The Formation of D-3-Phosphoglyceric Acid from Pentoses by *Escherichia coli*

By E. D. BERGMANN

Scientific Department, Israeli Ministry of Defence, Tel-Aviv

AND U. Z. LITTAUER AND B. E. VOLCANI

The Weizmann Institute of Science, Rehovoth, Israel

(Received 31 January 1953)

Whilst the breakdown of glucose to D-3-phosphoglyceric acid (PGA) has been observed in a number of micro-organisms (Werkman, Zoellner, Gilman & Reynolds, 1936; Stone & Werkman, 1936*a, b*, 1937; Wood, Stone & Werkman, 1937; Still, 1940), the formation of PGA from pentoses has so far only been observed in the case of *Propionibacterium pentosaceum* (Barker & Lipmann, 1949). The present investigation deals with the transformation of pentoses to PGA by *Escherichia coli*; it forms part of a study of the pathways of pentose fermentation. Since the fermentation of pentoses has been shown to be adaptive (Karström, 1938; Cohen & Scott, 1951; Cohen & Raff, 1951; Heald, 1952), the experiments were carried out with cells which had been grown upon media containing D-xylose and L-arabinose, respectively, and, for comparison, with cells which had been grown previously in the presence of glucose.

MATERIALS AND METHODS

Cultures. Cultures of *Esch. coli* (ATCC 9637) were maintained on 0.5% (w/v) Difco yeast extract slants with the addition of 0.2% (w/v) of the corresponding sugar. Cells from an 8-hr.-old agar slant culture were inoculated into 1.5 l. of a mineral medium (Cohen, 1949) contained in a 4 l. Erlenmeyer flask. Some experiments were carried out in a 0.5% Difco yeast extract liquid medium containing 0.05% sugar. The cultures were aerated vigorously and the cells harvested after 13–15 hr. and washed twice with 0.85% chilled KCl solution. Incubation temperature, 37°.

Materials. The following carbohydrates were used as substrates: D-glucose (C.P. analysed, Baker Chemical Co.); D-xylose, L-arabinose and D-lyxose (Pfanstiehl Chemical Co.); maltose, D-ribose and D-arabinose (Nutritional Biochemicals Corporation); glucose 1-phosphate (Bios Laboratories Inc.). Adenosine triphosphate (ATP) was used as the sodium salt, prepared from the commercial diarium salt (Delta Chemical Works).

Experimental conditions. The experimental conditions were similar to those described by Stone & Werkman (1937): wet cells, 0.35 or 0.70 g.; sugar, 100–500 μ moles; phosphate buffer (pH 6.8), 0.134 M; $MgCl_2$, 6H₂O, 0.00143 M; acetaldehyde, 360 μ moles; NaF, 0.0086 M; toluene, 0.1 ml.; total

volume, 3.5 ml. The mixture was shaken aerobically in a 25 ml. Erlenmeyer flask for 4 hr. at 37°. A 50% (w/v) trichloroacetic acid solution (0.4 ml.) was added and the precipitate removed by centrifuging. The supernatant was freed from inorganic phosphate by the addition of 0.4 ml. of magnesia mixture.

Analytical methods. Phosphoglyceric acid was determined polarimetrically (Meyerhof & Schultz, 1938), pentose by the orcinol reaction according to Drury (1948), and the total reducing sugar by the method of Folin & Malmros (1929).

RESULTS

Cells grown previously on pentose. The aerobic formation of PGA from pentose, glucose and maltose by cells which had been adapted to D-xylose and L-arabinose in mineral media is shown in Table 1. In all cases PGA was formed from maltose and glucose, whilst in the case of the pentoses, PGA was formed only from the sugar to which the cells were adapted; similar results were obtained with cells grown on yeast extract medium. Attempts to adapt the organism to grow in a synthetic medium containing D-ribose, by successive subculturing in the presence of this pentose, were unsuccessful.

