ments, it is possible that a high ratio of D- to L-lactate-ferricyanide reductases may be a convenient marker for respiratory deficiency due to cytoplasmic mutation. More work is required to delineate precisely the changes that have occurred in the lactate dehydrogenase system. Also, it is not known at the moment whether the quantitative differences in enzyme activity of the various petite strains shown in Table 1 are due to physiological variations during the growth cycle, or reflect more fundamental genetic differences.

The authors are grateful to Miss J. Jones and Mrs D. Collier for technical assistance. D.B.R. is grateful to the Medical Research Council and the Science Research Council for grants for equipment.

- Ephrussi, B. (1953). *Nuclear-Cytoplasmic Relations in Micro-organisms*. London: Oxford University Press.
- Horn, P. & Wilkie, D. (1967). *Heredity*, **21**, 625.
- Labeyrie, F. & Slonimski, P. P. (1964). *Bull. Soc. Chim. biol., Paris*, **46**, 1793.
- Mahler, H. R., Mackler, B., Grandchamp, S. & Slonimski, P. P. (1964). *Biochemistry*, **3**, 668.
- Ogur, M., St John, R. & Nagai, S. (1957). *Science*, **125**, 928.
- Roodyn, D. B. (1965a). *Nature, Lond.*, **206**, 1226.
- Roodyn, D. B. (1965b). In *Technicon Symp.: Automation in Analytical Chemistry*. p. 593. New York: Mediad Inc.
- Sherman, F. & Slonimski, P. P. (1964). *Biochim. biophys. Acta*, **90**, 1.
- Slonimski, P. P. (1953). *Formation des Enzymes Respiratoires chez la Levure*. Paris: Masson.

Biochem. J. (1967) **103**, 50

The Relation of Hydrogen Exchange between Deoxyadenosylcobalamin and Water to the Role of the Coenzyme in Ribonucleotide Reduction

By H. P. C. HOGENKAMP, R. K. GHAMBEER, CAROL BROWNSON and R. L. BLAKLEY
*Department of Biochemistry, John Curtin School of Medical Research,
Australian National University, Canberra, A.C.T., Australia*

(Received 10 January 1967)

5'-Deoxyadenosylcobalamin (coenzyme B₁₂) is required as a cofactor for the enzymic reduction of ribonucleotides to deoxyribonucleotides by cell-free extracts of *Lactobacillus leichmannii* (Blakley & Barker, 1964). Experiments with the partially purified enzyme have established that a major difference between the cobamide-dependent ribonucleotide reductase and a number of other cobamide-requiring enzymes such as glutamate mutase, methylmalonyl-CoA mutase and diol dehydrase is that in the reductase reaction solvent hydrogen atoms are incorporated into one of the products. Thus when the enzymic reduction of ATP is carried out in the presence of [³H]water, tritium is incorporated into dATP (Blakley, Ghambeer, Batterham & Brownson, 1966). To elucidate the role of deoxyadenosylcobalamin in the ribonucleotide reductase reaction of *L. leichmannii* we have now studied the exchange of hydrogen atoms between water and deoxyadenosylcobalamin in the presence of the ribonucleotide reductase.

Extracts of *L. leichmannii* grown under optimum conditions for the production of ribonucleotide reductase (Ghambeer & Blakley, 1966) were

prepared by the use of the Ribi pressure cell. After purification by protamine sulphate treatment, (NH₄)₂SO₄ fractionation and gel filtration, the reductase had a specific activity of 68 μmoles of ATP reduced/mg. of protein/hr. at 37° under standard conditions. Deoxy[5'-³H]adenosylcobalamin was synthesized as described by Frey & Abeles (1966), purified by column chromatography and crystallized from aqueous acetone (Barker, Smyth & Hogenkamp, 1963); the product had a specific activity of 630 000 counts/min./μmole. dITP was prepared by chemical deamination of dATP according to the method of MacNutt (1952).

A reaction mixture containing dimethylglutarate buffer, pH 7.3 (0.3M), dihydrolipoate (30mM), EDTA (1mM), deoxyadenosylcobalamin (0.8mM), ATP (0.5mM), enzyme (0.54mg.) and [³H]water (0.25c) in a total volume of 5ml. was incubated in the dark at 33°. After 1hr. deoxyadenosylcobalamin was isolated from the reaction mixture by phenol extraction, and re-extracted from the phenol into water by the method of Barker *et al.* (1963). Radioactivity measurements in a Packard Tri-Carb model 3003 liquid-scintillation spectrometer

indicated that 0.83 g. atom of labelled hydrogen had been incorporated/mole of deoxyadenosylcobalamin. To locate the position of the label an aqueous solution of deoxyadenosylcobalamin was photolysed and the photolysis products were separated by chromatography (Hogenkamp, 1964). The adenosine-5'-aldehyde contained 0.41 g. atom/mole, 8,5'-(cyclic)-adenosine 0.74 g. atom/mole and aquocobalamin 0.00 g. atom/mole. These data, as well as the loss of radioactivity from adenosine-5'-aldehyde on oxidation with NaOI, establish that one or both 5'-hydrogen atoms of deoxyadenosylcobalamin exchange with the solvent during the enzymic reduction of ATP. Subsequent experiments showed that no significant exchange takes place when either dihydrolipoate or ATP is omitted from the enzymic reaction.

