Stereochemistry of the Formation of Lactaldehyde and Acetoin produced by the Pyruvate Decarboxylases of Yeast (*Saccharomyces* sp.) and *Zymomonas mobilis*: Different Boltzmann Distributions Between Bound Forms of the Electrophile, Acetaldehyde, in the Two Enzymatic Reactions

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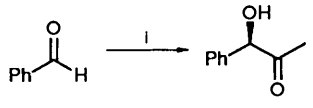
Pyruvate decarboxylase from *Saccharomyces* sp. catalysed the formation of acetoin of predominantly the opposite configuration than that obtained using the enzyme from *Zymomonas mobilis*. A similar result was obtained for the lactaldehyde formed in the pyruvate decarboxylase-catalysed decarboxylation of glyoxylate in the presence of acetaldehyde. The results are interpreted in terms of different Boltzmann distributions for the two enzymes between the enzyme–substrate complexes of acetaldehyde bound on its *re* and *si* faces respectively.

Acyloin formation from aldehydes catalysed by brewer's yeast has been studied ever since the original discovery by Neuberg[1] that phenyl acetyl carbinol [PAC, 1-hydroxy-1-phenylpropan-2-one, **1** (Scheme 1)] was produced following addition of



Scheme 1 *Enzyme:* i, brewer's yeast

benzaldehyde to fermenting yeast. The PAC produced was later shown to have the (*R*)-configuration. A patent describing the conversion of this optically active product into ephedrine in two chemical steps[2] established an industrial process by which ephedrine has been manufactured on a large scale until the present day. Although the enzyme responsible for the conversion of benzaldehyde into phenyl acetyl carbinol has long been considered to be pyruvate decarboxylase (PDC), it is only recently that experimental evidence has been obtained to support this contention.[3,4]

We have studied the substrate specificity of the PDCs from yeast (*Saccharomyces* sp.) and *Zymomonas mobilis* and have found that aromatic and heterocyclic aldehydes are strongly and specifically recognised, giving products of high optical purity. However, when aliphatic electrophilic acceptors of acyl anion equivalent transfer are involved, a different result is obtained. We describe here the results of studies with two microbial PDCs, two donors of acyl anion equivalents (pyruvate and glyoxylate) and one electrophilic acceptor, acetaldehyde.

Commercial yeast PDC was purified by FPLC to a specific activity of 23 U mg^{-1} and appeared to be of greater than 80% purity by denaturing polyacrylamide gel electrophoresis (PAGE) stained with Coomassie blue.

Escherichia coli strain DH1 (with no detectable PDC activity) was transformed[6] with a plasmid pLOI295 encoding the PDC gene from *Z. mobilis* strain CP4 ATCC 31821. The expressed PDC was purified by FPLC. The final activity was 150–186 U mg^{-1} which can be compared with activities of 120–181 U mg^{-1} reported for the pure enzyme.[7,8]

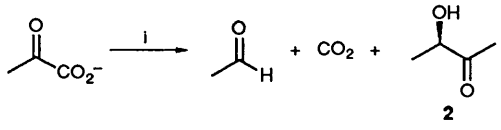
PDC catalyses the decarboxylation of pyruvate to give acetaldehyde and carbon dioxide with concomitant formation of acetoin **2** (Scheme 2).[9] The proportion of pyruvate decarboxylation diverted to acetoin production increases with increasing concentrations of acetaldehyde, approaching 100% at high concentrations of acetaldehyde.[9] Pyruvate was incubated with yeast PDC and the acetoin **2** produced was analysed by chiral GLC on a Lipodex A column. The major (*R*)-enantiomer[10] was eluted first. Samples from incubations were withdrawn after 4 h and 21 h. The decarboxylation was complete after 4 h. The incubation was continued for 21 h in order to obtain an indication of the degree of non-enzymatic epimerisation that might be occurring. Comparison of the results obtained for 4 h and 21 h incubations indicated that very little non-enzymatic epimerisation was occurring. Experiments were carried out in triplicate and gave remarkably consistent results (Table 1). Acetoin **2** of 53 ± 2 and 46 ± 1% ee was obtained after 4 and 21 h respectively. Identical results were obtained when acetaldehyde was added as a cosubstrate (Table 1).

Acetaldehyde alone is a very poor substrate for acetoin production catalysed by yeast PDC. Nevertheless, when acetaldehyde alone was incubated with yeast PDC, the optical activity of the acetoin produced was identical with that obtained with pyruvate alone and pyruvate plus acetaldehyde (Table 1).

