ELSEVIER

Journal of Molecular Catalysis A: Chemical 110 (1996) 141–151

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

Surface modification of carbon black by oxidation and its influence on the activity of immobilized catalase and iron–phthalocyanines

Rosario C. Sosa [b], Rudy F. Parton [a], Patricia E. Neys [a,*], O. Lardinois [b], Peter A. Jacobs [a], Paul G. Rouxhet [b]

[a] *Centrum voor Oppervlaktechemie en Katalyse, Department of Interface Chemistry, K.U. Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium*

[b] *Unité de Chimie des Interfaces, Université Catholique de Louvain, Place Croix du Sud 2 / 18, B-1348 Louvain-la-Neuve, Belgium*

Received 19 January 1996; accepted 15 May 1996

Abstract

A non-porous carbon black is oxidized using different reagents (H_2O_2 3 M, HNO_3 0.3 M at 95°C, O_2 at 400 and 600°C) and used as support for the enzyme catalase as well as for iron–phthalocyanines, enzyme mimics. Characterization of the oxidized carbons is done by X-ray photoelectron spectroscopy, gas-adsorption and electrophoretic mobility measurements. The treatments do not change the texture of the carbon black. Hydrogen peroxide and nitric acid increase the surface oxygen concentration, and make the charge of the carbon surface more negative, whereas oxidation with molecular oxygen has the opposite effect. The surfaces with a higher oxygen content and negative charge preserve a higher activity of adsorbed catalase, presumably because less deformation of the enzyme occurs as a result of higher repulsive electrostatic interactions and lower hydrophobic interactions. The same order is found for the dismutase-like activity of the iron–phthalocyanines supported on the carbon; the selective oxidation of hydrocarbons, i.e. the oxygenase activity follows the reverse order. Thus, sorption effects determine the selectivity of iron–phthalocyanine. Zeolite Y, which has a higher electrical charge and surface oxygen concentration compared to the oxidized carbons, is responsible for a higher ratio of dismutase-like activity with respect to oxygenase-like activity.

Keywords: Catalase; Cytochrome *P*-450; Carbon black; Iron–phthalocyanine; Zeolite Y; Oxidation; Dismutation; Hydrophilicity; Cyclohexane

1. Introduction

The manufacturing process is not always adequate to obtain the desirable surface properties of carbon black for certain applications. Post-treatments are often used to widen the applicability of these materials [1]. Oxidation is frequently used to enhance the hydrophilicity of the surface [2–4], however, a recent report shows that a decrease of hydrophilicity is also possible with oxidation [5]. Carbon surfaces can be characterized using classical methods as X-ray photoelectron spectroscopy (XPS), gas adsorption and electrophoretic mobility. Selective analyses have revealed the presence of different

* Corresponding author. Tel.: +32-16-321645; fax: +32-16-321998; e-mail: rudi.parton@agr.kuleuven.ac.be.

PII S1381-1169(96)00197-5

functional groups at the surface of carbon black, such as carboxyl, phenol, quinone, lactone and carbonyl groups [6]. The nature and the amount of these oxygen-bearing functions depend upon the nature of the carbon black and the method of oxidation [6]. Carbon blacks can be used as support to heterogenize homogeneous catalysts [7–9] and enzymes [5], but the immobilized systems can also be used to characterize the carbon surface [5].

Protein adsorption on solid surfaces is determined by electrostatic interactions, hydrophobic interactions, ion bridges and conformational stability of the protein. Adsorption of rigid proteins on hydrophilic surfaces is governed by electrostatic interactions, e.g. the adsorption of lysozyme or ribonuclease on α-Fe_2O_3 or apatite [10,11], while on hydrophobic surfaces, as polystyrene latex, apolar interactions are the driving force. The adsorption of large deformable proteins, such as human serum albumin, takes place over the whole pH range regardless the hydrophobicity of the adsorbent. The main driving force of the adsorption process seems to be a structural rearrangement in the protein molecule, although maximum adsorption is reached at the isoelectric point of the protein-covered surface [12]. When the protein is an enzyme, the deformations may be reflected directly in the catalytic activity, which is consequently informative about the adsorption process [13].

One aim of this paper is to show the relation between the characteristics of carbon black surface and the enzymatic activity of adsorbed catalase. Catalase (hydrogen peroxide oxidoreductase EC 1.11.1.6) catalyzes the decomposition of hydrogen peroxide into water and oxygen. *Aspergillus niger* catalase is a glycoprotein containing four subunits, each of which possesses a ferric protoporphyrin IX prosthetic group [14,15].

As a second goal, evidence is provided that a hydrophobic carbon as carrier of iron–phthalocyanines enhances their oxygenation activity. Iron–phthalocyanines (FePc) encapsulated in zeolites, mostly zeolite Y, are used as mimics of cytochrome *P*-450 [16–21]. The latter is an oxygenation enzyme which has also a ferric protoporphyrin IX as prosthetic group [22]. The iron–phthalocyanine imitates the prosthetic group, whereas the protein is modelled by the zeolite [20,21]. These models show a much higher stability than their homogeneous counterparts and consequently a higher final conversion [20,21]. However, the hydrophilicity of the zeolite support limits the activity for oxidation of apolar substrates [23]. The enzyme itself is hydrophobic, which makes it suitable to oxidize non-functionalized hydrocarbons as alkanes and olefins. The FePc–Y model is used for the oxidation of alkanes with peroxides; however, due to the high adsorption of peroxide on the zeolite the dismutase, or catalase-like function, i.e. the decomposition of peroxide, is favored over the oxygenase function, i.e. the selective oxidation of the alkane. To overcome this problem the FePc–Y catalyst can be embedded in hydrophobic membranes [24] or hydrophobic carbon supports instead of zeolites can be used to adsorb the iron–phthalocyanine, as will be shown here.