Enzyme reaction mixtures for experiments with deoxy[5'-³H]adenosylcobalamin contained 3,3-dimethylglutarate buffer, pH 7.3 (0.3M), dihydrolipoate (30mm), EDTA (1mm), deoxy[5'-³H]-adenosylcobalamin (0.15mm), enzyme (5.4 µg.) and ribonucleotide (2mm) or deoxyribonucleotide (2mm) in a total volume of 0.5ml. The reaction was initiated by the addition of deoxyadenosylcobalamin and, after incubation in the dark at 37° for 15min., terminated by freezing in liquid N₂. A portion of the ice was then sublimed and, after the sublimate had been thawed, 0.1ml. of it was used for determination of radioactivity in a Packard Tri-Carb model 3003 liquid-scintillation spectrometer.

When deoxy[5'-³H]adenosylcobalamin is incubated in a complete reaction mixture containing ATP, it was found that tritium is lost to the solvent, and that the dATP produced in the reaction contains no significant label. In the absence of either ATP or dihydrolipoate or when lipoate replaces dihydrolipoate no exchange takes place. Further, on prolonged incubation with 1.5mg. of enzyme all the label is lost from the deoxyadenosylcobalamin. These results confirm that in the complete ribonucleotide reductase system both 5'-hydrogen atoms of deoxyadenosylcobalamin are in rapid equilibrium with the solvent hydrogen atoms.

It has been shown (Blakley, 1966; Beck, Goulian, Larsson & Reichard, 1966) that the reduction of ribonucleotides by *L. leichmannii* reductase is specifically stimulated by the addition of certain deoxyribonucleotides. For example, the reduction of ATP is greatly stimulated by the addition of dGTP to the reductase reaction. It was therefore decided to investigate the effect of nucleotides on the rate of exchange between deoxy[5'-³H]adenosylcobalamin and water. The results of these experiments (Table 1) indicate that the nucleoside triphosphates promote the exchange reaction much

Table 1. *Effect of nucleotides and nucleosides on the exchange of hydrogen atoms between water and deoxyadenosylcobalamin.*

The composition of the complete system is given in the text.

Addition to complete system	³ H in solvent (counts/min.)	Addition to complete system	³ H in solvent (counts/min.)
None	130	dGTP	11 665
ATP	3545	dGDP	4760
ADP	815	dGMP	380
AMP	380	Deoxyguanosine	440
Adenosine	195	dATP	2500
GTP	5880	dITP	4000
ITP	5455	dUTP	1690
UTP	1278	dCPT	2995
CTP	1968	dTTP	895

more effectively than the corresponding diphosphates or monophosphates, the nucleosides being without effect. The high rate of exchange when dGTP is the only nucleotide present is particularly noteworthy because dGTP is not a substrate but rather a product and activator of the nucleotide reductase reaction. Exchange can therefore occur without the reduction of ribonucleotide. It has also been found that deoxyribonucleotides promote incorporation of label from [³H]water into deoxyadenosylcobalamin, and here also dGTP is the most effective nucleotide. Since deoxyribonucleotides do not appreciably inhibit the reduction of ribonucleotides (Blakley, 1966) it may be inferred that they bind poorly with the ribonucleotide site. Thus these observations suggest that all that is essential for the reductase to catalyse hydrogen exchange between deoxyadenosylcobalamin and water is the combination of a nucleoside triphosphate with the enzyme, in the presence of a suitable dithiol, at a site different from the ribonucleotide substrate site. These results differ from those of Beck, Abeles & Robinson (1966), who reported that exchange of hydrogen atoms between water and deoxyadenosylcobalamin is dependent on the complete reductase system including a ribonucleoside triphosphate. However, although they report the absence of exchange in the presence of a low concentration of dATP, which we find a poor activator (Table 1), they do not report the effect on exchange of other deoxyribonucleotides or of dATP at higher concentration.

Incubation of reductase, dihydrolipoate, deoxyadenosylcobalamin and dGTP in the presence of [³H]water does not result in the incorporation of label into dGTP over a 3 hr. period under conditions in which rapid exchange into deoxyadenosylcobalamin occurred.

