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Rhodium and Its Compounds in Amperometric Biosensors Based on Redox Enzymes

*Jan Soukup[1], Vojtěch Polan[1], Petr Kotzian[1] , Kurt Kalcher[2] and Karel Vytřas[1]**

[1] Department of Analytical Chemistry, University of Pardubice, Studentská 573, CZ-532 10 Pardubice, Czech Republic
[2] Institute of Chemistry – Analytical Chemistry, Karl-Franzens University, Universitaetsplatz 1, A-8010 Graz, Austria
*E-mail: karel.vytras@upce.cz

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Catalytic properties of rhodium and some of its compounds, namely, RhO_2, Rh_2O_3 and chlorobis(2-phenylpyridine)rhodium(III) dimer (CFPRD) were tested as mediators/catalysts of electron transfer in electrochemical biosensors for the determination of glucose. Measurements were realized using flow injection system and screen-printed carbon electrodes containing glucose oxidase as a model redox enzyme. From all of the above-mentioned matters, a sensor with RhO_2 was found the best exhibiting a linear increase of the amperometric signal glucose concentrations in the range of 5.5×10^{-5}-1.4×10^{-3} M with a detection limit of 2.2×10^{-5} M under optimized flow rate of 0.2 mL min^{-1} in 0.1 M phosphate buffer (pH 7.5) carrier. A metal rhodium-based sensor exhibited a linear increase in the range of 2.8×10^{-4}-1.4×10^{-3} M, a sensor modified with Rh_2O_3 showed similar linear dependence in the range of 1.4×10^{-3}- 5.5×10^{-3} M. Concerning sensors modified with CFPRD, a linear concentration dependence was observed in the range of 5.5×10^{-3}- 2.8×10^{-2} M.

Keywords: Amperometric biosensors, Screen-printed carbon electrodes, Rhodium and its compounds, Glucose, Glucose oxidase.

1. INTRODUCTION

Heterogeneous carbon materials have advantageously been used as supports for electrochemical biosensors because of their availability, low cost, low background currents, chemical inertness, ease of chemical derivatization and modification, as well as their suitability in diverse applications. Among the various carbon-based electrodes available for the construction of electrochemical sensors and biosensors with wide-spread popularity, carbon paste electrodes (CPEs)

and screen-printed carbon electrodes (SPCEs) captivate thanks of their ease of preparation and modification, ease of surface renewal and reproducibility in case of CPEs, and mass production of highly reproducible electrodes in case of SPCEs [1].

In construction of amperometric biosensors, the choice of mediators plays a very important role. Among the mediators that may lower the oxidation overpotential, numerous metal complexes, such as of iridium [2], rhodium [3], ruthenium [4] or osmium [5] and their oxides [6], ferrocene and its derivatives [7], Prussian blue and other metal hexacyanoferrates [8] but also some organic redox compounds, such as methylene blue and methyl viologen belong to the most often employed ones [9]. Among others, both rhodium [10] and rhodium dioxide [11] were tested recently and, as found, they showed an interesting electrocatalytical activity. Other similar rhodium-based sensors have not been already described. Therefore, other rhodium compounds were expected to show similar properties in amperometric measurements of hydrogen peroxide, which belongs – as well known – to products of many biocatalytic reactions when various oxidase enzymes are used. For that reason, both rhodium and some of its compounds were studied; the results are presented in this paper.

2. EXPERIMENTAL

2.1. Instrumentation

A modular electrochemical system AUTOLAB equipped with modules PGSTAT 30 and ECD (Ecochemie, Utrecht, Holand) was used in combination with a corresponding software (GPES, Ecochemie) under Windows$^{(P)}$. The flow injection system consisted of a peristaltic pump (Minipuls 3, Gilson SA., France), a sample injection valve (ECOM, Ventil C, Czech Republic), and a self-constructed thin layer electrochemical flow-through cell. The working electrode was fixed via rubber gaskets (thickness 0.6 mm) directly to the back plate of the thin layer cell with a Teflon support as a holder. The reference electrode was Ag/AgCl/3 M KCl (RE-6, BAS, USA), the stainless steel back plate represented the counter electrode of the cell.