Table 1 Production of (*R*)-acetoin by yeast PDC[a]

	%ee[b]	
Substrate (mmol dm^{-3})	4 h	21 h
Pyruvate (100)	53 ± 2	46 ± 1
Acetaldehyde (100)	*c*	44 ± 4
Pyruvate (100) + acetaldehyde (300)	50 ± 2	46 ± 1
Acetaldehyde (100) + pyruvamide (100)	41 ± 7	39 ± 2
Pyruvate (100) + glyoxylate (100)	*c*	35 ± 10

[a] Reaction mixtures contained sodium citrate buffer (0.1 mol dm^{-3}, pH 6.0), thiamin pyrophosphate (15 μmol dm^{-3}), $MgSO_4$ (0.1 mmol dm^{-3}), brewer's yeast PDC (7.55 U, 12 U mg^{-1}), incubated at 30 °C. [b] Ees were determined in triplicate. [c] Not detected.



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Scheme 2 *Enzyme:* i, yeast pyruvate decarboxylase

Table 2 Production of (*S*)-acetoin by *Zymomonas mobilis* PDC[a]

Substrate (mmol dm^{-3})	%ee[b] 4 h	21 h
Pyruvate (100)	29 ± 2	28 ± 3
Acetaldehyde (100)	25 ± 1	25 ± 3
Pyruvate (100) + acetaldehyde (300)	29 ± 1	28
Acetaldehyde (100) + pyruvamide (100)	26 ± 3	25 ± 2
Pyruvate (100) + glyoxylate (100)	*c*	24 ± 6

[a] Reaction mixtures contained sodium citrate buffer (0.1 mol dm^{-3}, pH 6.0), thiamin pyrophosphate (15 μmol dm^{-3}), $MgSO_4$ (0.1 mmol dm^{-3}), *Z. mobilis* PDC (7.55 U, 186 U mg^{-1}), incubated at 30 °C. [b] Ees were determined in triplicate. [c] Not detected.

Table 3 Production of lactaldehyde by pyruvate decarboxylase[a]

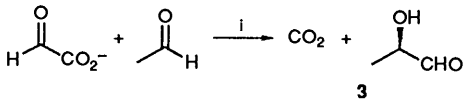
PDC (concentration/U cm^{-3})	%ee (config.)
Brewer's yeast (5.9)	22 (*R*)
Purified brewer's yeast (3.4)	19 (*R*)[b]
Purified brewer's yeast (3.4)	20 (*R*)[b]
Zymomonas mobilis (5.3)	52 (*S*)
Zymomonas mobilis (6.1)	56 (*S*)
Zymomonas mobilis (6.1)	55 (*S*)
Zymomonas mobilis (6.1)	61 (*S*)[b,c]

[a] Reaction mixtures contained pH 6.0 sodium citrate buffer (0.1 mol dm^{-3}), TPSS[d] (11.45 mmol dm^{-3}), thiamin pyrophosphate (15 μmol dm^{-3}), $MgSO_4$ (0.1 mmol dm^{-3}), sodium pyruvate (100 mmol dm^{-3}), sodium glyoxylate (100 mmol dm^{-3}) and either *Z. mobilis* PDC (186 U mg^{-1}), brewer's yeast PDC (12 U mg^{-1}) or purified brewer's yeast PDC (23 U mg^{-1}). The mixtures were incubated at 30 °C for 24 h. The optical purity of the lactaldehyde was determined by chiral HPLC of the corresponding 2,4-dinitrophenylhydrazone. [b] TPSS was omitted from the incubation mixture. [c] Determined after 17 h. [d] TPSS = 3-(trimethylsilyl)propane-1-sulfonic acid sodium salt.

It is well established that yeast PDC is activated by the substrate, pyruvate, but that this activation can also be induced by pyruvamide.[11] (The present experiments were conducted with pyruvate concentrations sufficiently high to ensure that reactions were catalysed by the activated complex.) When pyruvamide and acetaldehyde were incubated with the enzyme, only a slight diminution was observed in the optical activity of the acetoin produced after 4 h and 24 h (Table 1).

When a similar set of incubations was carried out with the PDC from *Z. mobilis* an extremely consistent set of data was obtained for all substrate concentrations (Table 2) except that in this case the *S*-enantiomer of acetoin predominated. The enantiomeric excess of the acetoin **2** produced was again remarkably consistent at about 28%.

Glyoxylic acid, in principle, could generate 'active formaldehyde' just as 'active acetaldehyde' is generated from pyruvate. Evidence that this can occur has been obtained using enzyme preparations from higher plants.[12] Glyoxylate alone is decarboxylated only stoichiometrically by the yeast enzyme since release of formaldehyde is very slow.[13] However, we have found that in the presence of acetaldehyde, turnover occurs with the formation of lactaldehyde **3** (Scheme 3). A similar



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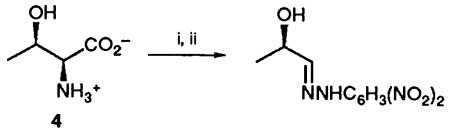
Scheme 3 *Enzyme:* i, yeast pyruvate decarboxylase

observation was made with the PDC from *Z. mobilis*. The lactaldehyde **3** produced in both cases was optically active. Chiral analysis was carried out by conversion of the lactaldehyde into its 2,4-dinitrophenylhydrazone followed by HPLC using a Chiralcel OB column. Standards were prepared by ninhydrin degradation of D,L- and L-threonine **4** (Scheme 4).[14] The results are given in Table 3. Once again, the