Although the data presently available do not

establish that the exchange of hydrogen atoms between water and deoxyadenosylcobalamin is an integral part of the reductase reaction the following facts are consistent with this view. Both the exchange reaction and the overall reduction of ribonucleotides have an essential requirement for dihydrolipoate. Further, deoxyribonucleotides that accelerate the reduction of specific ribonucleotides also cause much greater exchange to occur than that in the absence of nucleotides. The two reactions are catalysed by the same highly purified enzyme, and treatment of the enzyme preparation with protein reagents such as 1-fluoro-2,4-dinitrobenzene, *N*-bromosuccinimide and *N*-acetylimidazole decreases the rate of ribonucleotide reduction and of the exchange reaction to approximately the same extent. Finally, rate calculations show that the rate of the exchange reaction is comparable with that of ATP reduction under similar conditions. From these preliminary experiments it appears possible that deoxyadenosylcobalamin participates in hydrogen transfer between dihydrolipoate and the nucleotide substrate.

This investigation was supported in part by Grant 5R05TW00165-02 from the National Institutes of Health, U.S. Public Health Service. H.P.C.H. is on leave of absence from the University of Iowa and a recipient of a University of Iowa Research Council Award and a College of Medicine Compensation Plan Fellowship. R.K.G. is the holder of an Australian National University Scholarship.

- Barker, H. A., Smyth, R. D. & Hogenkamp, H. P. C. (1963). *Biochem. Prep.* **10**, 27.
 Beck, W. S., Abeles, R. H. & Robinson, W. G. (1966). *Biochem. biophys. Res. Commun.* **25**, 421.
 Beck, W. S., Goulian, M., Larsson, A. & Reichard, P. (1966). *J. biol. Chem.* **241**, 177.
 Blakley, R. L. (1966). *Fed. Proc.* **25**, 1633.
 Blakley, R. L. & Barker, H. A. (1964). *Biochem. biophys. Res. Commun.* **16**, 391.
 Blakley, R. K., Ghambeer, R. K., Batterham, T. J. & Brownson, C. (1966). *Biochem. biophys. Res. Commun.* **24**, 418.
 Frey, P. A. & Abeles, R. H. (1966). *J. biol. Chem.* **241**, 2732.
 Ghambeer, R. K. & Blakley, R. L. (1966). *J. biol. Chem.* **241**, 4710.
 Hogenkamp, H. P. C. (1964). *Ann. N.Y. Acad. Sci.* **112**, 522.
 MacNutt, W. S. (1952). *Biochem. J.* **50**, 384.

Biochem. J. (1967) **103**, 7c

Dicarboxylic Acid Catabolism by Bacteria

By P. J. CHAPMAN* and R. G. DUGGLEBY
Department of Biochemistry, University of Hull

(Received 16 January 1967)

A recent study of the bacterial metabolism of glutaric acid (Numa, Ishimura, Nakazawa, Okazaki & Hayaishi, 1964) prompted us to examine the routes by which bacteria degrade the higher members of the homologous series of straight-chain dicarboxylic acids. Acids of this series have been identified in cultures of bacteria utilizing *n*-paraffinic hydrocarbons (Ali Khan, Hall & Robinson, 1964; Kester & Foster, 1963) and alicyclic hydrocarbons (Colla & Treccani, 1960). Enzyme systems catalysing the ω -oxidation of fatty acids or their CoA thio esters to the corresponding dicarboxylic acids have been demonstrated in both bacteria (Kusunose, Kusunose & Coon, 1964) and mammals (Preiss & Bloch, 1964). The isolation of pimelic acid from cultures of azelaic acid-grown *Pseudomonas* sp. (Janota-Bassilik &

Wright, 1964) suggested that bacteria may degrade dicarboxylic acids by a pathway involving β -oxidation, as proposed by Verkade (1938) from the results of animal-feeding experiments. Accordingly experiments to examine this possibility were undertaken.

Two micro-organisms, *Moraxella lwoffii* (*Vibrio* O1, N.C.I.B. 8250) (Sebald & Véron, 1963) and *Pseudomonas aeruginosa* 8602 were selected for study. Cells were grown at 30° with shaking in a medium containing (g./l.): KH_2PO_4 , 1.8; K_2HPO_4 , 3.6; NH_4Cl , 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.40; with an appropriate dicarboxylic acid (0.05% w/v) neutralized with 5N-NaOH as sole carbon source. Initially experiments were conducted with *M. lwoffii*. Though oxalate and malonate did not support growth, cells grew readily at the expense of succinate and the higher dicarboxylic acid homologues up to and including dodecane-1,12-dicarboxylate.

Washed cells grown with pimelate immediately

* Present address: Department of Biochemistry, College of Biological Sciences, University of Minnesota, St Paul, Minn. 55101, U.S.A.