2. Experimental

2.1. Materials

A gas black was obtained from Degussa (Corax N 326). The announced properties are as follows: nitrogen surface area of 82 $m^2\ g^{-1}$, iodine adsorption of 82 mg g^{-1}, pore density of 465 g dm^{-3}, particle size (measured by electron microscope, arithmetic mean diameter) of 28 nm, volatile content of at most 1%, ash content of at most 0.5% and no detectable moisture. The carbon black (CB) was used without further purification.

The carbon black was oxidized with oxygen at 400°C (CB–$O_2$400) and 600°C (CB–$O_2$600). The weighed sample was placed in a tube furnace and heated in a flow of He at 100°C for

one hour. It was then heated up to the desired temperature and the oxygen flow started. After one hour it was cooled in a flow of He.

The carbon black (50 g l^{-1}) was oxidized with HNO_3 0.3 M (CB–HNO_3) or H_2O_2 3 M (CB–H_2O_2) at 95°C for 6 h. The suspensions were centrifuged and the carbon was washed by resuspension with water, shaken for 4 h and centrifuged. The samples were washed repeatedly to obtain a constant pH or a H_2O_2 concentration less than 2×10^{-4} M. This was estimated by measurement of O_2 release after injection of catalase into an aliquot of the supernatant. The samples were dried at 90°C for 12 h in a vacuum chamber at less than 1500 Pa.

Commercial NaY with silicon to aluminium ratio of 2.47 was acquired from Zeocat.

2.2. Chemicals

Cyclohexane (+99.9%), dichloromethane (+99%) and acetone (p.a.) were purchased from Janssen Chimica; FePc was acquired from Strem Chemicals and 1,2-dicyanobenzene (DCB) (+98%), dimethylformamide (99%), tertiary butyl hydroperoxide (*t*-BHP) (70% in water) and ferrocene (98%) were obtained from Aldrich. Water was purified by a Milli-Q plus system (Millipore). All other reagents were analytical grade.

2.3. Catalase

Aspergillus niger catalase was purchased from Merck in lyophilized state and stored at −20°C. Catalase from *Aspergillus niger* has been chosen because it is significantly more active and stable than the beef liver and *Penicillium* catalase when subjected to extreme conditions of pH, hydrogen peroxide concentrations and temperatures [25]. It has an average molecular weight of 385,000 Da [15]. The Stokes radius is 5.8 nm [26].

2.4. Surface area measurements

The BET surface area was determined by adsorption of nitrogen at liquid nitrogen temperature, using an ASAP 2000 instrument from Micrometrics. Complete adsorption and desorption isotherms were obtained with the same instrument.

2.5. X-ray photoelectron spectroscopy (XPS)

The surface chemical composition of the samples were determined by XPS using an SSI X-probe (SSX 100/206) photoelectron spectrometer of Fisons equipped with an aluminum anode (10 kV, 20 mA) and a quartz monochromator. The analysis chamber was maintained at approximately 7×10^{-7} Pa. The analyzer energy was 50 eV (FWHM of $Au_{4f_{7/2}}$: 1.0 eV). The samples being conductors, the surface charge compensator (flood gun) was turned off. In order to determine the binding energy (E_b) of the XPS peaks, the energy scale was calibrated by setting the $Au_{4f_{7/2}}$ peak at 83.98 eV. A tentative decomposition of the O_{1s} peak was performed using the software 8.3 D supplied by the manufacturer and maintaining a Gaussian/Lorentzian ratio of 85/15 for every component. The software allowed S-shaped background subtraction, considering that the background of any peak is proportional to the area of the peak at higher kinetic energy [27]. Surface atomic concentrations (atom fraction in %) were computed using sensitivity factors provided by the spectrometer software.

2.6. Electrophoretic mobility measurements

The electrical properties of carbon blacks were investigated by electrophoretic mobility measurements (Zetasizer III from Malvern instruments) in phosphate buffer (0.27 mM Na_2HPO_4–0.18 mM KH_2PO_4, pH 7, ionic strength 1 mM) following the procedure described in a previous work [5].

2.7. Catalase adsorption

Solutions of catalase were filtered on a 0.45 μm Millipore filter with low protein adsorption.

Samples of adsorbents were equilibrated for 4 h in phosphate buffer (24 mM Na_2HPO_4–16 mM KH_2PO_4, pH 7, ionic strength 88 mM); the operation was performed 4 times by centrifugation and resuspension, and the last pellet was dried at 90°C for 12 h in a vacuum chamber at less than 1500 Pa. The adsorption of catalase was performed at 30°C on 15 mg of carbon black suspended in 750 μl of the phosphate buffer. Before catalase injection, the carbon black was suspended in a tube placed in an ultrasonic bath. The tube containing the carbon black and catalase was stirred for 1 h at 9 rpm in a vertical plane perpendicular to the rotation axis. The amount adsorbed was 0.33 mg g^{-1}; under these conditions the total amount of catalase brought in contact with the carbon was irreversibly adsorbed.