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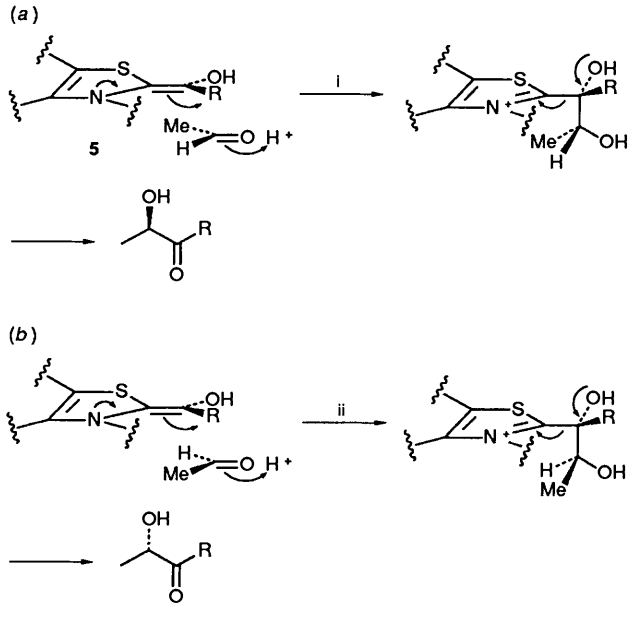
Scheme 4 *Reagents:* i, ninhydrin; ii, 2,4-dinitrophenylhydrazine

lactaldehyde **3** produced by each of the two enzymes was of consistent and reproducible optical purity but with the *R*-enantiomer predominating with yeast PDC and the *S*-enantiomer predominating with *Z. mobilis* PDC.

The finding that the pyruvate decarboxylases from yeast and from *Z. mobilis* catalyse the formation of acyloins of intermediate but consistent optical purity, mirrors the results obtained with the enzyme from wheat germ.[15] The present results indicate that nucleophilic attack of the hydroxyethyl adduct of the co-factor, thiamin pyrophosphate, attacks predominantly the *si*-face of bound acetaldehyde during catalysis by yeast PDC and predominantly the *re*-face during catalysis by the enzyme from *Z. mobilis* (Scheme 5, R = Me).



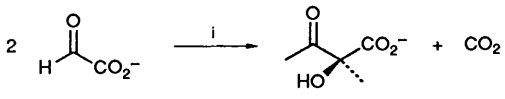
Scheme 5 *Enzymes:* i, yeast PDC; ii, *Z. mobilis* PDC

(The configuration of the hydroxyethyl intermediate **5** is unknown; the *Z*-configuration is arbitrarily chosen for purposes of illustration.) The stereochemistry of lactaldehyde formation reveals a consistency in the preferred stereochemical course of attack by either 'active acetaldehyde' or 'active formaldehyde' by the two enzymes (Scheme 5, R = H).

The reactions catalysed are essentially irreversible as indicated by the constant optical purities of acyloin products regardless of incubation time (Tables 1, 2). Although differences in k_{cat} for reactions proceeding *via* diastereoisomeric transition states are to be expected, the results obtained must reflect in part different binding energies for the *re*- and *si*-faces of acetaldehyde. If the differences in relative rates of formation of enantiomeric products were solely the result of the expression of different binding modes, these differences would reflect the Boltzmann distribution between the two binding modes (*re* or *si*) of the bound acetaldehyde. If this were so, for yeast PDC the energy differences for acetoin production and lactaldehyde

production would be 3.0 kJ mol^{-1} and 1.0 kJ mol^{-1} respectively, and for *Z. mobilis* PDC, −1.5 kJ mol^{-1} and −3.2 kJ mol^{-1} respectively. The net differences in energy between the binding modes would therefore be 4.5 kJ mol^{-1} for acetoin production and 4.2 kJ mol^{-1} for lactaldehyde production. The close similarity between these values provides further circumstantial evidence that the stereochemical differences in the reactions catalysed by the two enzymes largely reflect different binding modes. Such small differences, when the results were obtained for a single enzyme, would be virtually impossible to interpret. However, given the anticipated availability of a high resolution crystal structure for yeast PDC,[16] and amino acid sequences of both yeast PDC[17] and the homologous *Z. mobilis*[18] PDCs, an interpretation may be possible in this case by comparing the different architectures of the active sites of these enzymes.

The formation of acyloins by PDC is anomalous in the sense that the physiological role of the enzyme is the decarboxylation of pyruvate to acetaldehyde and carbon dioxide. However, light has been shed on the propensity of PDC to catalyse carbon–carbon bond formation by the finding that it is homologous with acetolactate synthase,[18] a thiamin pyrophosphate-dependent enzyme that catalyses the first common step in valine and isoleucine biosynthesis (Scheme 6).



Scheme 6 *Enzyme:* i, acetolactate synthase

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