig. 3). Similarly, glutamate was also immediately dissimilated by citrate-grown restored cells.

DISCUSSION

We have presented evidence that treatment of pre-induced cells of P. aeruginosa with EDTA and Tris buffer resulted in loss of the induced permease activity by restored cells. Similarly, uninduced restored cells in the presence of substrate did not exhibit induced permease activity after treatment with EDTA and Tris buffer. Whether the latter were unable to synthesize induced permeases or whether induced permeases were formed but their activity was not expressed could not be discerned from these experiments.

Cells incubated with EDTA and Tris buffer and then restored were able to dissimilate substrates to which they were constitutively permeable (e.g., glucose, alanine or glutamate) in a normal fashion.

Neither EDTA nor Tris buffer alone had this effect. Incubation with EDTA and phosphate buffer followed by restoration resulted in cells with the ability to form and to retain induced permease activity. Similarly, incubation of cells with Tris buffer in the absence of EDTA did not impair their ability

to form and to retain induced permease activity.

We cannot at this time offer an explanation for the mechanism of action of the EDTA-Tris combination. Goldschmidt and Wyss (1966) suggested that stronger chelation resulted from the EDTA-Tris complex which in turn resulted in a lethal effect on cells and cysts of Azotobacter. We have also observed that Tris buffer compounded the lethal effects of EDTA for P. aeruginosa.

Our evidence points to the cell membrane as the site of attack by the EDTA-Tris complex. It appears that induced permeases were displaced, removed or inactivated by this complex. Whether this is due to chelation effects cannot be discerned at this time. Our evidence indicated, moreover, a common site of attack toward all induced permeases.

SUMMARY

Incubation of P. aeruginosa with EDTA and Tris buffer resulted in a loss of induced permease activity of pre-induced restored cells and in the inability of such restored cells to form induced permease activity in the presence of inducers.

REFERENCES

Asbell, M. A. and Eagon, R. G. Biochem. Biophys. Res. Commun., 22, 664 (1966).
Barrett, J. T., Larson, A. D. and Kallio, R. E. J. Bacteriol., 65, 187 (1953).
Eagon, R. G. and Williams, A. K. J. Bacteriol., 79, 90 (1960).
Goldschmidt, M. C. and O. Wyss. J. Bacteriol., 91, 120 (1966).
Grelet, N. Ann. Inst. Pasteur, 81, 430 (1951).
Kepes, A. and Cohen, G. N. In The Bacteria, Vol. IV, Academic Press, N. Y., p. 179 (1962).
Kogut, M. and Podoski, E. P. Biochem. J., 55, 800 (1953).