2.8. Catalase activity

Catalase activity was assayed by a modification [29] of the procedure of Del Rio et al. [30]. A Clark oxygen electrode (OXI91, WTW) was standardized by setting 100% with the phosphate buffer saturated by air [31] at 30°C. 100 ml of the phosphate buffer was bubbled with nitrogen until the electrode gave a reading of less than 10%. The free or adsorbed catalase was then introduced into the solution and the rate of reoxygenation from the atmosphere was recorded. 100 μl of 10 M H_2O_2 solution was then added to the reaction vessel and the rate of oxygen production was recorded. The catalase activity was deduced from the change of slope [28].

2.9. Enzyme mimic synthesis

0.021 g FePc or approximately 50 m^2 (2.25 nm^2 was taken as surface area for one FePc molecule) was dissolved in dichloromethane (30 ml) and 1 g carbon black was added to the homogeneous solution. The suspension was continuously stirred. The solvent was removed by evaporation at 25°C and the carbon black with adsorbed iron phthalocyanines, FePc–C, was dried.

FePc–Y was synthesized and characterized as explained in a previous report [23]. It contains 1.14 FePc complexes and 3.2 Pc molecules per unit cell, which agrees with approximately 216 m^2 g^{-1} when the complexes would be adsorbed on a flat surface. On the curved surface of zeolite Y the blocked and occupied surface by phthalocyanines is higher. The total (inner and outer) surface area of zeolite Y is 792 m^2 g^{-1}, which has a pore volume of 0.276 ml g^{-1}. After synthesis of phthalocyanines the free surface is reduced up to 374 m^2 g^{-1} and the pore volume to 0.132 ml g^{-1}, which agrees well with the loading. No unchelated iron was left in the zeolite as shown by chemical analysis.

2.10. Oxidation activity with immobilized FePc complexes

The catalytic reactions were carried out in the liquid phase in a fed-batch reactor with continuous stirring at 25°C and atmospheric pressure. Acetone (50 ml) was used as a solvent with 0.5 g FePc–C or FePc–Y and 50 mmol cyclohexane. Tertiary butyl hydroperoxide (*t*-BHP) was added to the reaction mixture with a rate of 4.38 mmol h^{-1} until 100 mmol was added. Sampling was done after 100 h. Identification and quantification of products were performed by GC-analysis on a 50 m CP-Sil-5 capillary column from Chrompack, using appropriate sensitivity factors for the FID detector.

3. Results

The BET specific surface area of the untreated carbon black is 74.6 m^2 g^{-1} (Table 1). The oxidized carbon blacks all have a slightly higher surface area, the highest increase being obtained after treatment with nitric acid and hydrogen peroxide. The nitrogen adsorption isotherms are of type II according to the

Table 1
The BET specific surface area for the untreated and the oxidized carbon blacks

Carbon black	Surface area ($m^2\ g^{-1}$)
CB	74.6
CB–$O_2$400	78.7
CB–$O_2$600	82.9
CB–HNO_3	86.2
CB–H_2O_2	84.4

Brunauer classification, but the desorption isotherms are of type IV. The *t*-plot did not reveal any microporosity.

To obtain the pore size distribution curves, the desorption isotherms have been analyzed according to the BJH method [32]. Fig. 1 presents the variation of the cumulative surface area of CB–$O_2$600 with the pore diameter. The treated and untreated carbon give almost identical curves. The majority of pores have a size near 30 nm.

The O_{1s} peaks of carbon samples, untreated and treated with O_2, HNO_3 and H_2O_2, are shown in Fig. 2. The intensity scale is normalized in such a way that the peak area is proportional to the atom fraction of oxygen (O/(C + O + N)) at the surface. The shape of the peaks is not significantly influenced by oxidation; for all carbon blacks they are centered near 532 eV and have a width at half maximum of 3.1 ± 0.1 eV.

The variation of the oxygen concentration as a function of the treatment is shown in Fig. 3. The HNO_3 and H_2O_2 treatments increase considerably the oxygen concentration at the surface, whereas heating under oxygen flow decreases the oxygen concentration. The sample treated with HNO_3 shows a N_{1s} peak at 405 eV, which is typical of nitroxy groups [33]. The nitrogen surface concentration (around 0.1% of the atom fraction) is much below the oxygen concentration.

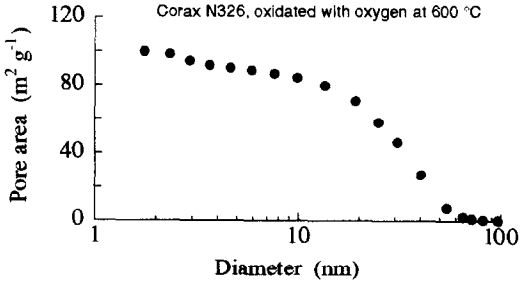


Fig. 1. A typical pore size distribution of Corax N326 (carbon black), in this case CB–$O_2$600. The pore area is shown as a cumulative pore area with decreasing pore diameter.

