ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 19–20 (2002) 399–403

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Lactate as an alternative to pyruvate in the yeast mediated preparation of PAC

Greg Laurence, Andrew J. Smallridge*, Maurie A. Trewhella

School of Life Sciences and Technology, Victoria University of Technology, P.O. Box 14428, Melbourne MC 8001, Australia

Received 7 September 2001; received in revised form 26 June 2002; accepted 29 July 2002

Abstract

The yeast mediated preparation of phenylacetylcarbinol (*l*-PAC) via the condensation of benzaldehyde with pyruvate has been conducted in petroleum spirit using lactic acid as the pyruvate precursor. The lactic acid is oxidised in situ to pyruvate, which then condenses with the benzaldehyde. The optimal conditions for the conversion of benzaldehyde to PAC were 5 g yeast/mmol benzaldehyde, 0.5 g lactic acid adjusted to pH 5.63 and no ethanol.

Keywords: Lactate; Yeast; Preparation of PAC; Organic solvent

1. Introduction

The yeast mediated preparation of *l*-phenylacetylcarbinol[1] (*l*-PAC) is an important commercial process as it forms the first step in the production of the vassopressor drugs ephedrine and pseudoephedrine [1]. The yeast mediated preparation of *l*-PAC utilises fermenting yeast to convert glucose into pyruvate which is condensed with added benzaldehyde to form *l*-PAC. One of the drawbacks to this process is the concomitant yeast mediated reduction of benzaldehyde to benzyl alcohol, which limits the yield of *l*-PAC [2]. In an attempt to minimise this side-reaction the yeast mediated reaction was carried out in an organic solvent using non-fermenting yeast [3]. This reaction gave *l*-PAC in low yields but with high optical purity in the absence of side-products such as benzyl alcohol. This was the first time a yeast mediated reaction of this type had been successfully conducted in an organic solvent. As with all yeast reactions utilising an organic solvent, the uncomplicated isolation of pure material from the reaction mixture was a feature. The drawback to this reaction is that since the yeast is not fermenting, glucose is not being converted to pyruvate and, hence pyruvate must be added to the reaction. The additional cost of the added pyruvate may limit the commercial applicability of this reaction.

It was thought that lactate may be a cheaper alternative to pyruvate in the yeast mediated production of *l*-PAC. The yeast could oxidise the lactic acid to pyruvic acid in situ and the pyruvate would then condense with the added benzaldehyde to produce *l*-PAC. The use of an organic solvent system for this reaction should allow the reduction of the unwanted side-products to be controlled and enable the ready isolation of the product.

* Corresponding author. Tel.: +613-9688-4758; fax: +613-9688-4995.
E-mail address: andrew.smallridge@vu.edu.au (A.J. Smallridge).

[1] The systematic name for *l*-phenylacetylcarbinol is (*R*)-3-phenyl-3-hydroxypropan-2-one. The precursor to ephedrine is almost universally know as *l*-PAC and, hence this terminology is used throughout the text.

1381-1177/02/$ – see front matter
PII: S1381-1177(02)00191-1

2. Experimental

2.1. Materials

Benzaldehyde was washed with 10% Na_2CO_3 solution until the evolution of CO_2 ceased, then washed with saturated Na_2SO_4 solution and dried over $CaCl_2$. The benzaldehyde was distilled under nitrogen at reduced pressure and stored under nitrogen in a desiccator.

The baker's yeast (*Saccharomyces cerevisiae*) used in the reactions was Mauripan instant dry yeast supplied by Kerry Pinnacle Bakery Products, Sunshine, Australia, and was stored at room temperature; no precautions were taken to exclude air or moisture as reproducible results were obtained even after months of storage under these conditions. BDH AnalaR petroleum spirit (40–60 °C) was used without purification; petroleum spirit is preferred to hexane as it is significantly cheaper, less toxic and gave identical results. Pyruvic acid and DL-lactic acid (85%) were purchased from Sigma–Aldrich.

2.2. Analytical methods

Optical rotations were measured with an Optical Activity PolAAr 2000 AA series polarimeter.

Gas chromatographic analysis of products was performed on a Shimadzu GC-17A using a BP1 column, 0.25 mm i.d., 15 m in length and with a phase thickness of 0.25 μm. The oven temperature was held at 40 °C for 3 min and then increased at 8 °C/min to 150 °C.

Chiral gas chromatography was conducted using a Chiraldex G-TA (30 m × 0.25 mm) column with a phase thickness of 0.125μm. The oven temperature was held at 95 °C.