Corresponding pH values were measured using a portable pH-meter (CPH 52, Elteca, Turnov, Czech Republic) equipped with a combined glass pH-sensor (OP-0808P, Radelkis, Budapest, Hungary). The measuring cell was calibrated with buffer solutions of the conventional activity scale [12].

2.2. Chemicals, Reagents and Solutions

Glucose oxidase (EC 1.1.3.4. from *Aspergillus niger*, specific activity 198 U mg^{-1}; GOx), Nafion (5% m/m solution in lower aliphatic alcohols), metallic rhodium and Rh(III) and Rh(IV) oxides, chlorobis(2-phenylpyridine)rhodium(III) dimer (CPPRD) were purchased from Aldrich. Hydrogen peroxide (30%) was obtained from Merck. Other chemicals used to prepare all buffer, stock and standard solutions were of analytical reagent grade and were purchased from Lachema (Brno, Czech Republic). Phosphate buffer was prepared by mixing aqueous solutions of sodium

dihydrogenphosphate and disodium hydrogenphosphate (both 0.100 M) to achieve solutions of the required pH values. The glucose stock solution (0.139 M) was prepared and diluted appropriately.

2.3. Electrode Preparation

The carbon ink (0.95 g, Gwent C50905D1, Pontypool, UK) and the corresponding catalyst (0.05 g) were thoroughly mixed manually for 5 min and subsequently sonicated for 5 min. The resulting mixture was immediately used for the fabrication of electrodes. The working electrodes were prepared by screen-printing of the modified ink onto an inert laser pre-etched ceramic support (113 × 166 × 0.635 mm, No. ADS96R, Coors Ceramics, Chattanooga, TN, USA). Thick layers of the modified carbon ink were formed by brushing the ink through an etched stencil (thickness 100 μm, electrode printing area 105 mm^2) with the aid of the squeegee of the screen-printing device (SP-200, MPM, Franklin, MA, USA and/or UL 1505 A, Tesla, CR) onto the ceramic substrates. The resulting plates were dried at 60 °C for 2 h.

2.3. Enzyme immobilization

In this paper, various immobilization methods of glucose oxidase were applied, comprising entrapment in Nafion, cross linking with glutaraldehyde, immobilization with cellulose acetate and electropolymerization using either pyrrol or *m*-phenylenediamine. Entrapment in Nafion was finally chosen as the best procedure. The enzyme (GOx, 1 mg) was dissolved in 20 μL of 0.1 M phosphate buffer (pH 7.50) and mixed with an equal amount of 0.05%, 0.5% or 5 % Nafion solution neutralized to pH ~ 7 with ammonia. The resulting mixture (5 μL) was applied directly onto the active area of the SPCE/RhO_2 surface and air-dried for 30 min.

2.4. Procedure

Measurements were performed by DC amperometry using both flow injection and batch mode arrangements. All operational variables were optimized, i. e., applied potential (from +0.6 to −0.3V *vs.* Ag/AgCl), pH of phosphate buffer (5 – 9) and flow rate (0.1 – 1.5 mL min^{-1}). Responses were evaluated using the peak heights (differences between background and response current of the analyte). Injections of analyte were repeated at least three times. The typical injection volume was 10 μL.

2.5. Sample Processing

A solid sample of instant tea (1.92 g, herbal tea for nursing mothers) was dissolved and diluted to 100 mL with 0.1 M pH 7.50 phosphate buffer. A volume of 500 μL of the prepared sample solution was mixed with 10 mL of the 0.1 M pH 7.50 phosphate buffer for analysis.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Response to Hydrogen Peroxide

All operational parameters (applied potential, pH of phosphate buffer and flow rate) influencing the H_2O_2 determination were optimized. Hydrogen peroxide is a frequent intermediate of many enzymatic reactions of oxidases. Therefore, the optimization of the conditions for H_2O_2 determination represented the first step in our investigations. The operating voltage, one of the most critical parameters of the amperometric response influencing the selectivity of the sensor, was studied in the potential window of -0.3 to +0.6 V *vs.* Ag/AgCl, where interferences from common redox species (ascorbic acid, uric acid, paracetamol) were almost negligible. Hydrodynamic voltammograms showing the dependence of the peak current on the potential applied in the range within -0.3 to +0.6 V *vs.* Ag/AgCl at 0.15 V intervals and recorded using SPEs modified with rhodium compounds are presented on Figure 1.