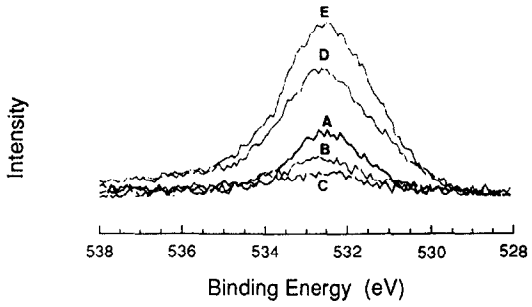


Fig. 2. The O_{1s} XPS peaks of carbon blacks and the oxidized carbon blacks: A = untreated; B = CB–$O_2$400; C = CB–$O_2$600; D = CB–HNO_3; E = CB–H_2O_2.

The electrophoretic mobility is correlated to the oxygen surface concentration (Fig. 4), the surface becoming more negative as a result of higher oxygen surface concentrations. Oxygen treatment at 400°C makes the surface about neutral at pH 7; burning at 600°C makes the surface positively charged at the same pH.

The specific activity of catalase was measured with H_2O_2 10 mM as substrate in order to be in the range where the activity is propor-

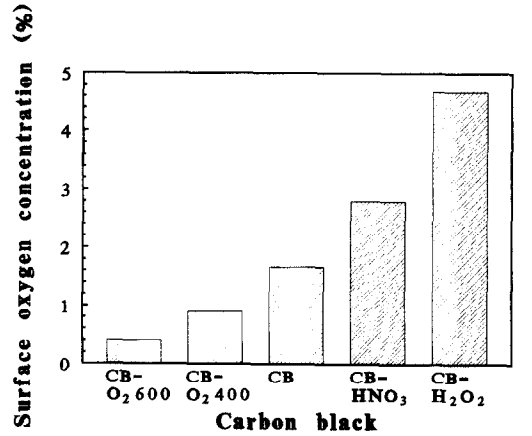


Fig. 3. The influence of oxidation on the surface oxygen concentration (atom fraction O/(C+O+N)) of carbon black.

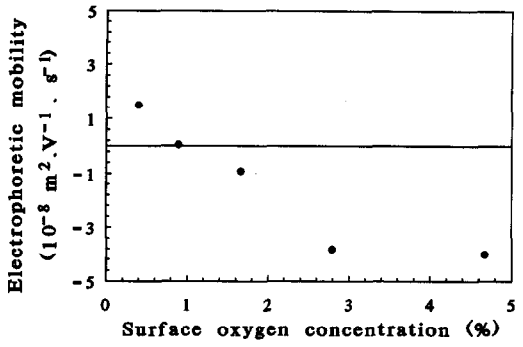


Fig. 4. The influence of the surface oxygen concentration (atom fraction O/(C+O+N)) of carbon black on its electrophoretic mobility (pH 7, ionic strength 1 mM).

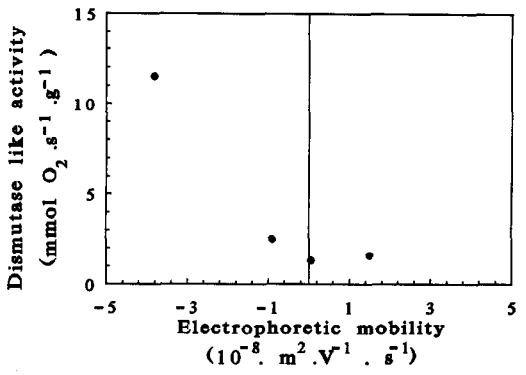


Fig. 6. Relation between the electrophoretic mobility of the carbon blacks and the activity of adsorbed catalase.

tional to the substrate concentration. It has been verified that, when measuring the activity of adsorbed catalase, no activity is observed in the liquid phase. The specific activity of catalase adsorbed on untreated carbon black is lower than the specific activity of the enzyme in solution, which is 47 mmol O_2 s^{-1} g^{-1}. The specific activity increases when the atom fraction of oxygen increases (Fig. 5) and when the electrophoretic mobility becomes more negative (Fig. 6).

The iron–phthalocyanine supported catalysts were tested in the oxidation reaction of cyclohexane with *t*-BHP. Information about two distinct actions was obtained. The conversion of cyclohexane to cyclohexanol and cyclohexanone provides an evaluation of the amount of *t*-BHP consumed by selective oxidation of cyclohexane, i.e. an oxygenase-like activity which mimics the activity of cytochrome *P*-450 (reaction (1)). The yield of *t*-butanol which is due to decomposition of *t*-BHP provides an evaluation of the dismutase-like activity mimicking the activity of catalase (reaction (2)).

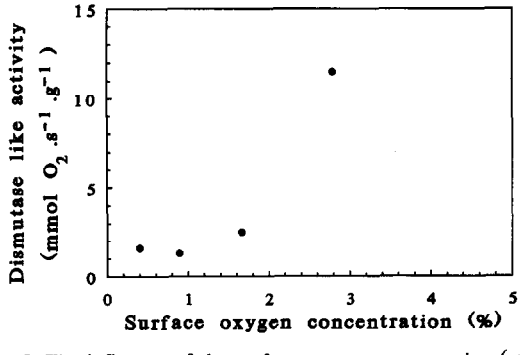
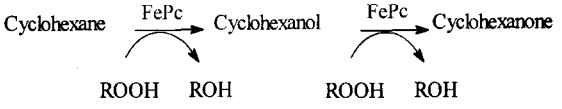


Fig. 5. The influence of the surface oxygen concentration (atom fraction O/(C+O+N)) of the carbon blacks on the activity of adsorbed catalase.



