RESEARCH ARTICLE
Eurasian J Bio Chem Sci, 6(2):77-80, 2023
https://doi.org/10.46239/ejbcs.1215182

EJBCS

Eurasian Journal of Biological and Chemical Sciences

Journal homepage: www.dergipark.org.tr/ejbcs

Peroxidase mimicking activity of macroporous carbon

Bekir Çakıroğlu*

**Sakarya University, Biomedical, Magnetic and Semiconductor Materials Research Center (BIMAS-RC), Sakarya, Turkey.*

*Corresponding author : bekircakiroglu@sakarya.edu.tr
Orcid No: https://orcid.org/0000-0001-5989-4545

Received : 06/12/2022
Accepted : 31/08/2023

Abstract: This study looked into the peroxidase-like activity of macroporous carbon produced using a silica template. Macroporous carbon's nanozyme activity was compared to that of commercial graphene oxide. The macroporous microstructure of carbon was visible in the field emission scanning electron microscopy image. By catalytically oxidizing the chromogenic substrate 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in the presence of hydrogen peroxide, the nanozyme activity was investigated. The oxidized form of ABTS, which has a green color, can be seen by the eye. The synthetic macroporous carbon developed a green hue without functionalization or enzyme use, indicating peroxidase activity. This is likely because of the enormous surface area and numerous active sites that are present on the surface. As active sites, the oxygen-containing functional groups created during carbonization can be crucial to the peroxidase-mimicking action.

Keywords: Nanozyme, Carbonaceous material, ABTS, Peroxidase-like activity, Sustainability

Makro gözenekli karbonun peroksidaz mimik aktivitesi

Özet: Bu çalışmada, silika şablonu kullanılarak üretilen makro gözenekli karbonun peroksidaz mimik aktivitesi araştırılmıştır. Makro gözenekli karbonun nanozim aktivitesi, ticari grafen oksit ile karşılaştırıldı. Karbonun alan emisyon taramalı elektron mikroskobu görüntüsü, makro gözenekli morfolojiyi ortaya çıkardı. Nanozim aktivitesi, kromojenik substrat 2,2'-azino-bis(3-etilbenzotiazolin-6-sülfonik asidin (ABTS) hidrojen peroksit varlığında katalitik oksidasyonu yoluyla incelenmiştir ve yeşil renkli ABTS'nin oksitlenmiş formu gözle görülebilecek şekilde oluştu. İşlevselleştirme ve enzim kullanımı olmadan, elde edilen makro gözenekli karbon yeşil renk gelişimi gösterdi, bu da muhtemelen geniş yüzey alanı ve dolayısıyla yüzeyde bulunan bol miktarda aktif bölge nedeniyle peroksidaz aktivitesini gösteriyor. Karbonizasyon sırasında oluşan oksijen içeren fonksiyonel gruplar, aktif bölgeler olarak davranabilir ve peroksidazı taklit eden aktivitede çok önemli bir rol oynayabilir.

Anahtar Kelimeler: Nanozim, karbonlu malzeme, ABTS, peroksidaz mimik aktivite, sürdürülebilirlik

1. Introduction

Enzymes have been extensively utilized owing to their efficient and specific catalytic activity on substrates under mild conditions (Hanefeld et al. 2013). However, some inherent problems with enzymes exist, such as expensive purification and poor working and storage stability (Q. Wang et al. 2021). They are also sensitive to pH, temperature, ionic strength, surfactants, and organic solvents, and their broad use is hampered by proteases' digestion (Wu et al. 2020). Since the exciting discovery of Fe_3O_4 MNPs with peroxidase-like activity in 2007(Gao et al. 2007), researchers have paid much attention to researching effective artificial enzymes "nanozymes" with intrinsic enzyme-like activities, to solve these problems (Q. Wang et al. 2018). Due to their impressive advantages, nanozymes have been at the forefront as a potential substitute to help with analyte detection (Ren et al. 2022). These include satisfying catalytic activity, high medium resistance, easy surface modification, cheap cost, and simple manufacturing (Liang and Yan 2019). However, unlike enzymes, nanozymes could not catalyze one substrate with high selectivity(Robert and Meunier, 2022). One possible challenging will be increasing the asymmetric selectivity of nanozymes (Yang et al. 2021). To date, morphology, heterogeneous atomic doping, particle size adjustment, and surface modification have all been used to modify the catalytic activity and selectivity of nanozymes (Jiang et al. 2019). The increased activity is a result of the bigger surface area exposing more active sites and the preferential exposure of catalytically active atoms. Surface defects including ledges, adatoms, vacancies, and kinks strongly attract substrates because they are coordinatively unsaturated reactive sites (Wang et al. 2016).