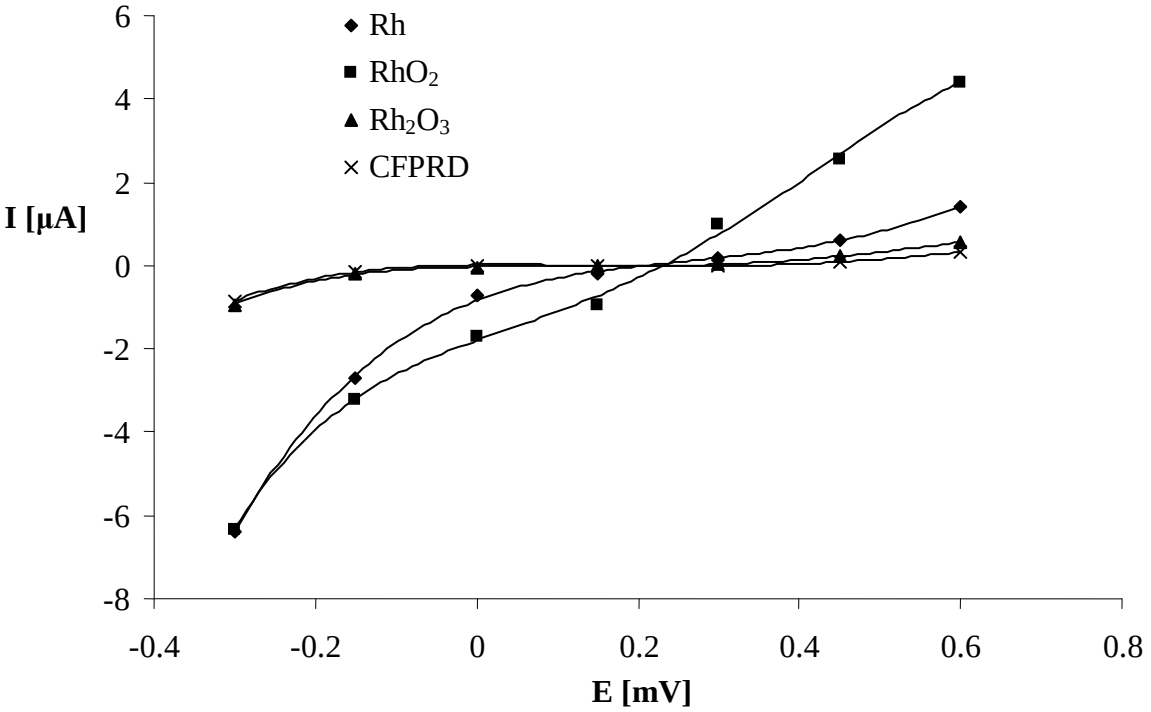

Figure 1. Optimization of the applied potential for determination of hydrogen peroxide using a flow injection analysis technique. Phosphate pH 7.50 carrying buffer; flow rate, 0.2 mL min^{-1}; potentials applied between -0.3 – 0.6 V *vs.* Ag/AgCl; H_2O_2 concentration, 2.9×10^{-3} M ; injected volume, 50 µL.

SPCE/RhO_2 sensors facilitated the detection of the H_2O_2 over the entire potential range examined, where its oxidation started at around +0.2 V and its reduction appeared at lower potentials. The SPCE/Rh sensor showed catalytic activity comparable with SPCE/RhO_2, particularly at more negative potentials. Although all the sensors exhibited enhanced activity when negative potentials were

applied, corresponding responses observed with both SPCE/Rh_2O_3 and SPCE/CPPRD was quite low. As evident from measurements, both the compounds containing trivalent rhodium did not exhibit sufficient catalytic activity. For further studies, a potential of -0.2 V was chosen taking into account measurable responses of all compounds as well as negligible effects of some interferants (ascorbic acid, etc.) at lower potentials.

The effect of pH was also investigated because many reactions, both electrochemical and enzymatic, are influenced by acidity of media. It was observed that the responses of both sensors, SPCE/RhO_2 and SPCE/Rh, increased with increasing pH values while the responses of SPCE/Rh_2O_3 and SPCE/CPPRD decreased (not shown). Considering practical applications and the reproducibility of the signals, a pH of 7.50, corresponding practically to a physiological buffer, was preferrable. The influence of the flow rate on the amperometric signal was also studied; a value of 0.2 mL min^{-1} was taken as optimum because it showed high response and time of analysis were not too long.