(1)

$$ROOH + ROOH \xrightarrow{FePc} 2ROH + O_2 \quad (2)$$

There is definitely a reverse relation (Table 2) between the dismutase-like activity and the oxygenase-like activity. As compared with carbon supported catalysts, FePc–Y gives a high decomposition activity relative to selective oxidation. Carbon black and zeolite Y without supported iron–phthalocyanines show no cyclohexane oxidation and only a slow decomposition of the peroxide, which does not alter the results shown in Table 2. Fe–Y shows a decomposition rate of the peroxide which is comparable to FePc–Y but the activity for oxidation of cyclohexane is one order of magnitude lower, however the contribution of Fe–Y to the activity is low since no unchelated iron could be detected in the catalyst. Carbon with impreg-

Table 2
The conversion of cyclohexane (oxygenase-like activity) and the decomposition of *t*-BHP to *t*-butanol (dismutase-like activity) in the reaction of cyclohexane and *t*-BHP in presence of FePc supported on carbon blacks and FePc encapsulated in zeolite Y

Catalyst	Dismutase-like activity [a]		Oxygenase-like activity [b]	
	(mmol)	TON [c]	(%)	TON [d]
FePc–CB–$O_2$400	5.0	285	68.0	3657
FePc–CB–$O_2$600	18.4	1047	34.8	1820
FePc–CB	17.0	964	37.2	1963
FePc–CB–HNO_3	32.6	1853	26	1368
FePc–CB–H_2O_2	59.8	3397	33.6	1772
FePc–Y	54.1	1406	16.34	408

[a] Yield of *t*-butanol (mmol) formed via the decomposition reaction from *t*-BHP, mimicking the catalase activity.
[b] Conversion of cyclohexane (50 mmol) to cyclohexanol and cyclohexanone, mimicking the cytochrome *P*-450 activity.
[c] Calculated as the amount of *t*-BHP converted to *t*-butanol (mmol) without oxidation of cyclohexane per mmol of FePc on the catalyst.
[d] Calculated as the amount of *t*-BHP converted to *t*-butanol (mmol) with oxidation of cyclohexane per mmol of FePc on the catalyst.

nated iron salts is not tested since its presence is unlikely as the carbons are impregnated with pure iron–phthalocyanines.

A reverse relation is observed between the ratio of dismutase-like to oxygenase-like activity and the electrophoretic mobility, as shown in Fig. 7. The highest ratios are obtained with the carbon blacks which are oxidized with hydrogen peroxide and nitric acid. These carbons have the most negative electrophoretic mobility and the highest oxygen content at the surface. An even higher ratio of dismutase-like to oxygenase-like activity (3.45) is obtained on FePc–Y. Zeolite Y, with a silicon to aluminum ratio of 2.46, bears a higher density of negative charges compared to carbon blacks.

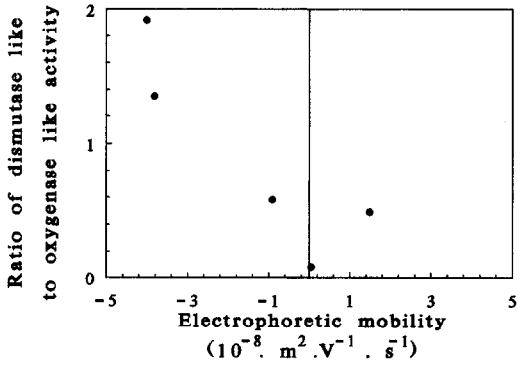


Fig. 7. Ratio of dismutase-like to oxygenase-like activity of iron–phthalocyanines supported on carbon blacks as a function of their electrophoretic mobility. The dismutase-like activity is defined as the amount of *t*-butanol (mmol) formed in the decomposition reaction of *t*-BHP with liberation of molecular oxygen. The oxygenase-like activity is defined as the amount of *t*-butanol (mmol) formed during the selective oxidation reaction of cyclohexane to cyclohexanol and cyclohexanone.

4. Discussion

The specific surface area expected for spheres with a diameter of 28 nm, characteristic for Corax N326, the carbon black used in these experiments, and a specific weight of 1.83 g cm^{-3} [34] is approximately 117 m^2 g^{-1}. This corresponds reasonably with the experimental surface area around 80 m^2 g^{-1} (Table 1) and a dominant apparent pore radius of 30 nm (Fig. 1). Consequently, there is no intraparticular porosity and the pores in dry carbon are due to aggregation of the particles. Oxidation of the carbon black increases the surface area slightly. According to the literature the increase depends on the nature of the carbon black and can be negligible or even absent [3,35–37]. None of the oxidation treatments reported here provoke any marked modification of the texture of the carbon black. In particular the oxidations do not create any significant microporosity.

