Les réactions d'oxydo-réduction bloquées au niveau des cytochromes b et c_1 par l'antimycine A sont restaurées par $K_3Fe(CN)_6$, lequel cède directement ses électrons au cytochrome c, P_1 et P_2 étant ainsi supprimées. Les mitochondries mises en présence de T_4 ou de CNI n'augmentent pratiquement pas de volume; P_2 apparaît donc comme indispensable, alors que P_1 ne l'est pas.

La suppression de P_3 est obtenue quand, après addition de KCN, on ajoute $K_3Fe(CN)_6$ qui accepte les électrons du cytochrome c réduit. Les produits iodés provoquent alors le gonflement mitochondrial; P_3 n'est donc pas indispensable à celui-ci (Fig. 1B).

L'importance de P_2 a été mise en évidence par les essais suivants. On a établi un flux d'électrons entre la région DPN-flavoprotéine et la portion de la chaîne respiratoire comprise entre les cytochromes a et a_3. On commence par isoler cette zone par action de l'Amytal et de KCN, on assure ensuite la respiration par la voie du succinate jusqu'au $K_3Fe(CN)_6$; l'addition de T_4 ou de CNI amène alors le gonflement des mitochondries. Cependant, comme celui-ci est supprimé par addition de dinitrophénol, on en déduit que la respiration seule, limitée à la région des cytochromes, s'avère inefficace, alors que la condition nécessaire et suffisante à l'hypertrophie mitochondriale par T_4 ou CNI semble être le couplage des réactions de phosphorylation à ce niveau (P_2).

Laboratoire de Biochimie générale et comparée, Collège de France, Paris (France)

JEAN ROCHE
RAYMOND MICHEL
JOSEPH E. RALL
ODETTE MICHEL
MARC GIRARD
STELIO VARRONE

[1] A. L. LEHNINGER, *J. Biol. Chem.*, 234 (1959) 2187.
[2] J. E. RALL, J. ROCHE, R. MICHEL, O. MICHEL ET S. VARRONE, *Biochem. Biophys. Res. Commun.*, 7 (1962) 111.
[3] J. E. RALL, J. ROCHE, R. MICHEL, O. MICHEL ET S. VARRONE, *Biochim. Biophys. Acta*, 62 (1962) 622.
[4] J. B. CHAPPEL ET G. D. GREVILLE, *Biochim. Biophys. Acta*, 38 (1960) 483.
[5] F. E. HUNTER, J. F. LEVY, J. FINK, B. SCHUTZ, F. GUERRA ET A. HURWITZ, *J. Biol. Chem.*, 234 (1959) 2176.
[6] T. E. CONOVER ET L. ERNSTER, *Biochim. Biophys. Acta*, 58 (1962) 189.
[7] J. ROCHE, J. E. RALL, R. MICHEL, O. MICHEL ET S. VARRONE, *Biochim. Biophys. Acta*, 56 (1962) 188.
[8] D. K. MYERS ET E. C. SLATER, *Biochem. J.*, 67 (1957) 558.

Reçu le 21 février, 1963

Biochim. Biophys. Acta, 71 (1963) 494–495

PN 1249

The enzymic glucosylation of polyglycerophosphate

In a previous communication we have reported the enzymic synthesis of polyglycerophosphate by a particulate enzyme from *Bacillus subtilis* (*Bacillus licheniformis* ATCC 9945)[1]. The enzymic formation of polyribitolphosphate by extracts of *Lactobacillus plantarum* has been demonstrated by GLASER[2]. Several glycerol- and ribitol-teichoic acids were shown to have sugar substituents and D-alanine on the polyol-phosphate backbone[3,4]. The transfer of *N*-acetylglucosamine from UDP-*N*-acetyl-

glucosamine to polyribitolphosphate has been reported[5]. Glycerolteichoic acid from *B. subtilis* (ATCC 9945) has been shown to contain glucose units[6]. We wish to report that the particulate enzyme preparation from this organism which synthesizes polyglycerophosphate from CDP-glycerol will also glucosylate the enzymically synthesized polymer using UDP-D-glucose as a glucosyl donor. As shown in Table I the glucosylation reaction is dependent on the presence of CDP-glycerol. The endogenous glycerolteichoic acid present in the particulate enzyme will not act as a glucosyl acceptor probably because it is already fully glucosylated.

TABLE I

SYNTHESIS OF GLUCOSYLATED POLYGLYCEROPHOSPHATE

The reaction mixture contained 10 μmoles of Tris–chloride buffer, 2 μmoles of $MgCl_2$, 0.2 μmole of EDTA and 0.1 ml of a particulate enzyme preparation[1] in a total volume of 0.5 ml. Where indicated 50 mμmoles of CDP-[^{14}C]glycerol or CDP-glycerol or 40 mμmoles of UDP-D-[^{14}C]glucose were added and the mixtures incubated at pH 8.0 at 37° for 1 h. The isolation procedure for the product was previously described[1].