2. Materials and Method

Graphene oxide (GO), sucrose, tetraethyl orthosilicate (TEOS), 25% ammonia solution, hydrofluoric acid, 98% sulfuric acid, and absolute ethanol were purchased from Sigma-Aldrich. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was purchased from Roche Diagnostics (Germany). Hydrogen peroxide solution (30%) was purchased from Merck. Acetate buffer solution (ABS) was prepared using glacial acetic acid (Merck) and sodium acetate (Sigma-Aldrich).

2.1. Preparation of SiO_2 Templates

SiO_2 (silica) NPs were synthesized via the classical Stöber Method (Han et al. 2017). Briefly, 1 mL of TEOS, 50 mL of ethanol, 1 mL of DW, and 5 mL of ammonia solution were magnetically stirred in a flask at 250 rpm and 25 °C in a water bath. After 6 h, SiO_2 NPs were purified by twice centrifugation at 10000 rpm for 20 min. The sedimented SiO_2 NPs were resuspended in ethanol (Figure 1), and the suspension was evaporated in an oven at 70 °C to obtain templates (Figure 2).

2.2. Preparation of Macroporous Carbon

The precursor of carbonaceous material contains 1.4 g of sucrose, 3.2 mL of deionized water, 14 mL of ethanol, and 40 µL of 98% sulfuric acid. The infiltration was carried under the vacuum by dropping carbon precursor solution onto the silica template at room temperature. The infiltration was repeated several times to fill the voids of the silica template. The sucrose-soaked templates were heated at 160 °C for 5 h in the air for the carbonization of sucrose catalyzed by sulfuric acid and annealed. Macroporous carbon was left behind by etching away silica templates with 4% HF solution overnight.

2.3. Peroxidase-like Activity Measurements

2.5 mL of 0.4 mM ABTS solutions were prepared in 100 mM ABS pH 3.8 with 4 mg of macroporous carbon. 30 µL of hydrogen peroxide (30%) was added to the above mixture and incubated for 10 min at room temperature. Then, macroporous carbon was removed from the reaction medium, and the absorbance spectra were recorded.

2.4. Characterization

The morphological features of nanozyme components were characterized by field emission scanning electron microscopy (FESEM) recorded on an FEI Quanta FEG 450. UV visible (UV-Vis) absorbance spectra were recorded using a Shimadzu UV-2600 spectrophotometer at 200-800 nm. The particle size distribution of silica NPs was obtained on a Nano Plus (Micromeritics). The functional group investigation of the nanocomposites was carried out by Perkin Elmer Fourier transform infrared (FTIR) Spectrometer (Spectrum Two).

