Biocatalytic Reduction of Aromatic Ketones Using Coenzyme Regeneration in An Electrochemical Process

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Abstract:

Electrochemically, halogenated aromatic ketones were reduced employing dried Geotrichum candidum cells as a highly enantioselective catalyst. A separate enzyme or co-substrate for coenzyme recycling was not required since enzymes in the dry cell catalysed both processes to decrease substrates and recycle the coenzyme using electricity.

KEYWORDS: Geotrichum candidum, electrochemistry, asymmetric reduction, and derivatives of acetophenone.

INTRODUCTION

A crucial step in organic synthesis, the reduction of aromatic ketones to their corresponding alcohols is essential for the creation of medicines, fine compounds, flavours, and perfumes. Chemical reducing agents are often used in traditional procedures for this transformation, although they frequently have problems such limited selectivity, difficult reaction conditions, and the production of toxic waste. Therefore, there has been an increase in interest in creating ecologically acceptable and sustainable options for this shift.

An appealing method for reducing aromatic ketones is biocatalysis, which uses enzymes as catalysts. High selectivity, gentle reaction conditions, and the capacity for stereoselective transformations are all characteristics of enzymes. However, obstacles including slow response speeds and the need for pricey coenzymes sometimes prevent their practical use in large-scale procedures.

The combination of electrochemistry and biocatalysis has shown promise as a solution to these problems. Unique benefits provided by electrochemical techniques include the capacity to replenish coenzymes on-site, moderate reaction conditions, and precise control over reaction parameters. It is conceivable to increase reaction speeds, boost selectivity, and achieve cost-effective coenzyme regeneration by combining electrochemistry with biocatalysis.

Here, using electricity, we demonstrate the asymmetric reduction of halogenated aromatic ketones by a crude dehydrogenase from the fungus Geotrichum candidum. A mediator, such as methylviologen

(MV2+), and enzymes, such as diaphorase, are required for the electrochemical recycling of the coenzyme's oxidised state. As indicated in Scheme 1, the biocatalyst can catalyse both reductions of NAD(P) + toNAD(P)Hand ketonesto the corresponding alcohols. We discovered that the crude dehydrogenase from Geotrichum candidum possessed diaphorase activity. With high efficiency, trifluoroacetophenone was decreased.

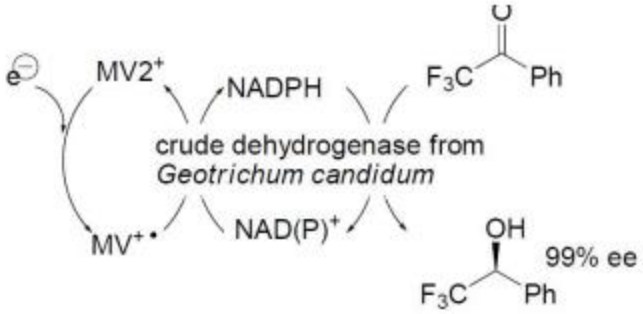

Figure 1: Trifluoroacetophenone is reduced symmetrically electrochemically.

REVIEW OF LITERATURE

The need for efficient and sustainable synthesis pathways in the pharmaceutical, flavour, and fragrance industries has spurred a lot of recent research on the reduction of aromatic ketones utilising biocatalytic methods. This section reviews the literature in the topic of biocatalytic reduction of aromatic ketones and the incorporation of electrochemistry for coenzyme renewal. It highlights significant discoveries and developments in these areas.

Enzymes and Coenzymes: A number of enzymes, such as alcohol dehydrogenases (ADHs), ketoreductases (KREDs), and ene-reductases (EREDs), have been investigated for the reduction of aromatic ketones. These enzymes allow for the stereocontrolled synthesis of chiral alcohols and show remarkable selectivity towards certain ketone substrates. Their practical use, however, is sometimes constrained by the need for pricey coenzymes that must be ingested in stoichiometric proportions, such NADH or NADPH.

Coenzyme Regeneration techniques: Several coenzyme regeneration techniques have been created in order to get around the problem of expensive coenzymes. To recycle or renew the coenzymes on-site, these methods combine biocatalytic reactions with other chemical or enzymatic activities. Examples include regenerating NADH from formate or glucose using formate dehydrogenase or glucose dehydrogenase, respectively. These methods still continue to depend on external chemicals or sacrificial substrates, which makes the procedure more difficult and expensive as a whole.

