Table 1. *Incorporation of aspartic acid into nicotinic acid by resting cell suspensions of Serratia marcescens*

The specific activities of aspartic acid and nicotinic acid were determined by total combustion and conversion into BaCO_3 . Nicotinic acid was also decarboxylated. Activities are expressed as counts/min. at infinite thickness of BaCO_3 . The activities of nicotinic acid are corrected for dilution with carrier. The activity of the pyridine ring was calculated from the difference between values for total combustion and decarboxylation.

Precursor	Specific activity of precursor (counts/min.)	Specific activity of nicotinic acid (counts/min.)	Percentage distribution of activity	
			Pyridine ring	Carboxyl group (C-7)
[2- ^{14}C]Aspartic acid	5.93×10^6	0.27×10^6	13	87
[3- ^{14}C]Aspartic acid	6.11×10^6	0.28×10^6	92.3	7.7
[4- ^{14}C]Aspartic acid	1.15×10^6	0.025×10^6	17	83

relative extents of incorporation of each carbon atom, although the percentage incorporation is of the same order in each case. The incorporation of C-4 and C-3 of aspartic acid into the carboxyl and ring carbon atoms respectively of nicotinic acid is in full agreement with the results reported by other authors with *Mycobacterium tuberculosis*. However, C-2 of aspartate contributes chiefly to the carboxyl group of nicotinic acid. This is not compatible with the hypothesis that C-2, C-3 and C-7 of nicotinic acid are derived from a unit composed of C-2, C-3 and C-4 of aspartate. It seems more likely that, at some stage in the incorporation, C-2 and C-4 of aspartate become equivalent and one of them is lost.

H. H. is in receipt of an Agricultural Research Council Studentship. We thank Carreras Ltd. for the purchase of the radioactive materials.

- Albertson, A. N. & Moat, A. G. (1965). *J. Bact.* **89**, 540.
 Barton-Wright, E. C. (1944). *Biochem. J.* **38**, 314.
 Crompton, C. E. & Woodruff, N. A. (1950). *Nucleonics*, **7**, 49.
 del Rio-Estrada, C. & Patino, H. (1962). *J. Bact.* **84**, 871.
 Gross, D., Schütte, H. R., Hübner, G. & Mothes, K. (1963). *Tetrahedron Lett.* no. 9, 541.
 Leete, E. (1965). *Science*, **147**, 1000.
 Mothes, E., Gross, D., Schütte, H. R. & Mothes, K. (1961). *Naturwissenschaften*, **48**, 623.
 Scott, T. A. & Happold, F. C. (1962). *Biochem. J.* **82**, 407.
 Van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509

Biochem. J. (1965) **96**, 10c

Succinyl-Phosphatides as Intermediates in the Succinate-Dehydrogenase Reaction of Yeast

By Y. H. ABDULLA and A. N. DAVISON

Departments of Pathology and Biochemistry, Guy's Hospital Medical School, London, S.E.1

(Received 20 May 1965)

It is now well established (see Green & Fleischer, 1964) that various preparations of succinate dehydrogenase (EC 1.3.99.1) may be blocked by the action of snake-venom phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4). We confirmed this observation by using ultrasonically treated baker's yeast (*Saccharomyces cerevisiae*) suspension incubated with phospholipase A in the presence of Ca^{2+} . After incubation, succinate-dehydrogenase activity was found to be inhibited, as determined by the addition of substrate with a suitable acceptor

system (Arrigoni & Singer, 1962). This experiment however, does not differentiate between the possibility that phospholipase A acts either by deacylation of an essential phosphoglyceride before the addition of substrate (Green & Fleischer, 1964) or that its action interferes more directly with the substrate-dehydrogenation reaction. Since phospholipase-A activity can be decreased by the removal of Ca^{2+} by EDTA (Long & Penny, 1957), this reagent was used to block phospholipase activity, before the addition of succinate, phenazine

Table 1. *Incorporation of [^{14}C]succinate into the lipid fractions of an ultrasonically treated yeast preparation*

[1,4- $^{14}\text{C}_2$]Succinate (4 μC) was incubated for 2 min. at 37° with 5 ml. of 10% (w/v) ultrasonically treated yeast prepared in 50 mM-tris-HCl buffer, pH 7.5, under the conditions described in the text. A portion of acetone and chloroform-methanol (2:1, v/v)-soluble material was run on thin-layer silicic acid G chromatograms as described by Davison & Graham-Wolfaard (1964). Results for typical control experiments are shown. Lipid areas have been identified by analysis and with pure lipids.

	Radioactivity of lipid spot (counts/100 sec.)					Origin (succinate)
	Neutral lipid	Phosphatidyl-ethanolamine	Phosphatidyl-choline	Sphingo-myelin	Lysolecithin	
Control: [^{14}C]succinate + boiled yeast preparation + KCN (1 mM)	127	131	135	130	140	31876
[^{14}C]Succinate + yeast preparation + KCN (1 mM)	150	158	3426	125	138	29887
Rechromatography of 'phosphatidylcholine' spot treated with phospholipase A	—	—	—	—	211	3315
Rechromatography of 'phosphatidylcholine' spot not treated with phospholipase A	—	—	3237	—	—	189

methosulphate and dichlorophenol-indophenol but after prolonged incubation of yeast with phospholipase A. Under these conditions succinate-dehydrogenase activity was largely restored.

