Adsorption of Protein on H-Beta Zeolite

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Abstract

The adsorption of two proteins, cytochrome c and α-chymotrypsin onto H-Beta zeolite was studied. The effect of pH on the adsorption capacity was studied at three different pHs, namely 3, 6, and 9. The adsorption capacity for cytochrome c and α-chymotrypsin was found to be the highest at pH 9 and pH 6 respectively. Increase in pH higher than their pI lead to the decrease in the adsorption capacity for both proteins. This is postulated to be due to electrostatic repulsion between protein and the surface of adsorbent. The adsorption characteristics of different proteins onto H-Beta zeolite depend, apart of experimental conditions used, on protein physical and chemical properties. The adsorption isotherm data of protein is well fitted to the Langmuir model. FTIR analysis was performed for both H-Beta zeolite with and without adsorbed protein to study the interaction between the protein and H-Beta zeolite surface. The observed decrease in the intensities of amide groups in protein structure is most likely a consequence of binding of protein onto H-Beta zeolite.

Keywords:

Protein, Cytochrome c, α-chymotrypsin, Adsorption, H-Beta Zeolite.

Introduction

Effective separation and purification of proteins has been an important issue in the biomedical and pharmaceutical industries. A novel protein adsorption has been developed in biotechnology to achieve highly efficient and economical separation processes. In many cases, proteins which have similar physical and chemical properties need to be separated, and thus highly selective adsorbents are desired [1]. Microporous molecular sieves, such as zeolite Y, ZSM-5 and zeolite Beta, have played important roles in acid catalysis because of their peculiar pore structures and strong intrinsic acidities [2].

Zeolite frameworks provide substrates that support the mobilities of the non framework cations that make them good ion exchangers. Due to the presence of never ending intricate pore and channel systems of controlled dimensions and accessibilities, zeolites become excellent sorbents and molecular sieves. Zeolitic material can offer extremely large specific surface area [3]. It has been reported that protein adsorption depends on the pore size of adsorbents, protein size, isoelectric point, hydrophobic interactions and the protein surface chemistry [4-6].

Recent research works are focusing into the development and improvement of mesoporous molecular sieve MCM-41 due to its larger pore volume, which is preferable in separation of larger biological molecule such as protein. However, Ernst et al. [7] reported that the adsorption of amino acids into MCM-41 is much lower than that of microporous adsorbents, H-Beta zeolite and HSZM-11. Research work by Cunman et al. [2] combined the benefits of mesoporous and microporous material to produce a meso-micro porous materials as a bimodal system and gives the benefits of each pore size regime which could potentially improve the efficiency of the zeolite catalysis. This clearly shows that, despite the development in synthesis of the new family of mesoporous molecular sieves, research work is still carried out to investigate and improve the potential of microporous molecular sieves.

In this work, the protein adsorption on H-Beta zeolite at various pHs was carried out using cytochrome c and α-chymotrypsin as model proteins. The interaction between protein and H-Beta zeolite surface was studied using Fourier Transform Infrared (FTIR) spectroscopy.

Materials and Methods

Materials

For the adsorption experiments, horse heart cytochrome c and α-chymotrypsin from bovine pancreas purchased from Sigma were used without further purification. H-Beta zeolite used was obtained from Zeolyst International. Aqueous phase were made up in freshly deionised water from Purite Select AN HP40 (Purite Ltd, England) with resistivity ~ 15 - 16 MΩcm. Buffers were prepared by using phosphoric acid (H_3PO_4), potassium dihydrogen orthophosphate (KH_2PO_4), di-potassium hydrogen phosphate anhydrous (K_2HPO_4), potassium hydrogen

carbonate ($KHCO_3$), and potassium carbonate anhydrous (K_2CO_3) obtained from Sigma and Merck. All the buffer solutions used were prepared from original chemicals without further purification.

H-Beta Zeolite Characterization

Nitrogen adsorption and desorption measurements were performed at 77K using a Micromeritics ASAP 2000 volumetric adsorption analyzer to determine the physical properties of H-Beta zeolite. The surface area was calculated from the adsorption isotherms by the BET method and the pore size distribution was obtained from the desorption branches of the isotherm using t-plot and Barret-Joyner-Halenda (BJH) method. Meanwhile, the calculation micropore volume and micropore surface area was using t-plot method.

