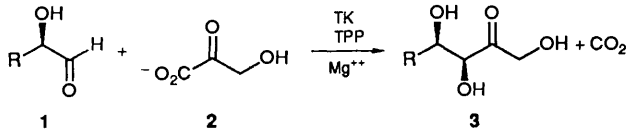
Enzyme-catalysed Carbon–Carbon Bond Formation: Use of Transketolase from *Escherichia coli*

Gordon R. Hobbs,[a] Malcolm D. Lilly,[b] Nicholas J. Turner,*[,a] John M. Ward,[c] Andrew J. Willets[d] and John M. Woodley[b]
[a] *Department of Chemistry, University of Exeter, Stocker Road, Exeter EX4 4QD, UK*
[b] *Advanced Centre for Biochemical Engineering, Department of Chemical and Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK*
[c] *Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK*
[d] *Department of Biological Sciences, University of Exeter, Prince of Wales Road, Exeter EX4 4QG, UK*

Transketolase has been obtained in greater quantities from an over-expressed *E. coli* transformant carrying the transketolase gene. Crude extracts of this organism are suitable for use in small scale biotransformations to provide mmol quantities of product. Initial results indicate that the transketolase from *E. coli* is relatively non-specific for the aldehyde component of the reaction.

In vivo transketolase (TK) (E.C. 2.2.1.1) catalyses a vital step in the pentose phosphate pathway, namely the transfer of a two carbon ketol group from D-xylulose-5-phosphate to D-ribose-5-phosphate thereby generating D-sedoheptulose-7-phosphate.[1] Consequently the enzyme plays an important role in the biosynthesis of aromatic compounds of industrial interest.[2] The enzyme requires magnesium(II) ions and thiamine pyrophosphate (TPP) as cofactors. However, the isolated enzyme also has synthetic potential. For this the reaction can be made more efficient by the use of hydroxypyruvate **2** as the ketol donor (Scheme 1). The resultant release of carbon dioxide ensures that the reaction is irreversible leading to trihydroxylated compounds **3** containing the D-*threo* configuration.



1 **2** **3**

Scheme 1

Until recently it was believed that only α-hydroxyaldehydes of the D-configuration were possible aldehyde acceptors **1** but a recent report[3] described the use of some simple α-unsubstituted aldehydes. Thus the low specificity of TK for the aldehyde component **1** together with the high stereoselectivity for the carbon–carbon bond forming step combine to make this enzyme a potentially useful catalyst for asymmetric synthesis. With this in mind we have initiated a programme whose aim is to study the fundamental principles required to develop an integrated process for carbon–carbon bond formation on a large scale using transketolase as the catalyst. This communication describes our initial work aimed at developing an efficient and reliable source of the biocatalyst.

Transketolase can be obtained from two sources (i) *Saccharomyces cerevisiae*[4] (commercially available from Sigma) and (ii) spinach.[5] However neither of these sources yields large quantities of the enzyme although the synthetic potential of these sources has been demonstrated on a small scale.[6] Therefore we sought to develop an organism that could be used to produce greater quantities of TK. A recent paper described the cloning of the gene for TK from *E. coli* back into *E. coli* BJ 502[7] and this proved to be a useful starting point for over-expression of the protein. The fragment of DNA (5Kb) encoding the TK gene (2Kb) was excised from the plasmid of *E. coli* (BJ502/pKD112A) and reintroduced into a high copy plasmid vector (pUC18) in strain JM107, generating two new transformants (JM107/pQR182 and JM107/pQR183), differing by the orientation of the gene with respect to the *lac* promoter sited on pUC18. These two new transformants showed increased specific activity as measured by assay of the crude cell free extracts (Table 1). Both JM107/pQR182 and JM107/pQR183 produced TK with approximately four fold higher specific activity than BJ502/pKD112A. Examination of polyacrylamide gels run under denaturing conditions reveal TK to be the dominant protein. Thus by the simple expedient of introducing the TK gene into a high copy plasmid we were able to overexpress the protein in *E. coli*.