The center of the O_{1s} peaks (Fig. 2) is near 532 eV and similar for all carbon blacks. They all have a width at half maximum of 3.1 ± 0.1 eV. A comparison with XPS spectra of standard compounds and polymers [38] shows that the band width is not due to a poor resolution of the spectrometer, but to a contribution of different chemical functions. Oxygen double bound to carbon is centered near 531.4 eV while oxygen single bound to carbon, is expected at 533.0 eV. The position and shape of the XPS peaks of carbon black and its oxidized forms is similar to those observed on graphite [39,40] and carbon

fibers [41]; clean electrochemical oxidation can vary the position of the peak [42]. Treatment with nitric acid and hydrogen peroxide enhances the oxygen concentration whereas oxidation with molecular oxygen lowers the concentration of oxygen (Fig. 3). The electrophoretic mobility reflects the potential at the slipping plane between the surface and the liquid, which results from the surface charge and adsorption of ions. It is made more negative by wet oxidizing treatments and becomes positive at pH 7 after oxidation with molecular oxygen (Fig. 4). The position and shape of the O_{1s} peaks and the surface electrical properties are consistent with carboxyl groups which are usually present on carbon black [34]. Oxidation with molecular oxygen removes part of the carboxylic acids as carbon dioxide or monoxide; however, yellow vapors in the exit gas indicate also the formation of volatile degradation products. It is not uncommon that oxidation decreases the surface oxygen concentration. Upon oxidation of carbon black with humid ozone the surface concentration of oxygen increased during a short reaction time and subsequently decreased; the release of CO_2 and CO was observed as well as the dissolution of condensed aromatic rings with carboxyl and phenol functions [43]. Extensive oxidation at high temperature does not increase the oxygen surface concentration but rather cleans the surface. It is not clear why the surface gets positively charged.

Adsorption often decreases the enzyme specific activity [44] as observed here. The loss of activity is lower when the surface oxygen concentration increases (Fig. 5). The higher hydrophilicity of the surface may decrease the deformation of the protein by hydrophobic interactions. However, the influence of surface oxidation may also be due to electrostatic interactions. The protein is negatively charged at pH 7 (isoelectric point 4.5 [45]) and the carbon surface becomes more negative as the oxygen concentration increases at the surface (Fig. 4 and Fig. 6). The increase of adsorbed catalase activity when the carbon surface is oxidized may thus be due to an increase of the repulsive enzyme-surface electrostatic interactions, which lowers the deformation of the enzyme in contact with the surface. A similar behaviour has been established for β-D-glucosidase. The latter is positively charged at pH 4 and adsorbs strongly on negatively charged clay [46] leading to enzyme deactivation. Above pH 6, both the enzyme and the clay have a negative charge and the enzyme remains active after adsorption.

Iron–phthalocyanines, used as models for protoporphyrin IX, are able to decompose peroxides, which is analogous to catalase (dismutase function, reaction (2)), but they can also oxygenate hydrocarbons, which are similar to cytochrome *P*-450 (oxygenase function, reaction (1)) [16–21]. The activities are not altered by eventual desorption of the FePc complex from the carbon surface, since activities of homogeneous iron–phthalocyanines are an order in magnitude lower than in case of the supported ones [23]. The direct comparison with catalase, shows that both the activity of catalase and the catalase-like activity of the complex increase as the carbon support surface contains more oxygen and is more negative. For catalase this has been attributed to a better preservation of the enzyme structure upon adsorption, due to a weaker affinity with the surface. For iron–phthalocyanine complexes the explanation is quite different; moreover, the oxygenase-like activity of the complexes decreases as the surface oxygen concentration of carbon black increases. This may be analyzed further through a broader comparison with hemoproteins.

The three general biological functions of hemoproteins are the transport of electrons (e.g., cytochrome C), the transport of oxygen (e.g., hemoglobin) and the catalysis of redox reactions (e.g., cytochrome *P*-450, catalase, peroxidase) [47,48]. All of these proteins have iron protoporphyrin IX (heme) as their prosthetic group. Redox catalysis by hemoproteins occurs in two distinct steps: the cleavage of the dioxygen bond to give a catalytic ferryl [$Fe^{IV}=O$] state and the oxidation of the substrate by oxygen

transfer or by electron abstraction [47,49]. However these enzymes show marked differences of selectivity:

catalase: $H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O$,

peroxidase: $H_2O_2 + 2X + 2H^+ \rightarrow 2X^+ + 2H_2O$,

cytochrome *P*-450: $RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$.

The factors which are involved in the control of the versatile reactivities of the iron–oxo species can be listed in three categories [50].

(i) Shape of the entrance channel to the iron porphyrin. Peroxidases are so constructed that the substrate is barred from contact with the oxygen of the catalytic active species by the protein structure. In the case of catalase, the heme is deeply buried inside the protein and is accessible via a narrow hydrophobic channel. This structure helps to explain the marked preference of catalase for a small substrate such as hydrogen peroxide.

(ii) Influence of amino acid residues close to the sixth coordination (distal) site of iron. The region of the active site of cytochrome *P*-450 in which the substrate and molecular oxygen are bound is composed entirely of lipophilic residues. In contrast, the activation of oxygen by peroxidases and catalases occurs in a highly polar environment. Salient among several polar residue are an imidazole and an arginine, which are thought to facilitate the peroxide cleavage and to stabilize the iron–oxo species in peroxidase and catalase.

(iii) Influence of the proximal ligand: an oxygen atom from tyrosine in catalase, a nitrogen atom from histidine in peroxidase or a sulfur atom from a cysteine residue in cytochrome *P*-450 or chloroperoxidase.