The combination of electrochemistry with biocatalysis provides a novel approach to the reduction of aromatic ketones. Electrochemical Processes in Biocatalysis. Coenzyme regeneration may be done effectively and sustainably using electrochemical techniques. These procedures allow for the regeneration of NADH or NADPH from their oxidised forms (NAD+ or NADP+) since electrons are transported directly to or from the electrode surface. This method is more economically feasible since it does not need external chemicals or sacrificial substrates.

Benefits of Electrochemical Processes: There are various benefits to combining electrochemistry with biocatalysis. First of all, electrochemical methods provide fine tuning of the reaction conditions for increased catalytic efficiency by allowing exact control over reaction parameters including potential, current, and temperature. Second, the gentle reaction conditions of electrochemistry reduce unwanted reactions and enhance the biocatalyst's stability and activity. Furthermore, the coenzyme regeneration takes place in-situ, lowering the coenzyme needs and raising the process' overall cost-effectiveness.

Experimental Development and Results: Recent research has shown that electrochemical techniques for the biocatalytic reduction of aromatic ketones may be successfully used. These investigations have shown better selectivity, reaction speeds, and coenzyme regeneration efficiency. The usage of immobilised enzymes on conductive electrodes, in particular, has shown remarkable results, allowing direct electron transport between the electrode and the biocatalyst, hence promoting effective coenzyme recycling.

Applications and Future Perspectives: Chiral alcohols are crucial building blocks for drug production, and the coupling of electrochemical and biocatalytic methods offers tremendous promise for applications in the pharmaceutical sector. The creation of certain enantiomers for desired sensory profiles is made possible by electrochemistry, which also offers the flavours and fragrances sector excellent control over reaction conditions. Additionally, the electrochemical techniques' sustainability fits with the increasing need for ecologically responsible chemical synthesis.

MATERIALAND METHODS

Materials

We bought NAD+ and NADP+ from KojinCo. According to the reference, crude alcohol dehydrogenase from Geotricum candidum IFO 4597 (APG4) was obtained. Aldrich was used to obtain methyl viologen, trifluoroacetophenone, o-chloroacetophenone, and other chemicals.

Electrolysis

A gold plate with a surface area of 2.0 cm2 was dipped in mercury, creating a gold-amalgam (Au/Hg) surface. The working electrode, an Au/Hg electrode, produced insignificant background currents at negative potentials, but further tests revealed the gold surface and led to increased background

currents brought on by the reduction of surface gold oxides.At this point, the old electrode was thrown away and a fresh one was made for more trials. Electrochemical studies were carried out in an H-type cell that was divided by agar (2% KCl), and the reaction was carried out in an argon atmosphere (argon gas was bubbled before the reaction).The working electrode was an Au amalgam electrode, while the reference electrode was an Ag/AgCl in a saturated KCl solution. In the instance of electrolysis, the Pt counter electrode was submerged in a different compartment. The supporting electorolyte solution was phosphate buffer (20 mM, pH 7.0). Three electrode system, Polarograph Model 311 and HECS 311B (Fuso Seisakusho Co.), were used to measure cyclic voltammograms.

General reduction technique

Under electrolysis (-0.72V versus SCE) in an environment of argon, a combination of ketones (1.0-2.8 mmol), APG4 (20 mg), NAD+ (1.6 mmol), and methyl viologen (1.7 mmol) in phosphate buffer (pH 7.0, 0.1M) was reacted. In order to prevent the substrate and product from vaporising, argon gas was bubbled before to electrolysis and ceased bubbling throughout the process.The combination was extracted using ether that included naphthalene as a GC internal standard after one day, and the resulting ether solution was then exposed to GC. Trifluoroacetophenone, Chirasil-DEX CB; 25 m; He 2 mL/min; 120 oC; ketone: 2.2 min; (S)-alcohol were used in the GC-analysis utilising a Shimadzu GC-17A instrument with a FID detector. O-chloroacetophenone, CP cycloxextrin, 130 oC, ketone, 9.4 min, (R)-alcohol, and 10.0 min. Alcohol: 9.1 minutes; 9.7 minutes.