Having established a source of substantial quantities of TK we next examined its substrate specificity with respect to the aldehyde acceptor. All experiments were carried out using JM107/pQR183 and the results obtained are shown in Table 2. It can be seen that the enzyme shows a relatively low specificity for the aldehyde substrate. Although α-hydroxyaldehydes were the best substrates, simple aldehydes (*e.g.* propionaldehyde, pyruvaldehyde) also reacted and could be converted into the corresponding products on a preparative scale (see below). Cyclic aldehydes were poor substrates presumably due to unfavourable steric interactions at the active site. Of particular interest is the similarity of the substrate specificity profile to that reported for yeast[3] and may indicate a similarity between TK from the two different sources. We are currently carrying out additional experiments to characterise the *E. coli* TK more fully.

In summary, we have established a new source of transketolase that is able to produce the enzyme in large quantities suitable for preparative biotransformations.

Experimental

Preparation of Biocatalyst.—Starter cultures were prepared by inoculating sterile media* (50 cm^3) with single colonies of the organism from an agar plate and incubated for 16 h on an orbital shaker (150 rpm) at 37 °C. Aliquots (5 cm^3) of these cultures were transferred to flasks containing sterile batch

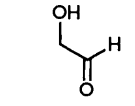
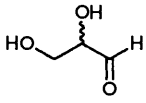
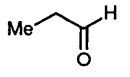
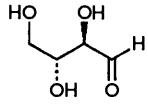
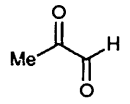
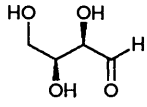
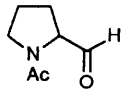
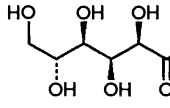
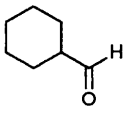
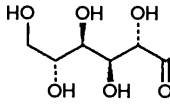
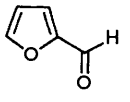
* The growth medium contained the following: $Na_2HPO_4 \cdot 12\ H_2O$ (15 g dm^{-3}), KH_2PO_4 (3 g dm^{-3}), NaCl (0.5 g dm^{-3} NH_4Cl (1 g dm^{-3}), $MgSO_4 \cdot 7H_2O$ (0.25 g dm^{-3}), shikimic acid (0.04 g dm^{-3}), thiamine (0.001 g dm^{-3}), ampicillin (0.1 g dm^{-3}) and either glucose (2.5 g dm^{-3}) or glycerol (2.5 g dm^{-3}).

Table 1 Activities of transketolase in cell-free extracts [8]

Transformant	Carbon source	TK activity U^a/cm^3	Protein mg cm^{-3}	Specific activity U^a/mg
BJ502/pKD112A	glucose	42.0	5.0	8.4
BJ502/pKD112A	glycerol	31.0	3.0	10.3
JM107/pQR182	glycerol	218.4	5.3	41.2
JM107/pQR183	glycerol	334.0	8.1	43.0

^{a} 1U = 1 μmol min^{-1}.

Table 2 Relative rates (V_{rel}) of aldehydes in their reaction[a] with hydroxypyruvate using crude extracts of *E. coli* JM107/pQR183

	V_{rel}		V_{rel}
	100		37
	20		89
	18		56
	5		14
	4		3
	3		

[a] The reaction mixture contained the following expressed as final concentration: TPP (0.2 mmol dm^{-3}), $MgCl_2 \cdot 6H_2O$ (0.9 mmol dm^{-3}), aldehyde (100 mmol dm^{-3}), hydroxypyruvate (7.5 mmol dm^{-3}), glycylglycine buffer (70 mmol dm^{-3}, pH 7.6), transketolase (3U) in a final volume of 1 cm^3. TK was added to initiate the reaction and aliquots (30 mm^3) were taken at 4 min intervals, deproteinised immediately and assayed for hydroxypyruvate.[9]

medium (50 cm^3 each). The batch cultures were incubated as above and then used to inoculate a fermenter (2 dm^3) containing the identical medium as the batch culture at 30 °C with an aeration rate of 6 dm^3 min^{-1}. Growth was allowed to proceed for 16 h after which the cells were harvested by centrifugation (15 min, 3800 rpm) and resuspended in glycylglycine buffer (0.1 mol dm^{-3}, pH 7.6, 20 cm^3). The cell free extract was prepared by sonication (3 × 1 min sonication followed by 1 min interval) and centrifugation (15 min, 3800 rpm) to remove cell debris.