Our interpretation of these experiments is that during succinate dehydrogenation a succinate ester intermediate is formed at the β -position of a phosphoglyceride associated with the enzyme, for the β -linkage is specifically hydrolysed by phospholipase A. Such a hypothesis accounts for the important observation that, even after prolonged incubation with phospholipase A, succinate-dehydrogenase activity can be restored by subsequently blocking phospholipase A.

This hypothesis has been tested by various experiments. First, [1,4- $^{14}\text{C}_2$]succinate was incubated for 2 min. with ultrasonically treated yeast under aerobic conditions in the presence of cyanide, as described in Table 1. Lipids were extracted from the preparation with acetone and then with chloroform-methanol (2:1, v/v). The combined extract was dried and chromatographed by thin-layer chromatography (Davison & Graham-Wolfaard, 1964). The spots were removed, suspended in the liquid-scintillator system of Snyder (1964) and counted in an Ecko scintillation counter. Table 1 shows that most of the ^{14}C incorporated into lipid was detected in a spot that ran with the same R_f as lecithin. This degree of incorporation could not be found in similar control experiments with [^{14}C]succinate with boiled yeast or yeast with excess of fumarate, with phospholipase A, or if yeast was incubated for up to 1 hr. in the absence of cyanide. As was expected, malonate depressed incorporation markedly.

In another experiment yeast was incubated for 1 hr. at 37° aerobically in the presence of excess of succinate. Lipid extract prepared as above was chromatographed in the same solvent system (see Table 1). The lipid running with the same R_f as lecithin was recovered from the chromatogram and a water-soluble fraction of this was found to be a phospholipid with an absorption maximum at 222 m μ and a smaller peak at 265 m μ . An absorption peak could just be detected at 222 m μ when potassium succinate (final concn. 1 mM) was added to a suspension of ultrasonically treated yeast, although in the presence of phospholipase A no such absorption was noted.

Synthesis of α -fatty acyl- β -succinylglyceryl-phosphorylcholine was next attempted. Egg-yolk lysolecithin (10 m-moles) was treated with succinic anhydride (13 m-moles) in chloroform at room temperature in the presence of 0.36 m-mole of HClO_4 . The product so obtained was purified by thin-layer chromatography and crystallized from hot benzene. The succinyl derivative was water-soluble and acetone-soluble and had absorption maxima in 0.1 M-phosphate buffer, pH 7, at 222 and 265 m μ . It was oxidized three times more rapidly than potassium succinate by the yeast system described by Arrigoni & Singer (1962). Added in excess its oxidation was not blocked by the presence of phospholipase A. After incubation of the succinyl derivative with phospholipase A in tris-HCl buffer, pH 7, saturated with ether in the presence of Ca^{2+} , thin-layer chromatography showed almost complete loss of the spot running with the same R_f as lecithin and the appearance of two spots, one similar to lysolecithin and one to succinate. Fumarate had no

Scheme 1.

inhibitory effect on the oxidation of the succinyl-phosphatide, and concentrations of malonate that completely inhibit oxidation of succinate only partially inhibited oxidation of equimolar amounts of succinyl-phosphatide (based on phosphorus content).

These results may be accounted for as follows. It is suggested that a first step in the succinate-dehydrogenase reaction is the formation of a succinate ester at the β -position of a phosphoglyceride close to or at the active site for dehydrogenation. The formation of this succinyl-phosphoglyceride intermediate from succinate may be competitively inhibited by fumarate and malonate.

The next step in the reaction is the dehydrogenation of β -succinyl-phosphatide to β -fumaryl-phosphatide, from which fumarate plus phospholipid are released (Scheme 1).

- Arrigoni, O. & Singer, T. P. (1962). *Nature, Lond.*, **193**, 1256.
 Davison, A. N. & Graham-Wolfaard, E. (1964). *J. Neurochem.* **11**, 147.
 Green, D. E. & Fleischer, S. (1964). In *Metabolism and Physiological Significance of Lipids*, p. 581. Ed. by Dawson, R. M. C. & Rhodes, D. N. London: John Wiley and Sons (Inc.) Ltd.
 Long, C. & Penny, I. F. (1957). *Biochem. J.* **65**, 382.
 Snyder, F. (1964). *Analyt. Biochem.* **9**, 183.

Biochem. J. (1965) **96**, 12c

Phenotypic Expression of the 'Relaxed' Genotype during Inhibition of *Escherichia coli* by β -2-Thienylalanine

By G. TURNOCK* and D. G. WILD
 Department of Biochemistry, University of Leeds

(Received 21 May 1965)

In bacteria, the synthesis of RNA normally depends on the presence of all the amino acids found in protein. Thus, when the essential amino acid is withdrawn from an amino acid auxotroph, neither protein nor RNA is made. Similarly, bacteria that require an amino acid for growth are unable to synthesize RNA in the presence of low concentrations of chloramphenicol unless trace amounts of the required amino acid are present (Pardee & Prestidge, 1956).

The dependence of RNA synthesis on amino acids is genetically determined, and mutants have been

described in which the normal 'stringent' control of RNA synthesis has become 'relaxed'; the characteristic feature of relaxed mutants that also require an amino acid for growth is that RNA continues to be made when the amino acid is withdrawn (Stent & Brenner, 1961). However, the relaxed genotype is not invariably expressed under conditions in which the rate of protein synthesis is varied. Neidhardt (1963) investigated the behaviour of relaxed strains during balanced growth in different media and found that the ratio of RNA to protein varied in an apparently normal manner with growth rate; similarly, a 'shift-down' transition, i.e. a decrease in growth rate produced by a change in

* Present address: University Institute of Microbiology, Copenhagen, Denmark.