Short Communications and Preliminary Notes

BREAKDOWN OF PENTOSE PHOSPHATES IN *ESCHERICHIA COLI*

by

ERNST D. BERGMANN
Scientific Department, Israeli Ministry of Defence

AND

U. Z. LITTAUER AND B. E. VOLCANI
Weizmann Institute of Science, Rehovoth (Israel)

Recent studies[1] on the degradation of pentose phosphates have shown that D-ribose-5-phosphate is degraded by animal tissues, red blood cells, plants and microorganisms, while D-arabinose-5-phosphate and D-xylose-5-phosphate have proved inert, at least towards bacteria[2] and yeast[3].

In continuation of an investigation on the degradation of pentoses in *E. coli*[4], the effect of sonic extracts of cells grown on pentoses, on D-ribose-5-phosphates, D-arabinose-5-phosphates, D-xylose-1- and D-xylose-5-phosphates was studied.

Sonic extracts were prepared from 16 to 19 hour-old cultures of *E. coli* K-12 grown at 37° on 0.5% Difco yeast extract supplemented with 0.05% of sugar (glucose, D-ribose, D-xylose or D-arabinose), and vigorously aerated.

The extracts were treated with manganous chloride according to HOFFMAN AND LAMPEN[5], and dialysed against 0.9% KCl for 3 hours at 2°. Each reaction mixture contained: 0.9 ml of extract (equivalent to 500 mg of wet cells); 1.5 to 2.0 μM of pentose phosphate; 0.5 ml of $M/10$ tris (hydroxymethyl)aminomethane buffer pH 7.0; 0.25 ml of 1 M NaF; and 0.2 ml of a 0.1% $MgCl_2.7H_2O$ solution in a final volume of 2.5 ml. Incubation was carried out at 30°, and samples were removed at 0, 30, 60, 120 and 240 minutes and deproteinized with 9 times their volume of 5% trichloroacetic acid. Pentose was determined in the protein-free filtrates using the orcinol test[6].

Ribose-5-phosphate is degraded, at a very fast rate, equally by extracts of cells grown on ribose, xylose, arabinose and glucose, while

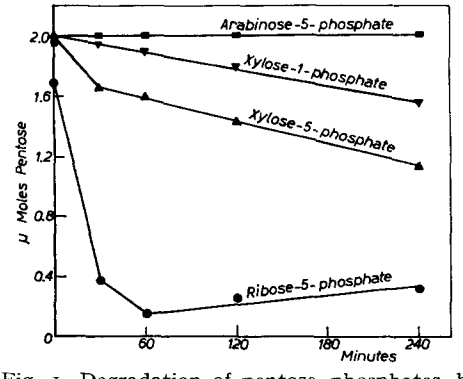

Fig. 1. Degradation of pentose phosphates by sonic extracts of *E. coli* K-12 grown on D-xylose. Endogenous pentose constant throughout the incubation, substracted.

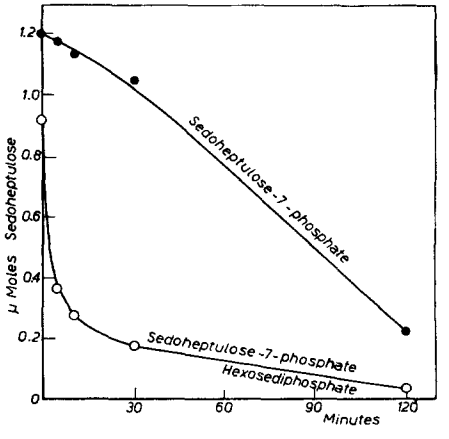

Fig. 2. Degradation of sedoheptulose-7-phosphate with and without hexosediphosphate (1.5 μM) by sonic extract of *E. coli* K-12 grown on D-xylose. The incubation mixture contained: 0.75 ml sonic extract not treated with manganous chloride (equivalent to 150 mg wet cells); $M/10$ phosphate buffer pH 7.0; other ingredients in quantities indicated in the text. Sedoheptulose-7-phosphate was determined in the protein-free filtrate using the orcinol test at 580 mμ and sedoheptulosan hydrate as a standard. Endogenous substracted.

D-arabinose-5-phosphate is practically not metabolised, even if the cells had been grown on arabinose. This inertness is in accord with the recent observation of COHEN[7] that the degradation of D-arabinose is preceded by conversion to D-ribulose and subsequent phosphorylation. Xylose-5-phosphate was degraded by all the extracts, though at a lower rate than D-ribose-5-phosphate; xylose-1-phosphate was less degraded (Fig. 1). On the whole, similar results were found with extracts of anaerobically grown cells. *E. coli* thus behaves differently from *L. pentosus*, sonic extracts of which did not affect D-xylose-5-phosphate, even when the organism was grown on xylose[2].