3. Results

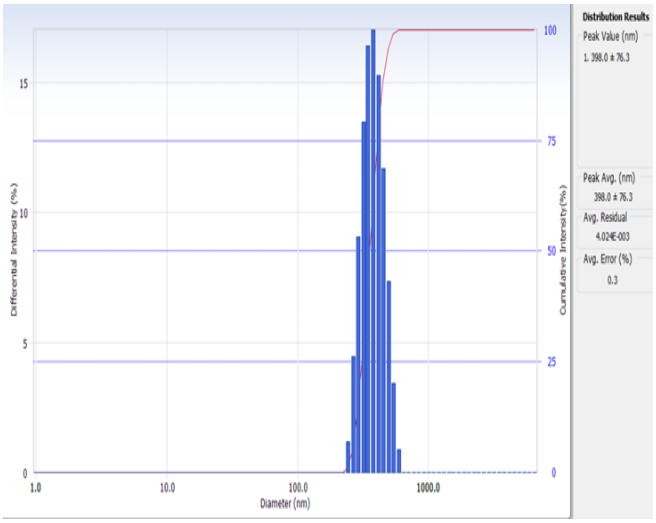

Fig. 1 Particle Size Dispersion of Silica NPs

Fig. 2 Silica Template for Macroporous Carbon Fabrication

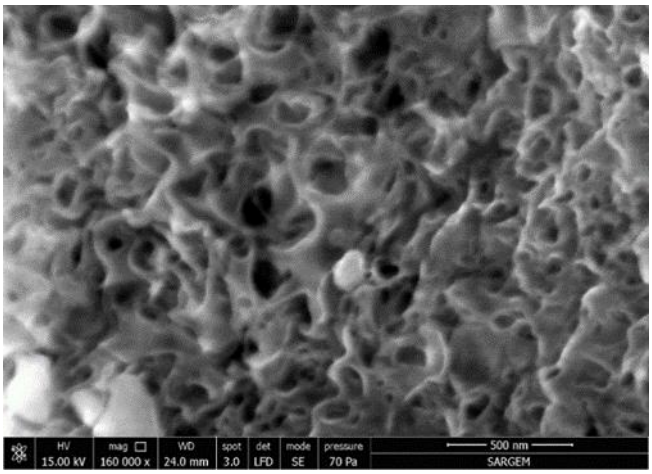

Fig. 3 FESEM Image of Macroporous Carbon

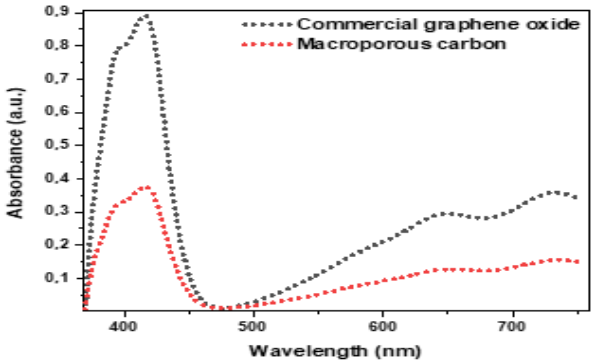

Fig. 4 Absorbance Spectrum of Oxidized ABTS Generated by Commercial Graphene Oxide and Macroporous Carbon

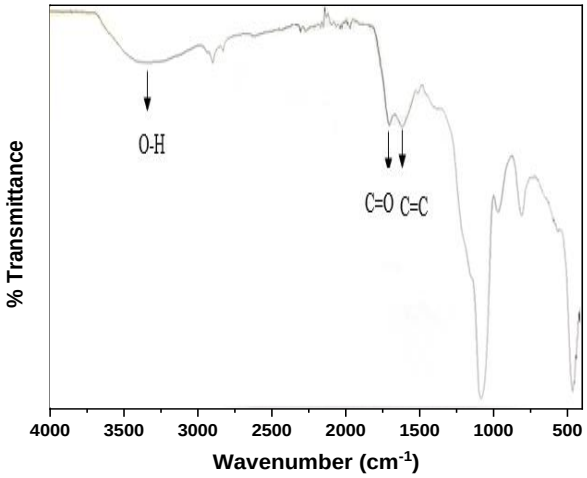

Fig. 5 FTIR Spectrum of Macroporous Carbon

4. Discussion

Carbon-based materials with enzyme-like activity have garnered great attention by researchers. Hitherto, numerous carbon-based nanozymes, including graphene oxide (Wang et al. 2020), carbon nanotubes (H. Wang et al. 2018), fullerenes (Hong et al. 2022), graphene quantum dots(Devi et al. 2021), and carbonaceous metal organic frameworks(Zhao et al. 2022), have been manufactured to investigate enzyme-mimicking activities. Carbon-based nanozymes, in contrast to natural enzymes, exhibit considerable potential in biomedical applications due to their plentiful active sites, outstanding stability, and good biological safety (X. Wang et al. 2021). In this context, low-cost carbonaceous nanozyme manufacturing is of great significance for the catalytic applications. This study reported the fabrication of an efficient carbon-based nanozyme as a peroxidase substitute.