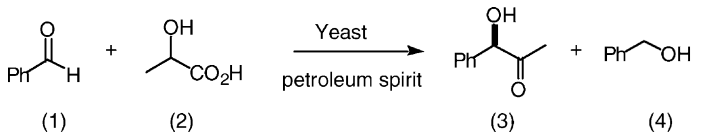
2.3. General procedure for the yeast reactions

Lactic acid (3.61 g, 0.04 mmol) was dissolved in water (50 ml) and the pH adjusted to 5.63 with ammonium acetate. Lactic acid solution (5 ml), benzaldehyde (106 mg, 1 mmol), petroleum spirit (50 ml) and yeast (5 g) were shaken for 24 h at room temperature. A sample was taken from the reaction mixture and subjected to gas chromatographic analysis. The conversion was calculated by comparing the amount of product with starting material in the GC trace and the values quoted represent an average of at least three reactions. The product was isolated by bulb-to-bulb distillation at 200 °C in a 15% yield. Chiral GC on the trifluoroacetyl derivative [3] of the product indicated a 83% ee.

3. Results and discussion

The yeast mediated condensation of benzaldehyde with lactic acid (Scheme 1) was conducted in petroleum spirit using the general procedure for the yeast catalysed production of *l*-PAC previously reported [3] with lactic acid used in place of pyruvate: 5 g yeast/mmol benzaldehyde, 1 g lactic acid (2), 45 ml petroleum spirit, 1 ml pH 6 citrate buffer per gram of yeast, 20 °C, 24 h. Analysis of the reaction mixture showed benzaldehyde (1; 17%), benzyl alcohol (4; 74%) and *l*-PAC (3; 9%). In comparison, the yeast mediated condensation of benzaldehyde and pyruvic acid in an organic system performed under similar conditions showed benzaldehyde (1; 70%), benzyl alcohol (4; 22%) and *l*-PAC (3; 8%) [3].

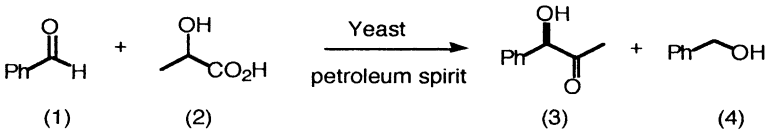
This result indicates that *l*-PAC can be synthesised using lactic acid and also indicates that the lactic acid is being oxidised by the enzyme(s) in the yeast to pyruvic acid. Interestingly, the lactic acid system yielded a higher amount of benzyl alcohol than the corresponding pyruvic acid reaction. The high conversion of benzaldehyde to the unwanted side-product, benzyl alcohol, shows that the reduction reaction is also taking place, but at a higher rate than in the pyruvic acid system.



(1) (2) (3) (4)

Scheme 1.

Table 1
The yeast mediated conversion of benzaldehyde to *l*-PAC using lactic acid with different amounts of added ethanol



(1) (2) (3) (4)

Ethanol (ml)	*l*-PAC (%)	Benzyl alcohol (%)
0.5	8	8
0.75	4	0
1.00	3	0

3.1. The addition of ethanol

Previous studies [3] have shown that the addition of small amounts of ethanol to the yeast mediated reaction inhibits the oxidoreductase enzymes which consequently limits the production of unwanted by-products, like benzyl alcohol, and thereby results in an increased conversion of benzaldehyde to *l*-PAC. A series of yeast mediated reactions using lactic acid was performed with the addition of a small amount of ethanol (0.5, 0.75 and 1 ml) and the results are as shown in Table 1.

The results show that although there is a significant drop in the amount of benzyl alcohol produced, compared with the reaction in the absence of ethanol, the amount of *l*-PAC produced has also decreased. It is most likely that the lower conversion of benzaldehyde to *l*-PAC is because the ethanol has inhibited the oxidation of lactate to pyruvate, thus reducing the amount of pyruvate available for the reaction.

3.2. The effect of pH

Since the addition of ethanol has a deleterious effect upon the production of both benzyl alcohol and *l*-PAC an alternative means was sought to selectively inhibit the reduction reaction. It has been demonstrated that different enzyme systems in yeast have different pH optima [4]. In order to optimise the pH of the system, the reaction was performed using lactic acid buffered to a range of pH values (1.95–7.2) using ammonium acetate and the results are as shown in Fig. 1. Ethanol was not used in these reactions in order to determine if pH alone could selectively inhibit the reduction of benzaldehyde without a decrease in *l*-PAC production.

The results show that as the pH is increased, the yeast is activated (pH 4) and the production of *l*-PAC is observed. Once the pH of the system becomes too basic (>7) the production of *l*-PAC ceases. Benzyl alcohol is also produced within similar pH ranges suggesting yeast is only active between pH 3 and 7. It is perhaps not surprising that the enzyme systems are active in the range of pH 3–7, since this is close to the physiological pH of yeast. Interestingly, the production of *l*-PAC and benzyl alcohol involve two different enzyme systems, yet both appear to exhibit two pH optima and at similar pH values. This would suggest that these two enzyme systems are interrelated, which is perhaps not surprising as both are required for the conversion of pyruvate to *l*-PAC.