The role the proximal oxygen atom in catalase has recently been investigated by Belal et al. [50] using Fe and Mn–porphyrin model system. This investigation showed that the 'true models' of catalase, e.g. iron porphyrins with a proximal oxygen atom, are not the most efficient dismutase catalysts. On the other hand, the best association for the dismutase activity gives also the highest oxygenase activity. However, the association between Fe and proximal oxygen still conserves a reasonable dismutase activity and has no oxygenase activity at all. These results have led Belal et al. [50] to propose that the role of the proximal tyrosine oxygen in catalase activity is not to enhance the dismutase activity, but to reduce the oxygenase activity.

The influence of oxidation of carbon black on the conformation of adsorbed catalase may affect the activity of the enzyme by acting on any of these factors. Its influence on the catalytic activity of adsorbed iron–phthalocyanine may be tentatively explained in the light of the information on hemoproteins.

The access of the complex is not expected to be appreciably affected by the oxygen concentration or the electrical properties of the carbon black surface. The environment of the coordination site involved in the catalytic process may be influenced by the support surface composition through the competitive adsorption between acetone solvent and the reagents, cyclohexane and *t*-BHP; this could be cleared out by adsorption measurements.

The nature of the out of plane proximal ligand has a key influence on the selectivity of hemoprotein and Fe and Mn–porphyrin model systems; oxygen present on carbon black may thus trigger the activity of iron–phthalocyanines by acting as proximal ligand. The experimental results would indicate that a stronger interaction between iron and the proximal out of plane ligand decreases the oxygenase-like with respect to the dismutase-like activity. However, the oxygen surface concentration may also influence the distribution of the electron density of the in plane ligands. It would be interesting to correlate the activity of the complexes with their affinity to the surface.

The turn-over of FePc–Y compared to FePc–CB may be attributed to a more difficult access to the active site. However, the oxygenase/dismutase activity ratio is clearly smaller. This is in line with a stronger interaction of iron with a

proximal ligand in zeolite compared to carbon black.

5. Conclusions

Oxidation of carbon black with nitric acid and hydrogen peroxide increases the oxygen content at the surface and makes the surface more negative. Oxidation of carbon black with molecular oxygen decreases the surface oxygen concentration and makes the surface less negative or slightly positive. The charge and the oxygen concentration at the surface have a prime influence on the catalytic properties of adsorbed catalase and its mimic. High ionic charges and oxygen concentrations make the surface hydrophilic, preserve a higher activity of adsorbed catalase and increase the dismutase-like activity of its mimic, the iron–phthalocyanine. The reverse relation is observed for oxygenase-like activity. Highest alkane conversions to oxygenates are found with iron–phthalocyanines supported on neutral carbon black, while the lowest are on negatively charged surfaces. Zeolite Y, which is more hydrophilic than the carbon blacks, shows the lowest ratio of hydrocarbon oxygenation with respect to peroxide decomposition. The hydrophilicity of the support controls the selectivity of adsorbed iron–phthalocyanine. Hydrocarbon oxidation is definitely increased when using a hydrophobic environment for the active site.

While the aim of the work was to mimic enzymes with adsorbed iron–phthalocyanines, comparison of the structures and activities of hemoproteins provide a guideline to analyze the relationship between the activity of phthalocyanine and the environment of the active site.

Acknowledgements

We are grateful to the Belgian Government for the support in the frame of a IUAP-PAI grant on Supramolecular Catalysis. R.C.S. thanks the same institution IUAP-PAI for a grant as Research fellow. R.F.P. thanks the Belgian National Fund of Scientific Research (N.F.W.O.) for a grant as a Post-Doctoral Research fellow. P.E.N. thanks "het Vlaams Instituut voor de bevordering van het wetenschappelijk-technologisch onderzoek in de industrie, het IWT" for a grant as Doctoral Research fellow. P.G.R. is a member of the Research Centre for Advanced Materials.