LAMPEN[8] interprets the inertness of xylose-5-phosphate to indicate that it is not an intermediate in the breakdown of xylose by *L. pentosus*, and that this sugar has first to be converted into D-xylulose before it is phosphorylated and degraded; the above experiments with *E. coli* suggest that *xylose-5-phosphate, and possibly xylose-1-phosphate, can be intermediates* in the breakdown of xylose by this organism and that this step is non-adaptive.

In view of the interrelationship between D-ribose-5-phosphate and sedoheptulose-7-phosphate described by HORECKER *et al.*[9] in yeast, liver and spinach, the breakdown of the latter by the various extracts of *E. coli* was tested. It was found that this compound was degraded by all the extracts, though slowly, and that addition of hexose diphosphate markedly hastened the degradation (Fig. 2). This activation by hexose diphosphate has been observed in other systems by HORECKER AND SMYRNIOTIS[10].

A full account of these results will be published.

The authors are indebted to Dr. B. L. HORECKER for the sedoheptulose phosphate, and to Dr. W. Z. HASSID for the xylose-1-phosphate used in this investigation.

REFERENCES

1 For a review see: B. L. HORECKER, *J. Cell. and Comp. Physiol.*, 41, Supplement 1 (1953) 37, and J. O. LAMPEN, *ibid.*, 183.
2 J. O. LAMPEN AND H. R. PETERJOHN, *J. Bact.*, 62 (1951) 281.
3 H. Z. SABLE, *Biochim. Biophys. Acta*, 8 (1952) 687.
4 E. D. BERGMANN, U. Z. LITTAUER AND B. E. VOLCANI, *Biochem. J.* (in press).
5 C. E. HOFFMANN AND J. O. LAMPEN, *J. Biol. Chem.*, 198 (1952) 885.
6 H. F. DRURY, *Arch. Biochem.*, 19 (1948) 455.
7 S. S. COHEN, *J. Biol. Chem.*, 201 (1953) 71.
8 J. O. LAMPEN in *Phosphorus Metabolism*, Vol. II (1952) 363 (W. D. MCELROY AND B. GLASS, eds. The Johns Hopkins Press, Baltimore).
9 B. L. HORECKER, P. Z. SMYRNIOTIS AND H. KLENOW, *Federation Proc.*, 12 (1953) 219.
10 B. L. HORECKER AND P. Z. SMYRNIOTIS, *J. Am. Chem. Soc.*, 75 (1953) 2021.

Received December 7th, 1953

ÜBER DEN NACHWEIS EINER WIRKUNG VON VITAMIN K_1 *IN VITRO* AUF DIE OXYDATIVE PHOSPHORYLIERUNG

von

CARL MARTIUS UND DAGOBERT NITZ-LITZOW

Physiologisch-Chemisches Institut der Universität Würzburg (Deutschland)

Wir berichteten vor kurzem[1] über den Einfluss von Mangel an Vitamin-K auf die oxydative Phosphorylierung von Lebermitochondrien, der sich in einem erniedrigten P/O-Quotienten äussert. In den nachfolgend beschriebenen Versuchen können wir nun zeigen, dass es gelingt, durch Zusatz von Vitamin K_1 *in vitro* zu atmenden Mitochondriensuspensionen von K-Mangelküken die Phosphorylierungsquote zu erhöhen. Die zu den Versuchen verwandten Tiere (weisse Leghornküken) waren durch längere Fütterung mit K-freiem Futter weitgehend avitaminotisch geworden und zeigten Gerinnungszeiten von ca. 20 Min.

Wie die Versuche 1–8 der Tabelle zeigen bewirkt Vitamin K_1 (es kam das Präparat Konakion der Fa. Hoffmann La Roche zur Verwendung, das ein racemisches Phyllochinon darstellt) in einer Konzentration von $5 \cdot 10^{-6}$ bis 10^{-5} *M* eine beträchtliche Steigerung des P/O-Quotienten. Diese ergibt sich aus einer durchweg erhöhten Phosphorylierung und in einigen Fällen besonders bei der höheren K_1-Dosierung aus einer leicht verringerten Atmung. Theoretisch sollte sich bei Zufuhr von Vitamin K_1 zu einem völlig K-freien System eine Steigerung der Phosphorylierung von 50% ergeben, wenn das Vitamin für eine der drei Reaktionsstufen benötigt wird. Dieser Wert wird in zwei Versuchen tatsächlich erreicht, bei den anderen bleibt er darunter. Das könnte einerseits auf nicht völlige Freiheit