Preparative TK Catalysed Reactions.—To a solution of magnesium chloride (1 mg, 6 mmol), thiamine pyrophosphate (11.5 mg, 0.025 mmol) and transketolase (50 U) in glycylglycine buffer (0.5 cm^3, 100 mmol dm^{-3}, pH 7.6) was added a solution of hydroxypyruvic acid (105 mg, 1 mmol) and the aldehyde (3 mmol, 3 equiv.) in glycylglycine buffer (20 cm^3, 100 mmol dm^{-3}, pH 7.6). The pH of the solution was then adjusted to pH 7.6 with sodium hydroxide (0.1 mol dm^{-3}). The reaction was allowed to proceed at 37 °C and monitored by TLC (ethyl acetate–methanol = 95:5). After the reaction was complete, silica (*ca.* 4 g) was added to the reaction mixture and the solvent removed under reduced pressure. Flash chromatography (ethyl acetate–petrol = 1:1) afforded the product.

(a) Using propionaldehyde the reaction yielded (3S)-1,3-*dihydroxypentan*-2-*one* (27 mg, 23%); δ_H(250 MHz; D_2O) 4.70 (1 H, d, J 19.0, HC*H*OH), 4.59 (1 H, d, J 19.0, *H*CHOH), 4.47 (1 H, dd, J 7.5 and 4.5, C*H*OH), 2.05–1.88 (1 H, m, *H*CHCH$_3$), 1.91–1.70 (1 H, m, HC*H*CH$_3$) and 1.07 (3 H, t, J 7.5, CH$_3$); δ_C(62.9 MHz; D_2O) 7.9 (CH$_3$), 25.7 (CH$_2$), 64.6 (CH$_2$), 75.3 (CH) and 213.7 (C=O). Treatment with benzoyl chloride in pyridine yielded the corresponding dibenzoate ester (25 mg, 45%); $[\alpha]_D$ = −5.6 (*c* 0.7, $CHCl_3$).

(b) Using pyruvaldehyde the reaction yielded (3S)-1,3-*dihydroxypentan*-2,4-*dione* (21 mg, 16%); δ_H(250 MHz; D_2O) 4.05–3.94 [1 H, m, C*H*(OH)], 3.66 (1 H, dd, J 4.3 and 11.6, HC*H*OH), 3.56 (1 H, dd, J 7.8 and 11.6, *H*CHOH) and 1.26 (3 H, d, J 6.5, C*H*$_3$CO); δ_C(62.9 MHz; D_2O) 17.5 (CH$_3$), 66.2 (CH$_2$), 67.4 (CH) and 217 (2 × C=O).

Acknowledgements

We thank Professor J. W. Frost for a gift of the strains *E. coli* BJ502 and BJ502/pKD112A and the SERC for the award of a studentship to G. R. H.

References

1 E. Racker, in *The Enzymes*, ed. P. D. Boyer, H. Lardy and K. Myrzback, vol. 5, p. 397, Academic Press, New York, 1961.
2 K. M. Draths, D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky and J. C. Lievense, *J. Am. Chem. Soc.*, 1992, **114**, 3956.
3 C. Demuynck, J. Bolte, L. Hecquet and V. Dalmas, *Tetrahedron Lett.*, 1991, **32**, 5085.
4 A. Datta and E. Racker, *J. Biol. Chem.*, 1961, **236**, 617.
5 J. Villafranca and B. Axelrod, *J. Biol. Chem.*, 1971, **246**, 3126.
6 D. C. Myles, P. J. Andrulis and G. M. Whitesides, *Tetrahedron Lett.*, 1991, **32**, 4835.
7 K. M. Draths and J. W. Frost, *J. Am. Chem. Soc.*, 1990, **112**, 1657.
8 Transketolase assayed as described by C. P. Heinrick, K. Noack and O. Wiss, *Biochem. Biophys. Res. Commun.*, 1972, **49**, 1427.
9 Hydroxypyruvate assayed as described by A. W. Holldorf, in *Methods in Enzymology*, vol. 3, Academic Press, New York, 1966, 578.

Paper 2/05949E
Received 6th *November* 1992
Accepted 20th *November* 1992