References

[1] G. Kühner and M. Voll, Carbon Black-Science and Technology, ed. J.-B. Donnet, R.C. Bansal and M.J. Wang, 2nd Ed. (Marcel Dekker, New York, 1993) p. 1.
[2] J.-B. Donnet and A. Voet, Carbon Black-Science and Technology, ed. J.-B. Donnet, R.C. Bansal and M.J. Wang, 2nd Ed. (Marcel Dekker, New York, 1993) p. 351.
[3] S. Hagiwara, K. Tsutsumi and H. Takahashi, Tanso 107 (1981) 139.
[4] H.P. Boehm, Angew. Chem. Int. Ed. Engl. 5 (1966) 533.
[5] R.C. Sosa, D. Masy and P.G. Rouxhet, Carbon 32 (1994) 1369.
[6] R.C. Bansal and J.-B. Donnet, Carbon Black-Science and Technology, ed. J.-B. Donnet, R.C. Bansal and M.J. Wang, 2nd Ed. (Marcel Dekker, New York, 1993) p. 175.
[7] A. Biloul, F. Coowar, O. Contamin, G. Scarbeck, M. Savy, D. van den Ham, J. Riga and J.J. Verbist, J. Electroanal. Chem. 350 (1993) 189.
[8] V. Iliev, A. Andreev, D. Wöhrle and G. Schulz-Ekloff, J. Mol. Catal. 66 (1991) L5–9.
[9] G. Schulz-Ekloff, D. Wöhrle and A. Andreev, Wiss. Z. Leuna-Merseburg 32 (1990) 649.
[10] T. Arai and W. Nordle, Colloids Surf. 51 (1990) 1.
[11] L. Barroug, J. Lemaire and P.G. Rouxhet, Colloids Surf. 37 (1989) 339.
[12] A.V. Elgersma, R.L. Zsom, W. Norde and L. Lykema, J. Colloid Interface Sci. 138 (1990) 145.
[13] W. Norde and J.P. Favier, Colloids Surf. 64 (1992) 87.
[14] A. Deisseroth and A.L. Dounce, Physiol. Rev. 50 (1970) 319.
[15] T. Kikuchi-Torii, S. Hayashi, H. Nakamoto and S. Nakamura, J. Biochem. 92 (1982) 1449.
[16] H. Herron, J. Coord. Chem. 19 (1988) 25.
[17] R.F. Parton, D.R.C. Huybrechts, Ph. Buskens and P.A. Jacobs, Stud. Surf. Sci. Catal. 65 (1991) 47.
[18] D.R. Huybrechts, R.F. Parton and P.A. Jacobs, Stud. Surf. Sci. Catal. 60 (1990) 225.
[19] R.F. Parton, L. Uytterhoeven and P.A. Jacobs, Stud. Surf. Sci. Catal. 59 (1991) 395.
[20] R. Parton, D. De Vos and P.A. Jacobs, Zeolite Microporous Solids: Synthesis, Structure and Reactivity, ed. E.G. Derouane et al. (Kluwer Academic Publishers, 1992) p. 555.

[21] D.E. De Vos, F. Thibault-Starzyk, P.-P. Knops-Gerrits, R.F. Parton and P.A. Jacobs, Macromol. Chem. Symp. Ser. 80 (1994) 157.
[22] R.E. White and M.J. Coon, Ann. Rev. Biochem. 49 (1980) 315.
[23] R.F. Parton, G.J. Peere, P.E. Neys, P.A. Jacobs, R. Claessens and G.V. Baron, J. Mol. Catal., accepted.
[24] R.F. Parton, I.F.J. Vankelecom, M.J.A. Casselman, C.P. Bezoukhanova, J.B. Uytterhoeven and P.A. Jacobs, Nature 370 (1994) 541.
[25] D. Scott and F. Hammer, Enzymologia 22 (1960) 229.
[26] A.A. Mosavi-Movahedi, A.E. Wilkinson and M.N. Jones, Int. J. Biol. Macromol. 9 (1987) 327.
[27] D.A. Shirley, Phys. Rev. B 5 (1972) 4709.
[28] O.H. Lowry, N.J. Rosebrough, A. Lewis Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[29] O.M. Lardinois and P.G. Rouxhet, Free Rad. Res. Commun. 20 (1994) 29.
[30] L.A. del Rio, M. Gomez Ortega, A. Leal Lopez and J. Lopez Gorgé, Anal. Biochem. 80 (1977) 409.
[31] M.L. Hitchman, Measurement of Dissolved Oxygen (John Wiley and Sons and Orbisphere Laboratories, Geneva, 1978) p. 255.
[32] E.P. Barrett, L.G. Joyner and P.P. Halenda, J. Am. Chem. Soc. 73 (1951) 373.
[33] S. Pignataro and G. Distefano, J. Electron. Spectrosc. Relat. Phenom. 2 (1973) 171.
[34] J.-B. Donnet and A. Voet, Carbon Black-Physics, Chemistry and Elastomer Reinforcement (Marcel Dekker, New York, 1976) p. 351.
[35] T. Murata and Y. Matsuda, Electrochim. Acta 27 (1982) 795.
[36] E. Papirer, S. Li and J.-B. Donnet, Carbon 25 (1987) 243.
[37] E. Papirer, J.-B. Donnet and A. Schultz, Carbon 5 (1967) 113.
[38] P.G. Rouxhet, A. Doen, J.L. Dewez and O. Heuschling, Progr. Org. Coat. 22 (1993) 327.
[39] B. Marchon, J. Carrazza, H. Heineman and G.A. Somorjai, Carbon 26 (1988) 507.
[40] E. Papirer, E. Guyon and N. Perol, Carbon 16 (1978) 133.
[41] E. Desimoni, G.L. Casella, A. Morone and A.M. Salvi, Surf. Interface Anal. 15 (1990) 627.
[42] C. Kozlowski and P.M.A. Sherwood, J. Chem. Soc. Faraday Trans. 181 (1985) 2745.
[43] J.-B. Donnet, P. Ehrburger and A. Voet, Carbon 10 (1972) 737.
[44] O.R. Zaborsky, Immobilized Enzymes for Food Processing (CRC Press, 1973) p. 175.
[45] J. Lavaenis and J. Masy, personal communications.
[46] H. Quiquampoix, Biochimie 69 (1987) 753.
[47] P.R.O. de Montellano, Acc. Chem. Res. 20 (1987) 289.
[48] P.R.O. de Montellano, Ann. Rev. Pharmacol. Toxicol. 32 (1992) 89.
[49] P.M. Wood, Biochem. Soc. Trans. 20 (1991) 349.
[50] R. Belal, M. Momenteau and B. Meunier, New J. Chem. 13 (1989) 853.