Since pH was unable to selectively inhibit the reductase enzymes, ethanol was added to the reaction. The reactions were repeated in the active pH range

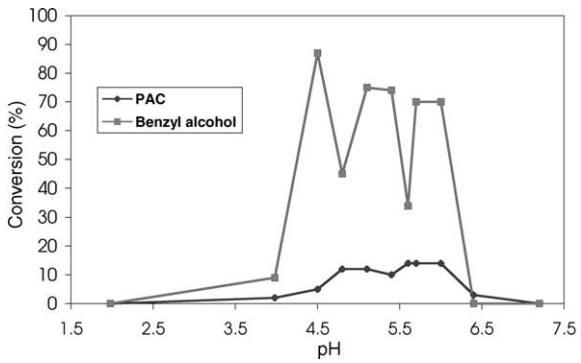


Fig. 1. Yeast mediated condensation of lactic acid and benzaldehyde at various pH values (0.3 g lactic acid, 1 mmol benzaldehyde, 50 ml petroleum spirit, 5 g yeast, 5 ml ammonium acetate buffer 20 °C).

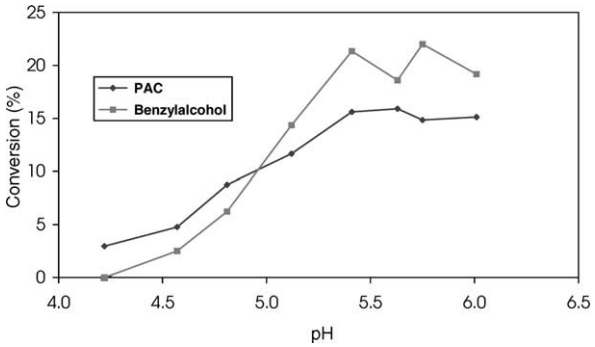


Fig. 2. Yeast mediated condensation of lactic acid and benzaldehyde at various pH values (0.3 g lactic acid, 1 mmol benzaldehyde, 50 ml petroleum spirit, 5 g yeast, 5 ml ammonium acetate buffer, 20 °C, 0.5 ml ethanol).

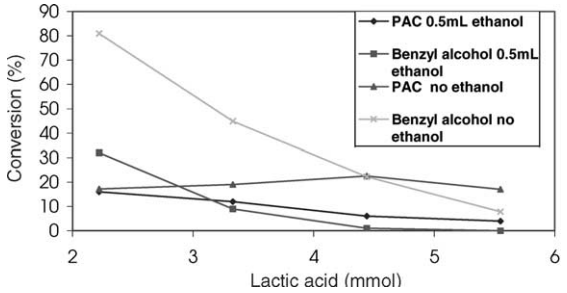


Fig. 3. Yeast mediated condensation of lactic acid and benzaldehyde with varying amounts of lactic acid in the presence and absence of ethanol (1 mmol benzaldehyde, 50 ml petroleum spirit, 5 g yeast, 5 ml water, 20 °C).

(pH 4–6) with the addition of 0.5 ml of ethanol and the results are as shown in Fig. 2.

The results show that, at similar pH values, the production of benzyl alcohol is significantly reduced in the presence of ethanol, e.g. at pH 5, from 75% down to 12%. The best result obtained for the production of *l*-PAC, in the presence of ethanol, is at pH 5.63 with 16% conversion to *l*-PAC and only 19% to benzyl alcohol. This was considered the optimum pH for this reaction.

The activation of the enzymes in yeast in an organic solvent is known to be dependent upon the amount of water present in the system [5]. The reactions in the current study used 1 ml water per gram of yeast which was the optimal amount determined for the yeast-mediated production of *l*-PAC from benzaldehyde and pyruvic acid [3]. The reaction involving lactic acid may have a different optimum and this was investigated by varying the amount of water added to the reaction. The yeast-mediated acyloin condensation was carried out using 0.8–1.2 ml water per gram yeast. Very little change in the amount of *l*-PAC or benzyl alcohol produced was observed, therefore, 1 ml water per gram yeast was considered optimal.

3.3. Optimum lactic acid concentration

Previous investigations of the yeast-mediated production of *l*-PAC using pyruvic acid showed that the amount of *l*-PAC formed was dependant upon the amount of pyruvic acid added to the reaction [3]; it was also demonstrated that increased levels of pyruvic acid inhibited the reduction reaction and reduced the amount of benzyl alcohol formed. In order to examine if a similar dependence on lactic acid existed, a range of reactions involving different amounts of lactic acid was conducted.