Under operational parameters described above (pH 7.50; potential, -0.2 V; flow rate, 0.2 mL min^{-1}, injection volume 50 µL), calibration plots for H_2O_2 were constructed; corresponding parameters are listed in Table 1. All electrodes retained constant responses after 100 injections and responses also did not change after one month; all of the electrodes used were stored in the fridge during experiments for long term stability.

Table 1. Parameters of calibration plots for hydrogen peroxide determination

Catalyst	**Linearity [M]**	**LOD [M]**	**Regression equation**	r_c
SCPE/RhO_2	2.9×10^{-5}-1.2×10^{-2}	0.95×10^{-5}	$y = 823.05\,x + 0.2354$	0.9953
SCPE/Rh	1.5×10^{-4}-7.4×10^{-3}	5.9×10^{-5}	$y = 774.27\,x - 0.1517$	0.9961
CPE/Rh_2O_3	2.9×10^{-3}- 1.5×10^{-2}	9.7×10^{-4}	$y = 32.35\,x + 0.1438$	0.9851
SCPE/CPPRD	1.5×10^{-3}- 2.9×10^{-2}	7.4×10^{-4}	$y = 49.01\,x + 0.1532$	0.9970

Conditions: potential aplied, –0.2V (vs. Ag/AgCl); carrier, phosphate pH 7.50 buffer (0.1 mol L^{-1}); flow rate, 0.2 mL min^{-1}; injection volume, 50 µL. LOD – limit of detection; r_c – correlation coefficient; x – concentration [mol L^{-1}]; y – current response [µA].

As seen from Table 1, linear segments of all calibration dependences start practically from zero amperometric responses.

3.2. Biosensors for Glucose Determination

In basic studies of biosensors based on oxidases, glucose oxidase is very often applied because of its relatively low price, stability (even when heated to 60 °C), and satisfactory production of H_2O_2 for electrochemical measurements. In Fig. 2, the dependence of the peak current of the glucose biosensor, containing the mediators under consideration, on the applied potential is shown. While at

positive potentials applied (higher than +0.1 V) the dependences of the peak current to glucose concentrations were analogous to those to hydrogen peroxide, an increase of positive anodic current was observed at negative potentials. Generally, catalysts showed better responses at potentials higher than +0.45 V or lower than -0.2 V.