Protein Adsorption Measurement

Cytochrome c solutions with concentrations ranging 0.13 g/L to 1.3 g/L was prepared by dissolving different amounts of cytochrome c into deionized water. 10 mg of H-Beta zeolite was placed into test tubes containing a mixture of 2 mL of cytochrome c solution and 1 mL of 0.1 M buffer solution at various pH. The samples were continuously shaken at 293K until equilibrium is reached. Samples were centrifuged for about 20 minutes at 2000 rpm. Centrifugation was made to avoid the interference from scattering particles in the UV-VIS analysis [8]. After centrifugation, a sample of supernatant was withdrawn, and the cytochrome c concentration was analysed by Lambda 35 UV/VIS Spectrophotometer (PerkinElmer, Inc) at 280 nm. The amount of protein adsorbed onto H-Beta zeolite was calculated based on mass balance. Experimental work was conducted at room temperature. The batch adsorption experiment was repeated for α-chymotrypsin at concentration ranging from 0.25 g/L to 2.5 g/L.

FTIR Measurement

The experiments were carried out using a Perkin Elmer Spectrum 2000 Explorer FTIR (Fourier Transform Infrared Spectroscopy). Fresh H-Beta zeolite was ground and then diluted in pure KBr. The sample is pressed to form a pellet. The sample was scanned 10 times per spectrum at $4cm^{-1}$ resolution. Infrared spectra for pure proteins sample were also obtained using similar procedure. Sample of adsorbed protein was prepared using a mixture of 6 mL of 5 mM cyotchrome c and 3 mL of 0.1 M buffer contacted with 1 g of H-Beta zeolite at pH 9. For α-chymotrypsin, adsorption experiment was made by immersing 0.5 g of H-Beta zeolite into 1 mM buffered protein solution at pH 6. After equilibrium, the samples were chilled at -20 °C and allowed to dry overnight in the Freeze Dryer (Heto FD 4.0). Freeze drying technique was used to minimize the protein denaturation. FTIR spectra for the later samples were obtained by the method mentioned previously.

Results and Discussions

Protein being a large biological molecule has a molecular weight of 12300 and 25000 for cytochrome c and α-chymotrypsin, respectively. The isoelectic point of cytochrome c was found to be 9.8 and 8.0 for α-chymotrypsin [8]. From structural data cytochrome c has a unit cell of dimensions a = b = 58.34 Å, c = 41.83 Å and a hydrodynamic diameter of 30 Å, giving the protein an average diameter of ~ 30 Å [6], which is equivalent to 3.0 nm while the diameter for α-chymotrypsin is 4.5 nm. In general, proteins with hydrodynamic diameter larger that the pore diameter adsorb on the outer surface of H-Beta zeolite, while relatively small proteins interact with the microporous and show high loadings. It has been previously reported that the protein size is a limiting factor for adsorption to occur [6]. Based on the characterization made on H-Beta zeolite, it was found that the surface area is 494 m^2/g. Pore volume of H-Beta zeolite is 0.131 m^3/g for micropore and 0.730 m^3/g for mesopore while the average pore diameter was found to be 6.97 nm.

Figure 1 shows the effect of solution pHs on the adsorption capacity of both proteins onto ZSM-5 [9]. The amount adsorbed for cytochrome c increases as the pH of the solution increases, from pH 3 to pH 12, and reached a maximum adsorption at pH 9. This observed increase is most likely a consequence of the strong electrostatic interaction between the negative charges on the aluminum sites and the positively amino acid residues of cytochrome c [8]. Similarly, for α-chymotrypsin, the amount adsorbed was found to be increasing as the pH increases but decreases as the pH solution is greater that pH 6. The maximum loading for both proteins was found to be 0.002 mmol/g.

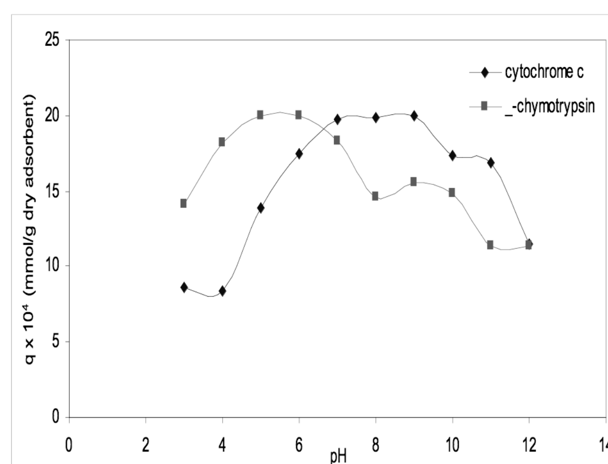

Figure 1: Influence of pH on the adsorption of cytochrome c and α-chymotrypsin onto ZSM-5 [9].