Cells grown previously on glucose. Table 1 shows that cells grown in glucose-containing medium produced, under aerobic conditions, considerable amounts of PGA from glucose and maltose, whilst only small quantities were formed from L-arabinose, D-ribose, and D-lyxose. The results obtained with D-xylose and D-arabinose were surprising: not only was no PGA formed when small amounts of these sugars were used, but addition of larger amounts reduced the quantity of PGA formed to below that produced by the blank (PGA formed in absence of added substrate). Furthermore, in the case of L-arabinose-grown cells addition of D-arabinose and D-xylose decreased the endogenous PGA formation, whilst with xylose-grown cells only D-arabinose showed this effect. When the yeast extract medium was employed, the blank values were much higher, and consequently the effect was even more pronounced.

Table 1. *Aerobic formation of D-3-phosphoglyceric acid by cells of Esch. coli grown on D-glucose, L-arabinose and D-xylose*

(0.7 g. wet cells grown on mineral medium, buffer containing 0.134M sodium and potassium phosphates, pH 6.8, 0.00143M-MgCl₂, 6H₂O, 0.0086M-NaF, 360 μmoles acetaldehyde, 0.1 ml. toluene. Total volume, 3.5 ml. Incubation time, 4 hr. Temp. 37°.)

Substrate	Concn. (μmoles)	Cells grown on D-glucose		Cells grown on L-arabinose		Cells grown on D-xylose	
		PGA (μmoles)	ΔPGA* (μmoles)	PGA (μmoles)	ΔPGA (μmoles)	PGA (μmoles)	ΔPGA (μmoles)
Maltose	120	157.5	146.6	144.5	137.6	160.6	156.4
D-Glucose	120	83.3	72.4	79.0	72.1	80.0	76.8
D-Ribose	120	15.0	4.1	10.0	3.1	8.4	4.2
L-Arabinose	120	13.7	2.8	83.9	77.0	7.4	3.2
D-Arabinose	120	10.0	-0.9	9.5	2.6	3.0	-1.2
D-Arabinose	600	4.9	-6.0	0.7	-6.2	0.0	-4.2
D-Xylose	120	9.4	-1.5	6.9	0.0	35.2	31.0
D-Xylose	600	1.0	-9.9	0.0	-6.9	117.9	113.7
D-Lyxose	120	11.4	0.5	10.3	3.4	12.9	8.7
No addition	—	10.9	—	6.9	—	4.2	—

* ΔPGA = difference between PGA formed and control (incubated without sugar).

Table 2. *Formation of phosphoglyceric acid from glucose 1-phosphate in the presence or absence of D-xylose by glucose-grown cells of Esch. coli*

(0.18 g. wet cells grown on yeast extract medium; buffer containing 0.134M sodium and potassium phosphates, pH 6.8, 0.00143M-MgCl₂, 6H₂O, 0.0086M-NaF, 360 μmoles acetaldehyde, 0.1 ml. toluene. Total volume, 3.5 ml. Incubation time, 4 hr. Temp. 37°.)

Substrate	Concn. (μmoles)	PGA (μmoles)	ΔPGA* (μmoles)
None	—	2.6	—
D-Xylose	500	0.0	-2.6
Glucose 1-phosphate	7.8	8.5	—
Glucose 1-phosphate + D-xylose	7.8 500	0.0	-8.5
Glucose 1-phosphate	39	30.1	—
Glucose 1-phosphate + D-xylose	39 500	10.0	-20.1

* ΔPGA = difference between PGA formed and control (incubated without xylose).

Table 3. *Anaerobic formation of D-3-phosphoglyceric acid by D-xylose-grown cells of Esch. coli*

(0.35 g. wet cells grown on mineral medium, buffer containing 0.134M sodium and potassium phosphates, pH 6.8, 0.00143M-MgCl₂, 6H₂O, 0.055M-KF, 360 μmoles acetaldehyde, 25 μmoles ATP, 0.1 ml. toluene. Total volume, 3.5 ml. Incubation time, 4 hr. Temp. 37°.)

Substrate	Concn. (μmoles)	PGA (μmoles)	ΔPGA* (μmoles)
Maltose	120	89.0	88.5
D-Glucose	120	69.8	69.3
D-Ribose	120	2.9	2.4
L-Arabinose	120	3.9	3.4
D-Xylose	120	80.7	80.2
D-Xylose, no ATP	120	8.0	—
No sugar and ATP added	—	0.5	—

* ΔPGA = difference between PGA formed and control (incubated with ATP and without sugar).