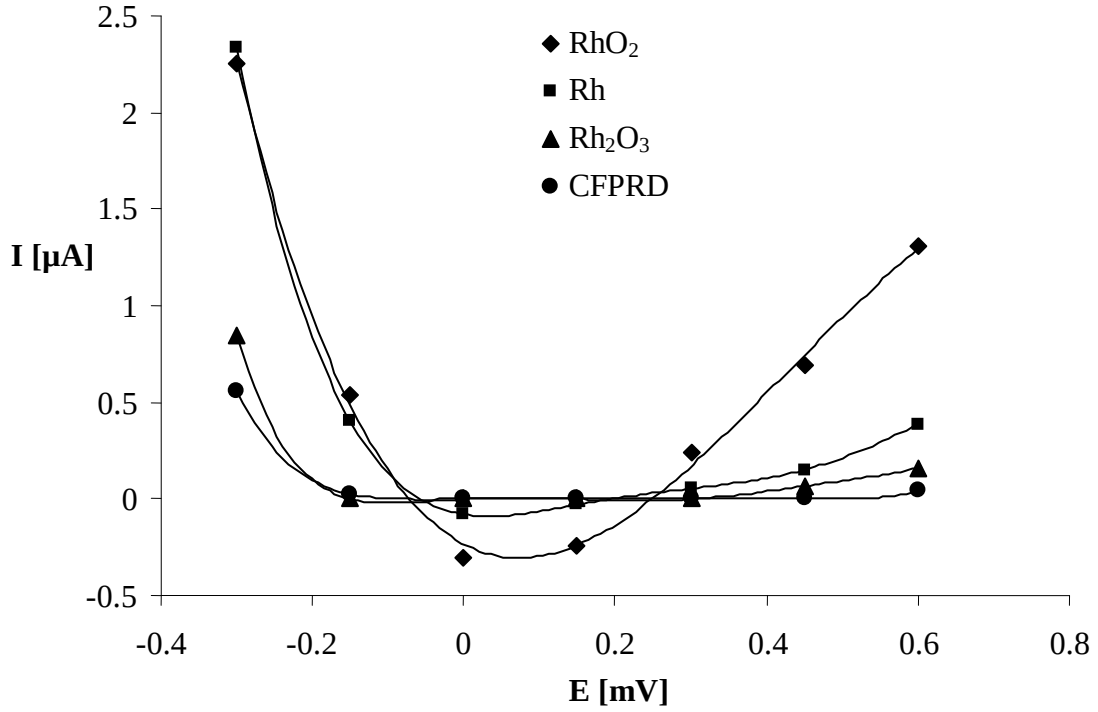

Figure 2. Amperometric response for glucose at different potentials applied. Phosphate pH 7.50 buffer; flow rate, 0.2 mL min^{-1}; potentials applied between -0.3 – 0.6 V *vs.* Ag/AgCl; glucose concentration, 1.4×10^{-3} M (SPCE/Rh/GOx), 2.8×10^{-4} M (SPCE/RhO_2/GOx), 5.5×10^{-3} M (SPCE/Rh_2O_3/GOx and SPCE/CPPRD/GOx); injection volume, 50 µL, FIA mode.

The progress of biocatalytic reactions of glucose oxidase as well as of other enzymes depends strongly on the pH value of particular media. As regards corresponding pH-dependences of amperometric signals, SPCE/RhO_2/GOx and SPCE/Rh_2O_3/GOx sensors showed a maximum around pH 6 which is close to the pH for maximum activity of GOx. Similar results were achieved for SPCE/Rh/GOx and SPCE/CPPRD/GOx sensors at pH 7. For further studies, a pH of 7.5 (a physiological value) was selected due to easy applicability for various biological as well as food samples.

Concerning the flow rate, all catalysts showed a decrease by 50% of the response towards glucose when increasing from 0.1 to 1.0 mL min^{-1}. As a compromise between time of analysis and response sensitivity, a flow rate of 0.2 mL min^{-1} was selected. Calibration plots for glucose were registered under the selected operational parameters (potential, -0.2 V; flow rate, 0.2 mL min^{-1}; pH 7.50) (Figure 3.). Proposed biosensors retained their activities after more than 40 injections or 24 h and they showed 0.67 % of begginig responses after one month. It should be mentioned that because the SPCEs are designed as one-shot sensors, their behavior was not investigated in longer periods. Linear

relationships between peak heights and glucose concentrations are listed in Table 2. As can be seen in the table, the SPCE/RhO_2/GOx sensors showed the highest signals with the widest dynamic range and therefore, they were used the determination of glucose in tea samples.

In comparison with characteristics of other mediators used in glucose sensors [13, 14], rhodium-based sensors (especially that with rhodium dioxide) disposed of sufficient long term stability and wide linear range of the calibration curve.