The maximal loading is clearly a function of solution pH. The amount adsorbed for cyochrome c is maximal at pH 9 which is close to pI of cytochome c. pH 6 gives the highest adsorption capacity for α-chymotrypsin because it's the closest to the pI value which is 8.0. The protein is positively charged at a pH below pI and negatively charged at a pH a pH above pI [8]. At pH solution higher than the pI value, α-chymotrypsin adsorption decreases. Electrostatic repulsion between the negatively charged α-chymotrypsin molecule and the negatively charged of the silanol groups of the adsorbent results in a reduced amount of adsorption. Figure 1 shows a pronounce effect of the pH towards adsorption capacity as the adsorbed amount increases as the pH increases and reduces as the pH exceeds the pI value. The experimental work was carried out at pHs below the pI value of cytochrome c, therefore the reduced amount of adsorption is not evident in the investigation.

The adsorption isotherms of cytochrome c and α-chymotrypsin on H-Beta zeolite at various solution pHs are shown in Figures 2 and 3, which clearly show that the hydrophobic solute is adsorbed strongly than the less hydrophobic ones. At higher molar concentration, it appears that the uptake of cytochrome c approaches a maximum around 0.0119 mmol/g, meanwhile it is expected that the adsorption for α-chymotrypsin increases at higher concentration. Figure 2 shows a sharp initial rise of the isotherm, suggesting a high affinity between cytochrome c and the adsorbent surface. This observation is consistent with those of Vinu and Hartmann [8], who found the same adsorption characteristics for adsorption of cytochrome c onto MCM-41. In this work, the maximum loading for α-chymotrypsin is found to be at 0.007 mmol/g. The adsorption isotherm was shown to depend strongly on the molecular size of the adsorbate relative to the size of the matrix pores, with the highest adsorption obtained is for cytochrome c followed by α-chymotrypsin. This postulates that the adsorption capacity is affected by the difference in molecular weight and size of two proteins.

The adsorption isotherms obtained for both proteins were found to be confirm well to the ideal Langmuir model equation, which is given by [10]

$$q = \frac{q_s bC}{1 + bC} \qquad (1)$$

where q_{sb} is the Henry's law constant, and q_s and b are the Langmuir parameter constants. The transformed form of Langmuir equation is given by Equation 2 [11], which will be used to calculate the Langmuir parameter constants by fitting adsorption isotherm data obtained for both proteins.

$$\frac{q}{C} = \frac{1}{q_s b} + \frac{1}{q_s} C \qquad (2)$$

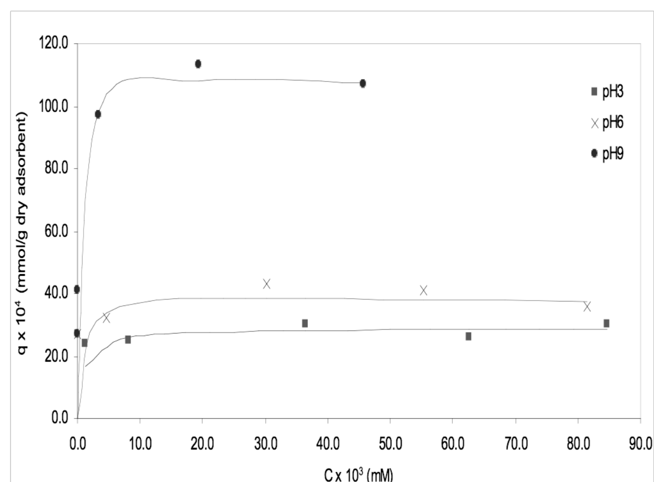

Figure 2: Adsorption isotherm at 293K of cytochrome c on H-Beta zeolite at various pHs.

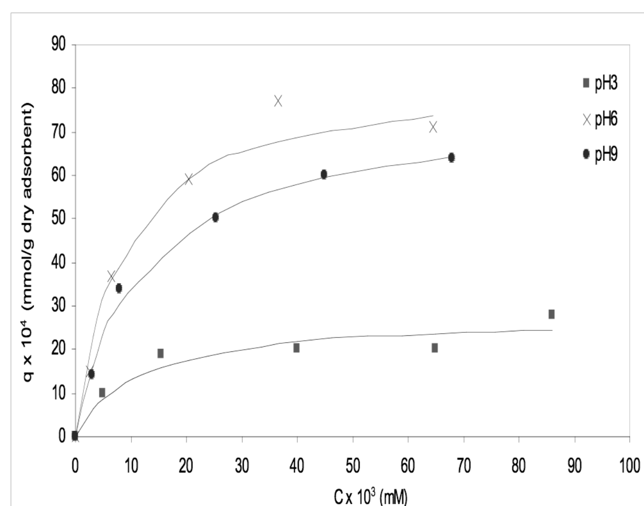

Figure 3: Adsorption isotherm at 293K of α-chymotrypsin on H-Beta zeolite at various pH.

The Langmuir parameter constants were calculated by nonlinear regression analysis of adsorption isotherm data using Langmuir transformed equation (Equation 2), which give a good correlation coefficient (R^2 > 0.99). The Langmuir parameter constants, q_s and b obtained is given in Table 1, which was found to depend strongly on pH and types of protein used.

