Isothermal Calorimetry to Monitor Enzymatic Oxidation

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Abstract

When tyrosine is exposed to the enzyme tyrosinase in the presence of oxygen, the phenolic moiety is oxidized to an ortho-quinone. This oxidation can be monitored in-situ by using isothermal calorimetry. A TAM Air isothermal calorimeter with motorized admix accessory was used to monitor this reaction at 25°C. Heat flow from the reaction was dependent on the oxygen content inside the ampoule. There was a larger total heat detected for the reaction when ambient air filled the ampoule head space as compared to that when the sample ampoule was purged with dry nitrogen. There was still some oxidation observed from the nitrogen purged sample due to dissolved oxygen in the aqueous solution. Isothermal calorimetry is an excellent technique to monitor enzymatic reactions.

Introduction

Tyrosinase is a copper-containing enzyme that catalyzes the production of melanin and other pigments which result from the oxidation of the tyrosine derivatives in plant and animal tissues.[1-2] Tyrosine (also known as hydroxyl phenylalanine) has a molecular weight of 181.19 g/mol and is most commonly found in nature as the para- or L- isomer. When L-tyrosine is exposed to tyrosinase in the presence of oxygen the benzene ring is oxidized at the hydroxyl group and is converted to an ortho-quinone molecule (Figure 1). An example of this oxidation reaction is most commonly observed as the blackening of peeled potatoes. By using isothermal calorimetry and the appropriate sample accessories, the oxidation reaction can be monitored in real time.

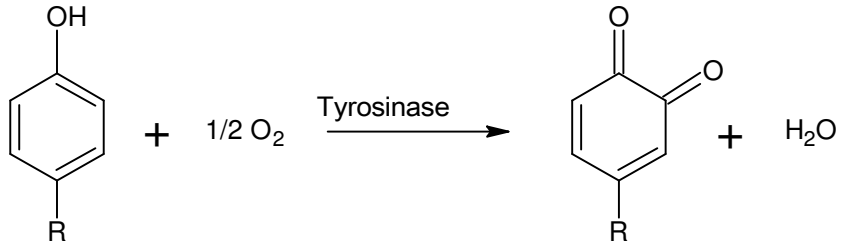

Figure 1: Reaction equation for the enzymatic oxidation of tyrosine

Experimental

Lyophilized tyrosinase from mushroom and reagent grade L-Tyrosine (≥98%) powders were purchased from Sigma-Aldrich (St. Louis, MO). Solutions of approximately 60 units/mL and 1 mM were prepared, respectively, with reagent grade deionized water (Ricca Chemical Co., Arlington, TX). The TAM Air thermostat was set to 25 °C. A disposable glass ampoule was filled with 10 mL of L-tyrosine solution and 1.0 mL of the tyrosinase solution was filled into the syringe of the admix accessory. Further instructions on the preparation of the admix accessory can be found in Experimental and Technical Note EN 302.[3] The entire admix accessory with loaded solutions was then equilibrated in the calorimeter for ~2 hrs. A generic experimental wizard in TAM Air Assistant was initiated and data was collected for a few minutes to establish a heat flow baseline and time zero of each experiment was considered when the tyrosinase solution was injected into the tyrosine. Samples were stirred continuously at a rate of 30-60 RPM.

Results and Discussion

The oxidation reaction of tyrosine can be detected by the TAM Air calorimeter. As shown in Figure 2, the oxidation reaction is exothermic and when more oxygen was present there was a larger heat flow signal detected. The solid maroon curve in Figure 2 represents the sample where the head space above the tyrosine solution was ambient air (approx. 21% oxygen) and the blue dashed curve represents a sample that was purged or bubbled with dry nitrogen. A blank experiment of tyrosinase injected into deionized water with ambient air in the head space is shown by the green dash-dot curve in Figure 2. As expected, the amount of oxygen present dictates the magnitude of the reaction and thus the heat flow measured. Slight exothermic drift noticed in the baseline may be attributed to heat of friction of constant stirring. Additional kinetic information may also be deduced from the heat flow signal by modeling the curve or performing the experiment at multiple temperatures (not shown). The picture in Figure 3 compares the solution color change after the reaction with respect to oxygen content. On the left is the clear blank sample, in the middle is the nitrogen purged sample, and on the right is the ambient air sample, which is the darkest in color.

Conclusions

Isothermal calorimetry is a universal technique used to monitor heat flow. The TAM Air calorimeter was used here to monitor the enzymatic oxidation of tyrosine by tyrosinase. With the appropriate sample preparation and experimental considerations, both enthalpic and kinetic information can be determined from the heat flow results. Increased sensitivity of the TAM III could provide the ability to detect the same reaction at lower solution and oxygen concentrations.

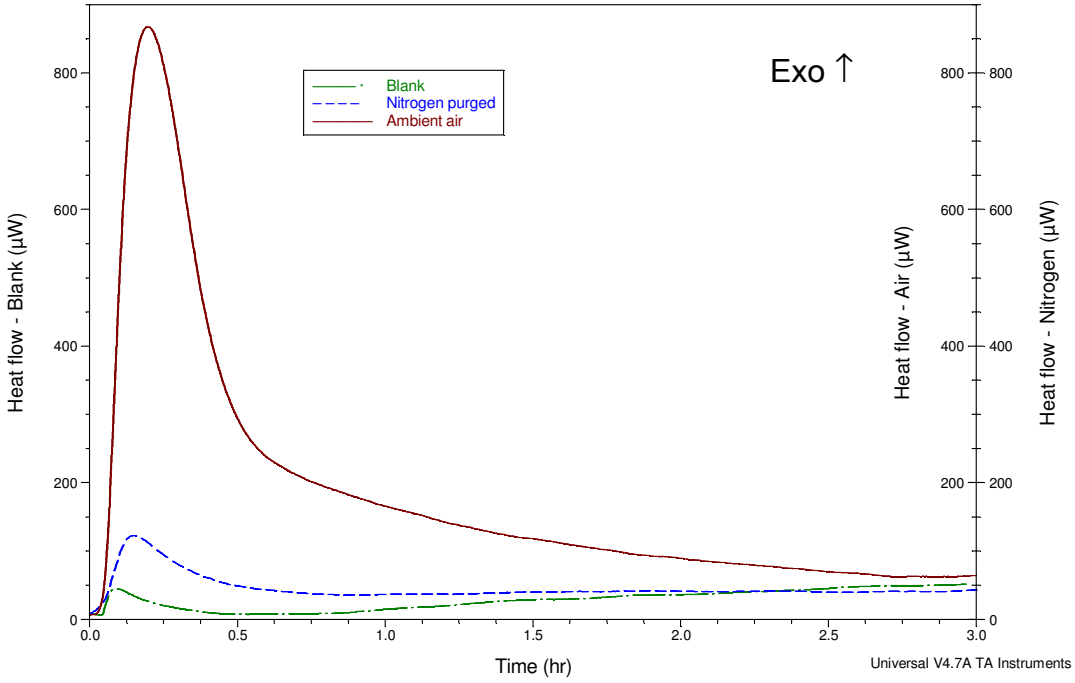

Figure 2: Heat flow results at 25℃. Solid – ambient air, Dashed – nitrogen purged, Dash-dot – blank experiment with ambient air

Figure 3: Left - blank experiment with ambient air, Middle - nitrogen purged, Right - ambient air

References

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