Particle size distribution of silica NPs was exhibited in Figure 1. The average diameter of silica NPs was found to be 398 nm with a narrow particle distribution value. Figure 2 shows the opalescence of silica NPs deposition owing to its opal photonic crystal behavior.

The FESEM image of carbon material revealed a porous morphology, which implies abundant active sites (Chen et al. 2020) (Figure 3). Up to now, carbonaceous materials have mirrored peroxidase, oxidase, superoxide dismutase and catalase-like activities (Sun et al. 2018). Depending on their morphology and active groups, the enzyme mimicking activities have been found to change. Fullerenes with ball-shape morphology display SOD-like activity, while graphene oxide with leaf-like morphology mostly displayed substantial peroxidase mimicking activity.

Peroxidase mimicking activities were investigated using chromogenic substrate ABTS in the presence of hydrogen peroxide (Figure 4). Graphene oxide is a well-known peroxidase-like nanozyme in the literature owing to its carboxyl groups, which play a crucial role in the catalytic oxidation of chromogenic substrates in hydrogen peroxide containing solution (Song et al. 2010). Commercial graphene oxide revealed peroxidase-like activity that can oxidase ABTS in the presence of H_2O_2 by producing a green-colored product. On the other hand, the macroporous carbon has also demonstrated peroxidase-mimicking activity, although it led to less absorbance intensity than graphene oxide. At the absorbance wavelength of 646 nm, the fabricated carbon showed half the absorbance intensity generated by graphene oxide (Figure 4). FTIR analysis revealed the existence of O-H (3395 cm^{-1}) and C=O (1720 cm^{-1}) functional groups (Çakıroğlu et al. 2018), implying that carboxyl groups are introduced on the microporous carbon during carbonization (Figure 5). The peroxidase-like catalytic activity of carbon can be attributed to the carboxyl groups (-COOH) on the surface (Wang et al. 2020).

According to the literature, the reaction mechanism of peroxidase mimicking activity was realized by reduction of hydrogen peroxide on carboxylic acid moiety with the concomitant oxidation of ABTS, as can be seen in the equations below (Lin et al. 2019).

$$\mathbf{H_2O_2 + ABTS \rightarrow 2\,H_2O + oxABTS}$$

Herein, radicals are probably formed during nanozyme catalysis, and these radicals can facilitate the oxidation of ABTS on the active sites of carbonaceous material (X. Wang et al. 2021).

Thus, it is confirmed in this study that oxygen containing functional groups on carbonaceous material are responsible for the chromogenic substrate oxidation in the presence of hydrogen peroxide.

5. Conclusion

In this study, a macroporous carbon was reported for peroxidase-mimicking activity. Its peroxidase-like activity was examined in the presence of a chromogenic substrate ABTS. Carbon material was synthesized with favorable morphology for reactants and products. The rational design of material grants access to abundant catalytically active sites and enhances the catalytic activity. This study may find its unique niche in the sensor area as a biomimic peroxidase for hydrogen peroxide monitoring.

Acknowledgements

The author would like to thank Prof. Dr. Ayşe Ogan and Assoc. Prof. Dr. Emrah Bulut for their valuable contributions.

References

Çakıroğlu B, Çiğil-Beyler A, Ogan A, Kahraman MV, Demir S. 2018. Covalent immobilization of acetylcholinesterase on a novel polyacrylic acid-based nanofiber membrane. Eng Life Sci. 18:254-262

Chen Y, Jiao L, Yan H, Xu W, Wu Y, Wang H, Gu W, Zhu C. 2020. Hierarchically Porous S/N Codoped Carbon Nanozymes with Enhanced Peroxidase-like Activity for Total Antioxidant Capacity Biosensing. Anal Chem. 92:13518–13524