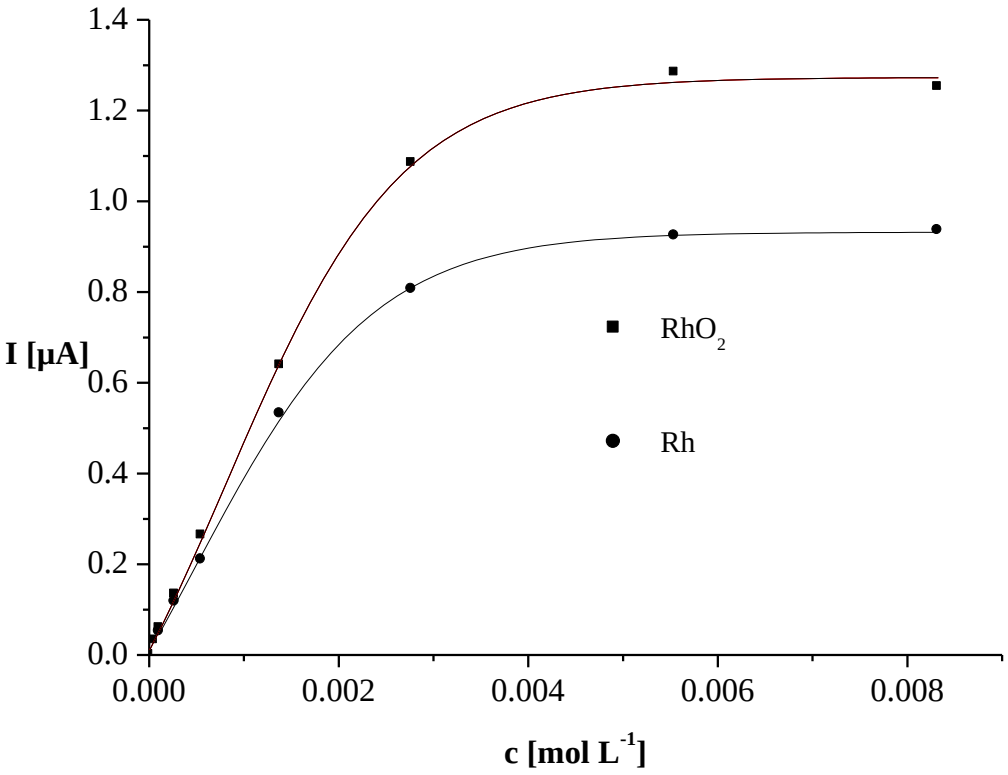

Figure 3 Calibration curves for determination of glucose. Phosphate pH 7.50 carrying buffer; potential applied, -0.2V *vs.* Ag/AgCl; flow rate, 0.2 mL min^{-1}; injection volume, 50 µL.

Table 2. Parameters of calibration plots in glucose determination

Catalyst	**Linearity [M]**	**LOD [M]**	**Regression equation**	r_c
SCPE/RhO_2/GOx	5.5×10^{-5}-1.4×10^{-3}	2.2×10^{-5}	$y = 454.97\,x + 0.01$	0.9999
SCPE/Rh/GOx	2.8×10^{-4}-1.4×10^{-3}	8.3×10^{-5}	$y = 375.27\,x + 0.01$	0.9994
SCPE/Rh_2O_3/GOx	1.4×10^{-3}-5.5×10^{-3}	3.9×10^{-4}	$y = 14.60\,x + 0.012$	0.9999
SCPE/CFPRD/GOx	5.5×10^{-3}-2.8×10^{-2}	1.7×10^{-3}	$y = 5.40\,x + 0.0065$	0.9994

Conditions and symbols as given under Table1.

3.3. Interferences

There are many easily oxidizable species in real symplex (e.g., blood, foodstuff), the main important of which are both ascorbic and uric acids. It was observed that while both of them were electroactive when potential of +0.5 V was applied, negligible or very low responses only were indicated at -0.2 V. All of the sensors tested rhodium embodied similar behavior. Thus, the potential of -0.2 V vs. Ag/AgCl was applied for consequent experiments [11].

3.4. Analytical Applications

The glucose biosensor with RhO_2 as a mediator was applied to determine the glucose content in a sample of instant tea for nursing mothers. Applied conditions were as described above, samples were injected in 50 µL portions. Producer declared that product contained 52.08 % of glucose, the found content of glucose by measurement with a SPCE/RhO_2/Gox sensor was determined to be 52.16 %. In that way, the suitability of the sensor use for glucose determination in similar types of samples was verified.

5. CONCLUSIONS

Novel biosensors for determination of glucose with rhodium-based mediators were investigated in flow injection mode and compared. Results confirmed that RhO_2 is the best mediator of electron transfer of all rhodium compounds tested but satisfying results can also be achieved using electrodes modified with metallic rhodium. Electrodes modified with either Rh or RhO_2 yield significantly higher response toward H_2O_2 than Rh_2O_3 and CPPRD.

The highest response of the amperometric signal to glucose occured in +0.6 V (it would probably be even higher at more positive potential, but not investigated), but the choice of this potential for determination of glucose in real samples cannot be recommended because of possible interferences (ascorbic acid, uric acid, etc.). For that reason, potential of -0.2 V was applied. Finally, it should be pointed out that although glucose oxidase was used as a representative enzyme in all studies, SCPEs based on rhodium compounds could also be modified with other oxidases to allow determinations of various types of substances undergoing biocatalytic reactions.

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