RESULTS AND DISCUSSION

According to a cyclic voltammogram, the electrochemically catalytic current was seen in the presence of APG4 as the biocatalyst (both diaphorase and dehydrogenase), MV2+ as the mediator, and NAD+ as the coenzyme. The cathodic peak potential of methyl viologen was found to be at -0.72 V versus SCE. The matching (S)-alcohol was produced when trifluoroacetophenone (TFA) was introduced to the system and the electrolysis was carried out for 24 hours as stated in TABLE 1. Dipaphorase (DP) and methyl viologen were often employed in electrolysis to reduce NAD+ to NADH. Two moles of the resulting reduced form of methyl viologen use a diaphorase to reduce one mole of NAD+ from the oxidised form of methyl viologen after receiving an electron from the cathode. Dehydrogenase, which catalyses the reduction of a substrate ketone to the product, and diaphorase are then needed for the reduction. The crude alcohol dehydrogenase, however, has considered diaphorase activities, therefore the current reduction mechanism does not need any diaphorase from outside.Without DP, the reduction might continue, and the product was produced in 34% yiled (entry 2). On the other hand, the yield was lowered when DP was added to the combination (entry 5: 22% yield with DP). As a result, too much DP may speed up NADH oxidation while preventing ketone reduction.Although glucose was added to the reaction mixture, it was not used to reduce NAD+ but rather to remove active oxygen [5], which was created when the reduced form of methyl viologen, MV+, reacted with oxygen (even though the reaction was carried out in an argon atmosphere, it is still challenging to

completely remove oxygen).The reduced form of the coenzyme may react with active oxygen. The reduction of the substrate must next proceed with the total elimination of oxygen.As shown in entry 1, the addition of glucose could not alter the decrease of ketone without electrolysis. However, the reduction that took place during electrolysis when glucose was present produced a good output of (S)-alcohol. Galactose and inositol are examples of other sugars that may be utilised as additions.In contrast to glucose, the (S)-alcohol had poor chemical yields (galactose = 44%, inositol = 23%), and when galactose was added as an additive, the enantioselectivity fell to 95% ee. Since the reaction with galactose and without electrolysis produced the (S)-alcohol of 48%ee with 7% chemical yield, the low ee seen in the reaction with galactose results from the presence of a small dehydrogenase that converts the ketone to the (R)-alcohol. Galactose may work as a reductive agent to turn the substrate into a low-ee (S)-alcohol. However, compared to other electrochemical processes, such as oxidations, the addition of glucose to electrochemical reduction is a handy and practical strategy since it does not need the nervous system's total elimination of oxygen.

TABLE1 :Enzymatic electrochemical reduction ofTFAa,b)

Entry	MV2+(mM)	NAD(mM)	Glucose(mM)	Yield(%)	ee.(%)	Config.
1	0	1.6	56	4		
2	1.6	1.6	0	34	>99	*S*
3	1.6	0	56	0		
4	1.6	1.6	56	72	>99	*S*
5[C)]	1.6	1.6	56	22	>99	*S*
6	1.6	1.6	56	4		

a)APG4 (20 mg) was used, b) The electrolysis was performed at -0.7V vs SCEfor 17 h and maximum 0.1 mAof electrolysiscurrent was observed during the electrolysis, c) Diaphorase (10 mg) was added to the reaction mixture.

Trifluoroacetophenone was subsequently reduced in 72% chemical yield with >99% efficiency. Calculations show that the reaction's current efficiency is 28.1%. The reduction of the second substrate, o-chloroacetophenone, using the same procedure yielded a 13% yield with 46% ee. This ketone was also electrochemically reduced, but the yield and enantioselectivity were poor. We cannot now explain the bad outcome since the interaction of o-chloroacetophenone with the fungus Geotricumcandidum IFO 4597 produced outstanding ee [4].It's conceivable that an additional enzyme, which ordinarily doesn't react with ketone, will contribute to the reduction. To adapt the current reduction technique to additional ketones, further research is required.

CONCLUSION

The bio-electro-chemical reduction of trifluoroacetophenone was good.The advantage of the current approach is the addition of a crude enzyme with diaphorase activities to the dehydrogenase that generates trifluoroacetophenone. Thus, the coenzyme recycling process for the reduction proceeded without the need for an extra enzyme or co-substrate.The second merit is the current system's activity. The ketone was reduced within a day, although the electrolytic reduction using methylviologen often needs extensive reaction periods, like a week [3g].

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