Table 4. *Effect of phosphate and potassium ions on the formation of D-3-phosphoglyceric acid from xylose by xylose-grown cells of Esch. coli*

(0.35 g. wet cells grown on yeast extract medium, 0.00143M-MgCl₂, 6H₂O, 0.055M-NaF, 360 μmoles acetaldehyde, 25 μmoles ATP, 0.1 ml. toluene. Total volume, 3.5 ml. Incubation time, 4 hr. Temp. 37°.)

Substrate	Concn. (μmoles)	Sodium and potassium phosphates (μmoles)	PGA (μmoles)
None	—	469	4.5
Xylose	120	117	34.6
Xylose	120	234	40.6
Xylose	120	469	62.3
Xylose	120	469*	81.9

* Sodium and potassium phosphates and NaF were replaced by 469 μmoles KH₂PO₄ and K₂HPO₄ and 0.055M-KF.

It was thought that xylose might have been converted under these conditions into a phosphate ester. However, determination of the amount of pentose phosphate (orcinol reaction) in the barium-soluble, alcohol-insoluble fraction (Umbreit, Burris & Stauffer, 1945) showed that even less phosphorylated pentose was formed from xylose than in the blank. D-Xylose also inhibited the formation of PGA from glucose 1-phosphate (Table 2). In these experiments the quantity of cells used was decreased in order to reduce the formation of endogenous PGA.

Anaerobic PGA formation. The cells were grown in a mineral medium containing D-xylose as above, but the experiments were carried out under anaerobic conditions in evacuated Thunberg tubes. Table 3 shows that PGA was formed from xylose, glucose and maltose in the presence of ATP. In the case of xylose, 8 μmoles of PGA were formed without ATP as compared with 80 μmoles with ATP.

Table 5. *Pentose formation from glucose by glucose-grown cells of Esch. coli*

(0.5 g. wet cells grown on yeast extract medium, buffer containing 0.35 M sodium and potassium phosphates at pH 6.8, 0.00143 M-MgCl₂, 6H₂O, 0.0086 M-NaF, 300 μ moles sodium fumarate, 0.1 ml. toluene. Total volume, 3.5 ml. Incubation time, 4 hr. Temp. 37°.)

Substrate	PGA (μ moles)	Pentose (μ moles)				Pentose phosphate (μ moles)	Glucose utilized (μ moles)
		0 hr.	4 hr.	Pentose formed	Δ Pentose*		
None	24.1	1.5	10.8	9.3	—	2.1	—
Glucose, 160 μ moles	37.6	1.5	19.4	17.9	8.6	2.7	21.2

* Δ Pentose = difference between pentose formed from glucose and in the control (incubated without glucose).

Table 6. *Determination of free and protein-bound pentose in glucose-grown cells of Esch. coli*

(Bacterial cell suspension as in Table 5.)

Substrate	(1)		(2)	(3)		(4)	(5)
	Free pentose (μ moles)		Change in free pentose (μ moles)	Protein-bound pentose* (μ moles)		Change in protein-bound pentose (μ moles)	Increase of pentose formation (μ moles) ((2) - (4))
	(0 hr.)	(4 hr.)		(0 hr.)	(4 hr.)		
None	1.5	18.0	16.5	12.0	0.0	-12.0	4.5
Glucose, 120 μ moles	1.4	24.2	22.8	12.2	0.3	-11.9	10.9

* Protein-bound pentose = total pentose - free pentose.

Effect of potassium and phosphate. In the anaerobic experiments with xylose as substrate, replacement of the sodium ion of the buffer (Na₂HPO₄) by potassium (K₂HPO₄) increased PGA formation by 28%. A relationship was found to exist between the increase of the phosphate concentration and the production of PGA (Table 4).