Reactions were conducted both in the presence and absence of ethanol to investigate whether varying the amount of lactic acid could selectively inhibit the production of benzyl alcohol. Various solutions of lactic acid were prepared (2.22–5.55 mmol) and adjusted to pH 5.63 using ammonium acetate. Results are as shown in Fig. 3.

The results show, both in the absence and presence of ethanol, that as the amount of lactic acid added to the reaction is increased the amount of benzyl alcohol decreases dramatically. It has been shown, using ^{13}C labelled pyruvate, that in the yeast mediated reactions pyruvate is decarboxylated to acetaldehyde which is then reduced to ethanol [3]. The reduction of acetaldehyde to ethanol involves the concomitant oxidation of NADH to $NAD^{\oplus}$. The reduction of benzaldehyde to benzyl alcohol also involves NADH. In the reactions conducted in an organic solvent there are no added nutrients available to permit the recycling of $NAD^{\oplus}$ back to NADH. The amount of NADH available in these reactions is, therefore, limited and with both the reactions consuming NADH at the same time, it is quickly exhausted. As the amount of lactic acid is increased, more ethanol is formed, which has a two-fold effect on the yeast system. First, the consumption of NADH is elevated which limits the amount of

reductant available to convert benzaldehyde to benzyl alcohol and second, the ethanol produced inhibits the reductase enzyme(s). Overall this has the effect of decreasing benzyl alcohol formation without altering the production of *l*-PAC.

The level of *l*-PAC is less dependent upon the amount of lactic acid added to the reaction. In the absence of ethanol, the amount of *l*-PAC produced remains virtually constant. When ethanol is added to the reaction, the production of *l*-PAC decreases as the amount of lactic acid increases. This suggests, as discussed previously, that the oxidation of lactic acid to pyruvic acid has also been inhibited by the added ethanol.

These results show that the optimal level of lactic acid with the addition of ethanol to be 2.22 mmol (16% *l*-PAC, 32% benzyl alcohol) as at this level the amount of *l*-PAC produced is greatest. In the absence of ethanol, the optimal level of lactic acid was 4.5 mmol; slightly higher conversion to *l*-PAC (22%) was achieved with a lower amount of benzyl alcohol (22%) being produced, indicating that the reaction is more efficient in the absence of ethanol.

3.4. Enantioselectivity

The *l*-PAC formed from the yeast mediated acyloin condensation reaction using the optimum reaction conditions was isolated and purified. *l*-PAC was isolated in 15% yield and with 83% ee. As with all yeast mediated reactions in an organic solvent, the isolation and purification process is considerably simpler and quicker than that required for corresponding reactions conducted using fermenting yeast in water. The yield and ee obtained compare favourably with the reaction conducted in an organic solvent using pyruvate (16% yield and 86% ee). Analysis of the *l*-PAC present in the crude reaction mixture gave a similar ee value indicating that the ee is representative of the reaction selectivity and is not influenced by the isolation or purification procedures.

4. Conclusion

The results obtained from various reactions performed under a variety of conditions showed that lactic acid can be used in place of pyruvic acid for the biotransformation of benzaldehyde to *l*-PAC. The optimal conditions for the conversion of benzaldehyde to *l*-PAC were 5 g yeast per mmol of benzaldehyde, 0.5 g lactic acid adjusted to pH 5.63 and no ethanol. The relatively low yield of *l*-PAC and the significant quantity of benzyl alcohol produced, indicates that this reaction is less attractive than the system using pyruvate and that further advances in *l*-PAC formation and side-product suppression are required before the organic solvent system can compete with the current commercial production of *l*-PAC. The use of yeast in an organic solvent for the preparation of *l*-PAC is, however, a simpler system than processes employing isolated enzyme preparations in an aqueous medium. These results complement the previously reported use of an organic solvent system for yeast reduction reactions and emphasise the applicability of an organic solvent as a medium for yeast mediated reduction reactions.

References

[1] G. Hildebrandt, W. Klavehn, US Patent 1,956,950 (1934).
[2] Y.S. Chow, H.S. Shin, A.A. Adesina, P.L. Rogers, Biotechnol. Lett. 17 (1995) 1201.
[3] M. Kostraby, A.J. Smallridge, M.A. Trewhella, Biotechnol. Bioeng. 77 (2002) 827.
[4] K. Nakamura, Y. Kawai, N. Nakajima, A. Ohno, J. Org. Chem. 56 (1991) 4778.
[5] L. Jayasinghe, D. Kodituwakku, A.J. Smallridge, M.A. Trewhella, Bull. Chem. Soc. Jpn. 67 (1994) 2528.