Table 1: Langmuir equation parameters for the adsorption of proteins upon H-Beta zeolite.

Adsorbate	**pH**	**$q_s b$ (l/g)**	**b (l/mmol)**	**q_s (mmol/g)**
cytochrome c	3	3.0	1015.8	0.0029
	6	44.4	16420.4	0.0027
	9	384.6	245653.8	0.0016
α-chymotrypsin	3	0.2	87.6	0.0028
	6	1.0	115.9	0.0084
	9	0.6	85.7	0.0075

In Figure 4, according to Speight [12], the spectra for pure H-Beta zeolite shows a band at around 3467 cm^{-1} which is assigned to hydroxyl group. The results are similar to those reported for cytochrome c and α-chymotrypsin adsorbed into H-Beta, in which the same compound type was found in both spectra at around 3467 cm^{-1}. Figures 4 (d) and (e) also show the presence of a very broad band in the 3200 – 3500 cm^{-1} region, which is a characteristic of OH group for H-Beta. The result is also consistent as previously reported by Camblor et al. [13]. Proteins, one of the most important and versatile classes of biological compounds, are made from nitrogen-containing molecules called α-amino acids.

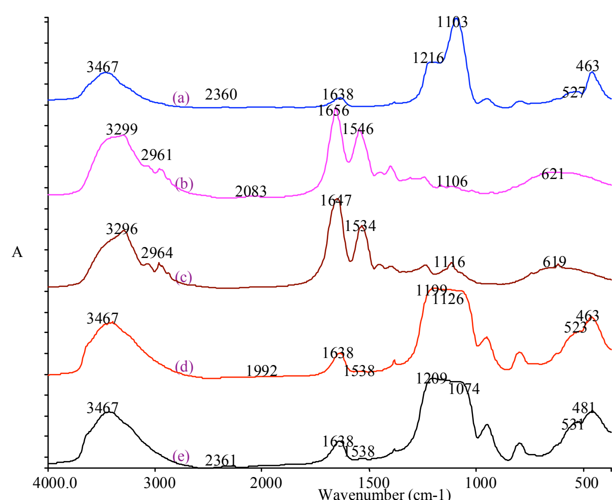

Figure 4: FTIR analysis on: (a) H-Beta zeolite; (b) Cytochrome;, (c) α-chymotrypsin; (d) Cytochrome c adsorbed on H-Beta; and (e) α-chymotrypsin adsorbed on H-Beta zeolite.

The amine functional group of one α-amino acid reacts with the carboxyl group of another α-amino acid to form amide bond [14]. As observed Figure in 4 (b), the presence of band at 1656 and 1546 cm^{-1} is expected to show that the amine group exists in the sample. The 1660-1700 cm^{-1} spectral region is dominated by the amide I mode of the polypeptide backbone of cytochrome c. The analysis of the 1656 and 1546 cm^{-1} in Figure 4 (b) correspond to amide I and amide II modes, respectively. The same result can be observed for α-chymotrypsin, where the hydrogen bonded in regular external α-helix and weakly hydrogen bonded NH represented by 1647 and 1634 cm^{-1} band. From Figures 4 (d) and 4 (e), the changes in structure of both proteins adsorbed onto H-Beta zeolite are shown by the ~70 % decrease in the band intensities at 1656 and 1647 cm^{-1} for amide I mode, meanwhile amide II band experienced almost 90 % decrease in intensities suggested that protein structure has changed due to adsorption process. Most of the amide groups were exchanged under the equilibrium condition. The adsorption of proteins onto H-Beta zeolite modifies its structure, resulting in the spreading of proteins adsorbed onto the hydrophobic surface because of the unfolding of the protein and increase self-association. The binding of protein can be explained by electrostatic interaction between the positively charged lysine and the negatively charged H-Beta zeolite.

Conclusion

The molecular sieve H-Beta zeolite has been explored for its ability to adsorb cytochrome c and α-chymotrypsin, from aqueous solution in batch experiment. Protein size, isoelectric point and also protein surface chemistry are important parameters influencing the adsorption process. The maximum adsorption occurs near the isoelectic point of cytochrome c at pH 9 and chymotrypsin at pH 6. Increase in pH exceeding the protein pI value decreases the adsorption capacity due to electrostatic repulsive forces. The binding of the proteins and H-Beta zeolite can be explained by interaction between protein and H-Beta zeolite surface shown by the FTIR analysis in which the amides group intensities in protein structure reduced after adsorption.

Acknowledgements

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Notation

b	Langmuir adsorption parameter, l/mmol
C	equilibrium concentration, mM
q	solute concentration in adsorbent, mmol/g adsorbent
q_s	Langmuir isotherm parameter, mmol/g dry adsorbent

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