Isolation of PGA. From experiments with D-xylose, D-3-phosphoglyceric acid was isolated in the form of its acid barium salt, [α]_D²⁰ - 754° in the presence of molybdate (Meyerhof & Schultz (1938) give [α]_D²⁰ - 745°). (Found: P, 8.5. C₃H₅O₇PBa, 2H₂O requires 8.7%.)

Formation of pentose from glucose. The formation of PGA from glucose by glucose-grown cells of *Esch. coli* is not the only process occurring. In addition to PGA, small amounts (0.1-0.4 μ moles) of pentose were formed when glucose-grown cells were incubated in the presence of glucose with fumarate as the oxidizing agent. Part of this pentose was found in the barium-soluble, alcohol-insoluble fraction (Umbreit *et al.* 1945) and probably occurs as a phosphate ester. The results of a typical experiment are given in Table 5.

The possibility that the net formation of pentose (Δ pentose in Table 5) was due to breakdown of the nucleic acids of the cell, was eliminated by an experiment using the method of Schneider (1945). Samples of the suspensions (0.1 ml.) with and without glucose, as described in Table 5, were removed before the incubation and 4 hr. later. To each sample, 4.9 ml. of a 5% (w/v) trichloroacetic acid solution was added. The acid-soluble pentose was determined in the filtrate after treatment with tri-

chloroacetic acid at 5° for 30 min., and the total pentose (acid-soluble plus nucleic acid pentose) was determined after the treatment at 90° for 15 min.

The data given in Table 6 show that the net increase of 6.4 μ M of the acid-soluble pentose formed in the presence of glucose (increase of pentose formed in the presence of glucose minus increase of pentose formed in its absence) (column 5) did not derive from the nucleic acids of the bacterial cells.

DISCUSSION

In the experiments described here, PGA is formed from pentoses, although not to the extent of 1 mole per mole of pentose utilized; the maximum yield obtained was 80% of that quantity. The difference may be attributed to the incompleteness of the inhibition caused by fluoride, rather than to a mechanism of pentose degradation different from the fission into C₂ and C₃.

Stone & Werkman (1936a, b, 1937) have shown that toluene-treated cells of *Esch. coli* form substantial amounts of PGA from glucose in the presence of hexose diphosphate (HDP) under both aerobic and anaerobic conditions. In the present investigation, it was found that the anaerobic breakdown of xylose by toluene-treated suspensions of *Esch. coli* requires the addition of small quantities of ATP, whilst in the case of the aerobic breakdown no additional ATP is required. It is assumed that the aerobic system can generate energy-rich phosphate bonds and thus dispense with an extraneous source of them.

In its ability to transform pentoses into PGA, *Esch. coli* resembles *Propionibacterium pentosaceum* (Barker & Lipmann, 1949). The enzyme system involved in this process (or at least one enzyme of this system) is adaptive in nature, as the conversion of a pentose to PGA was only observed in cells which had been grown previously in presence of that pentose (see Ley & Cornut, 1951; Heald, 1952).

In striking contrast to this result stands the observation that glucose-grown cells of *Esch. coli* do not convert D-xylose and D-arabinose into PGA (L-arabinose and D-ribose give small amounts of the acid); moreover, when D-xylose and D-arabinose are used in large enough amounts, the quantity of PGA formed is less than that which the cells produce in absence of any added substrate. L-Arabinose-grown cells behave similarly; but in the case of D-xylose-grown cells, only D-arabinose inhibits the production of PGA. It has been shown that this effect is not due to a phosphorylation of the inhibitory pentose. The endogenous PGA is presumably formed from the reserve carbohydrate of the bacterial cells by glycolysis. The fact that xylose inhibits the formation of PGA from glucose 1-phosphate tends to indicate that at least one of the effects of the xylose consists in interference in a reaction subsequent to the phosphorylation of the cell carbohydrate by phosphorylase.

This inhibitory action of pentose recalls the finding of Doudoroff, Hassid, Putman, Potter & Lederberg (1949) that the amylomaltase of *Esch. coli* (Monod & Torriani, 1948) is inhibited by D-xylose. However, whilst amylomaltase is not affected by D-arabinose, in the present investigation this sugar behaved like D-xylose. Thus, the resemblance between the two phenomena is incomplete, and no definite conclusion can be drawn as to the point at which the pentose molecule blocks the pathway of the formation of endogenous PGA.