Substrates	*mμmoles of ^{14}C-labeled substrate incorporated*	
	Glycerol	*Glucose*
CDP-[^{14}C]glycerol	8.0	—
UDP-D-[^{14}C]glucose	—	0.21
UDP-D-[^{14}C]glucose + CDP-glycerol	—	2.66

Identification of the glucosylated product is based on the following observations. Enzymically glucosylated teichoic acid was synthesized from UDP-D-[^{14}C]glucose and CDP-[^{14}C]glycerol and isolated by a previously described method[1]. Alkaline hydrolysis (0.5 N KOH, 2 h, 100°), followed by chromatography on Dowex-1 X8 formate yielded [^{14}C]glycerophosphate and a phosphorylated compound (I) which has been identified as glucosylglycerophosphate on the basis of the following observations. On paper chromatography in neutral ammonium acetate solvent[7] it moved as a single spot with $R_{a\text{-}glycerophosphate} = 1.15$. On acid hydrolysis (1 N, 3 h, 100°) Compound I gave rise to an equimolar mixture of glucose and glycerophosphate. After dephosphorylation with *Escherichia coli* alkaline phosphatase Compound I was converted to Compound II which has been identified as glucosylglycerol. The latter moved as a single spot with $R_{glucose} = 1.15$ in propanol–ammonia–water (6:3:1)[8] and could be clearly distinguished from glucose and glycerol by chromatography on charcoal-Celite columns[9]. Acid hydrolysis of Compound II (1 N $HClO_4$, 3 h, 100°) gave rise to equimolar quantities of glucose and glycerol. Compound II was not attacked by glucose oxidase. After acid hydrolysis, however, glucose in Compound II became available to oxidation by glucose oxidase and was quantitatively converted to gluconic acid. The enzyme preparation will incorporate glycerol and glucose into teichoic acid in a ratio of 3:1, and as expected, the ratio of glycerol to glucosylglycerol in the isolated polymer is 2:1. This heavily glucosylated polymer thus resembles the glycerolteichoic acid isolated from the cell wall of *Staphylococcus albus*[10] in which the ratio of *N*-acetylgalactosamine to glycerol is also 1:3.

The enzyme system does not synthesize a glucosylated CDP-glycerol. Attempts to use enzymically synthesized "isolated"[1] polyglycerophosphate as a glucosyl

acceptor have so far been negative, suggesting that externally added polyglycerophosphate, unlike the material synthesized "*in situ*" by the particulate preparation, is not available to the glucosylating enzyme. Work is in progress to determine the structure of the enzymically synthesized polymer and the mechanism of these reactions.

The author wishes to thank Dr. L. GLASER for his continued interest in this work and helpful discussions.

This work was supported by Grant No. G-20027 from the National Science Foundation. The author is the recipient of a postdoctoral fellowship from the U.S. Public Health Service.

Department of Biological Chemistry, MAX M. BURGER
Washington University School of Medicine,
St. Louis, Mo. (U.S.A.)

1 M. M. BURGER AND L. GLASER, *Biochim. Biophys. Acta*, 64 (1962) 575.
2 L. GLASER, *Biochim. Biophys. Acta*, 71 (1963) 237.
3 J. BADDILEY, *Federation Proc.*, 21 (1962) 1084.
4 P. CRITCHLEY, A. R. ARCHIBALD AND J. BADDILEY, *Biochem. J.*, 85 (1962) 420.
5 S. G. NATHENSON AND J. L. STROMINGER, *J. Biol. Chem.*, 237 (1962) PC 3839.
6 M. M. BURGER AND L. GLASER, unpublished observation.
7 A. C. PALADINI AND L. F. LELOIR, *Biochem. J.*, 51 (1952) 426.
8 C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
9 R. L. WHISTLER AND D. F. DURSO, *J. Am. Chem. Soc.*, 72 (1950) 677.
10 D. C. ELLWOOD, M. V. KELEMEN AND J. BADDILEY, *Biochem. J.*, 86 (1963) 213.

Received March 9th, 1963

Biochim. Biophys. Acta, 71 (1963) 495–497

PN 1244

Zur Umbildung der 2-Desoxyhexosen in höheren Pflanzen

Im Gegensatz zu den zahlreichen Arbeiten über die Umbildung und Wirkungsweise von 2-Desoxyhexosen im tierischen Gewebe, in Hefepilzen und Mikroben gibt es nur wenige analoge Studien, die sich mit dem Stoffwechsel dieser Zucker im Pflanzengewebe befassen[1–7]. In unseren Untersuchungen, die an die interessanten Experimente von BARBER[6] und SÜSS[7] mit 2-deGlc an Tabakpflanzen anknüpfen, wurden als Versuchsobjekte sowohl erwachsene als auch 2–3 Monate alte Tabakpflanzen (12–18 g), erwachsene Sonnenblumen und Keimpflanzen von Erbsen, Weizen, Mais und Sonnenblumen verwendet. Die Verabreichung der 10^{-4} bis 10^{-6} M wässrigen Lösungen von 2-deGlc und 2-deGal erfolgte bei intakten Pflanzen durch Absorption durch die Wurzeln, bei den abgeschnittenen Pflanzen durch die Achsen. Nach 24 h wurden die ganzen Pflanzen im 70 % Äthanol homogenisiert und der abgetrennte Gewebsbrei noch zweimal mit Äthanol extrahiert. Die vereinten eingeengten Extrakte wurden papierchromatographisch analysiert.

Bei allen Versuchen, ohne Rücksicht auf die Dauer, Versuchsbedingungen, Alter

Abkürzungen: 2-deGlc, 2-Desoxy-D-glucose; 2-deGal, 2-Desoxy-D-galaktose; 2-deGlcA-lacton, 2-Desoxy-D-gluconolacton; 2-deGalA-lacton, 2-Desoxy-D-galaktonolacton.