Devi M, Das P, Boruah PK, Deka MJ, Duarah R, Gogoi A, Neog D, Dutta HS, Das MR. 2021. Fluorescent graphitic carbon nitride and graphene oxide quantum dots as efficient nanozymes: Colorimetric detection of fluoride ion in water by graphitic carbon nitride quantum dots. J Environ Chem Eng. 9:104803

Gao L, Zhuang J, Nie L, Zhang J, Zhang Y, Gu N, Wang T, Feng J, Yang D, Perrett S, Yan X. 2007. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. Nat Nanotechnol. 2:577–583

Hanefeld U, Cao L, Magner E. 2013. Enzyme immobilisation: fundamentals and application. Chem Soc Rev. 42:6211–6212

Hong SJ, Chun H, Hong M, Han B. 2022. N- and B-doped fullerene as peroxidase- and catalase-like metal-free nanozymes with pH-switchable catalytic activity: A first-principles approach. Appl Surf Sci. 598:153715

Jiang D, Ni D, Rosenkrans ZT, Huang P, Yan X, Cai W. 2019. Nanozyme: new horizons for responsive biomedical applications. Chem Soc Rev. 48:3683–3704

Liang M, Yan X. 2019. Nanozymes: From New Concepts, Mechanisms, and Standards to Applications. Acc Chem Res. 52:2190–2200

Lin S, Zhang Y, Cao W, Wang X, Qin L, Zhou M, Wei H. 2019. Nucleobase-mediated synthesis of nitrogen-doped carbon nanozymes as efficient peroxidase mimics. Dalt Trans. 48: 1993–1999

Ren X, Chen D, Wang Y, Li H, Zhang Y, Chen H, Li X, Huo M. 2022. Nanozymes-recent development and biomedical applications. J Nanobiotechnology. 20:92

Robert A, Meunier B. 2022. How to Define a Nanozyme. ACS Nano. 16:6956–6959

Song Y, Qu K, Zhao C, Ren J, Qu X. 2010. Graphene Oxide: Intrinsic Peroxidase Catalytic Activity and Its Application to Glucose Detection. Adv Mater. 22:2206–2210

Sun H, Zhou Y, Ren J, Qu X. 2018. Carbon Nanozymes: Enzymatic Properties, Catalytic Mechanism, and Applications. Angew Chemie Int Ed. 57:9224–9237

Wang D, Song X, Li P, Gao XJ, Gao X. 2020. Origins of the peroxidase mimicking activities of graphene oxide from first principles. J Mater Chem B. 8:9028–9034

Wang H, Li P, Yu D, Zhang Y, Wang Z, Liu C, Qiu H, Liu Z, Ren J, Qu X. 2018. Unraveling the Enzymatic Activity of Oxygenated Carbon Nanotubes and Their Application in the Treatment of Bacterial Infections. Nano Lett. 18:3344–3351

Wang Q, Liu S, Tang Z. 2021. Recent progress in the design of analytical methods based on nanozymes. J Mater Chem B. 9:8174–8184

Wang Q, Wei H, Zhang Z, Wang E, Dong S. 2018. Nanozyme: An emerging alternative to natural enzyme for biosensing and immunoassay. TrAC Trends Anal Chem. 105:218–224

Wang X, Hu Y, Wei H. 2016. Nanozymes in bionanotechnology: from sensing to therapeutics and beyond. Inorg Chem Front. 3:41–60

Wang X, Wang H, Zhou S. 2021. Progress and Perspective on Carbon-Based Nanozymes for Peroxidase-like Applications. J Phys Chem Lett. 12:11751–11760

Wu W, Huang L, Wang E, Dong S. 2020. Atomic engineering of single-atom nanozymes for enzyme-like catalysis. Chem Sci. 11:9741–9756

Yang W, Yang X, Zhu L, Chu H, Li X, Xu W. 2021. Nanozymes: Activity origin, catalytic mechanism, and biological application. Coord Chem Rev. 448:214170

Zhao J, Gong J, Wei J, Yang Q, Li G, Tong Y, He W. 2022. Metal organic framework loaded fluorescent nitrogen-doped carbon nanozyme with light regulating redox ability for detection of ferric ion and glutathione. J Colloid Interface Sci. 618:11–21