In the experiments with glucose, it was found that *Esch. coli* transforms this sugar not only into PGA, but also into significant amounts (10–40 μ moles/100 μ moles of glucose utilized) of pentose, partly in the form of its phosphate. This fact is in accord with the observation of Cohen (1951) and Scott & Cohen (1951) that glucose can be oxidized via glucose 6-phosphate and 6-phosphogluconic acid to a pentose phosphate and carbon dioxide. It is likely that the amount of pentose found does not represent the total quantity formed from glucose, as part of the pentose will be transformed into PGA under the present experimental conditions.

SUMMARY

1. *Escherichia coli*, adapted to D-xylose or L-arabinose, produces under aerobic conditions D-3-phosphoglyceric acid (PGA) from maltose, D-glucose and from the pentose to which it had been adapted.

2. *Esch. coli*, grown previously in the presence of glucose, gives significant amounts of PGA when incubated aerobically with D-glucose and maltose, and very small amounts when incubated with L-arabinose, D-ribose, D-xylose, or D-lyxose.

3. D-Xylose and D-arabinose decrease the quantity of endogenous PGA formed by cells grown on D-glucose and L-arabinose; for D-xylose-grown cells, only D-arabinose shows a similar effect.

4. A large amount of PGA is formed from xylose by xylose-grown cells under anaerobic conditions only when adenosine triphosphate is added.

5. In the presence of potassium ions, more PGA is formed than in the presence of sodium ions.

6. *Esch. coli* transforms glucose partly into a pentose and pentose phosphate.

This paper forms part of a thesis presented by Uriel Z. Littauer to the Hebrew University, Jerusalem, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

REFERENCES

- Barker, H. A. & Lipmann, F. (1949). *J. biol. Chem.* **179**, 247.
 Cohen, S. S. (1949). *J. biol. Chem.* **177**, 807.
 Cohen, S. S. (1951). *Nature, Lond.*, **168**, 746.
 Cohen, S. S. & Raff, R. (1951). *J. biol. Chem.* **188**, 501.
 Cohen, S. S. & Scott, D. B. N. (1951). *Fed. Proc.* **10**, 173.
 Doudoroff, M., Hassid, W. Z., Putman, E. W., Potter, A. L. & Lederberg, J. (1949). *J. biol. Chem.* **179**, 921.
 Drury, H. F. (1948). *Arch. Biochem.* **19**, 455.
 Folin, O. & Malmros, H. (1929). *J. biol. Chem.* **83**, 115.
 Heald, P. J. (1952). *Biochem. J.* **52**, 378.
 Karström, H. (1938). *Ergebn. Enzymforsch.* **7**, 350.
 Ley, J. de & Cornut, S. (1951). *Nature, Lond.*, **168**, 515.
 Meyerhof, O. & Schultz, W. (1938). *Biochem. Z.* **297**, 60.
 Monod, J. & Torriani, A. M. (1948). *C.R. Acad. Sci., Paris*, **227**, 240.
 Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
 Scott, D. B. N. & Cohen, S. S. (1951). *J. biol. Chem.* **188**, 509.
 Still, J. L. (1940). *Biochem. J.* **34**, 1374.
 Stone, R. W. & Werkman, C. H. (1936a). *Iowa St. Coll. J. Sci.* **10**, 341.
 Stone, R. W. & Werkman, C. H. (1936b). *Iowa St. Coll. J. Sci.* **11**, 1.
 Stone, R. W. & Werkman, C. H. (1937). *Biochem. J.* **31**, 1516.
 Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1945). *Manometric Techniques and Tissue Metabolism*, p. 185. Minneapolis: Burgess Publishing Co.
 Werkman, C. H., Zoellner, E. A., Gilman, H. & Reynolds, H. (1936). *J. Bact.* **31**, 5.
 Wood, H. G., Stone, R. W. & Werkman, C. H. (1937). *Biochem. J.* **31**, 349.