where E is the heat of adsorption for the first layer, and E_v is that for the second and higher layers and is equal to the heat of liquefaction.According Eq. (1), by using the obtained experimental results, an isotherm can be plotted as a straight line with $1/v[(P_0/P) - 1]$ versus P/P_0 in the range of $0.05 < P/P_0 < 0.35$. The value of the slope and the y-intercept of the line are used to calculate the volume of gas adsorbed at monolayer coverage v_m and the BET constant c.

A total surface area S_t and a specific surface area S are evaluated by the following equations:

$$S_t = (v_m Ns)/M \quad (3)$$

$$S = S_t/a \quad (4)$$

where N, s, M and a are, respectively, Avogadro's number, adsorption cross section, molecular weight of adsorbate and weight of sample solid. In the BET measurements the absorbate was liquid N_2 at 77 K as the adsorbate with an adsorption cross section $s = 16.2$ Å^2 and absoebent was HA.

In this work, we also studied the adsorption of BSA on HA along with the effect of pH and presence of KCl and ion PO_4^{3-} in solution on BSA adsorption by ultraviolet (UV) spectroscopy. The obtained results indicated that surface energy of HA predominates the effect of size and charge of BSA.

References

Andrade, J.D., 1985. Surface and Interfacial Aspects of Biomedical Polymers, Surface Chemistry and Physics. Plenum Press, New York.
Antonelli, M.L., Capalbi, A., Gente, G., Palacios, A.C., Sallustio, S., La Mesa, C., 2004. Colloids Surf. A: Physicochem. Eng. Aspects 246, 127–134.
Creighton, T.H., 1993. Proteins. Freeman, New York.
Kandori, K., Fudo, A., Ishikawa, T., 2002. Colloid Surf. B Biointerfaces 24 (2), 145–153.
Kazuhiko, K., Yumi, U., Tatsuo, I., 2002. J. Colloid Interface Sci. 252, 269–275.
Norde, W., 1986. Adv. Colloid Interface Sci. 25 (4), 267–340.
Qilie, L., Joseph, D., Andrade, J., 1998. J. Colloid Interface Sci. 200, 104–113.
Sharpe, J.R., Sammons, R.L., Marquis, P.M., 1997. Biomaterials 18, 471–476.
Shi, D., Jiang, G., Wen, X., 2000. J. Biomed. Mater. Res. Appl. Biomater. 53 (35), 457–466.

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2.
Cloning, expression and characterization of a chitin-binding domain

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Chitosan and chitin represent a family of biopolymers, made up of β(1 → 4)-linked *N*-acetyl-D-glucosamine and D-glucosamine subunits. Chitin can be found in exoskeletons of insects, fungal cell walls and shells of crustaceans and is one of the most abundant natural polysaccharides in nature, second only to cellulose. On the other hand, chitosan is mainly obtained industrially by alkaline hydrolysis of chitin (Tsigos et al., 2000). Due to their biodegradability and biocompatibility, chitin and chitosan, are widely studied for biomedical applications (Tsigos et al., 2000; Dodane and Vilivalam, 1998; Felt et al., 1998; Madihally and Matthew, 1999; Koide, 1998).

Chitinases are enzymes that degrade chitin and, although chitin has not yet been found in humans, a protein with homology to fungal, bacterial or plant chitinases, have been identified in humans. This enzyme, later identified as a chitotriosidase, is expressed by macrophages and its activity is dramatically elevated in patients with Gaucher disease (Renkema et al., 1995; Boot et al., 1995).

The DNA encoding for the chitin-binding domain of the human chitinase was synthesised by GenScript Corporation (USA) with optimized codons for bacterial expression. Then, the DNA was cloned in pET25b(+) vector of the pET expression system (Novagen). In order to obtain soluble and active protein several expression strains of *Escherichia coli* and culture mediums were tested. Soluble protein was obtained using *Escherichia coli Tuner(DE3)* cultured in minimal medium M9 and we showed that it bound to chitin but not to chitosan, cellulose or starch, as it was expected. Using its affinity to chitin, the recombinant protein was purified and then tested to evaluate its specificity. Affinity tests were made using several polysaccharides and we confirmed that, after purification, the human chitin-binding domain was still bound to chitin but not to other polysaccharides.

The results showed that the chitin-binding domain of the human chitinase was successfully produced in *E. coli* in a soluble and active form as it bound to chitin and not to other polysaccharides. They also showed that the protein purification was possible using chitin and that, after purification, the recombinant protein maintained its affinity and specificity towards chitin as it did not bind to other polysacchrides, including chitosan, cellulose or starch.

References

Boot, R.G., et al., 1995. J. Biol. Chem. 270, 26252–26256.
Dodane, V., Vilivalam, V., 1998. Pharm. Sci. Technol. Today 1, 246–253.
Felt, O., et al., 1998. Drug Dev. Ind. Pharm. 24, 979–993.
Koide, S.S., 1998. Nutr. Res. 18, 1091–1101.
Madihally, S.V., Matthew, H.W.T., 1999. Biomaterials 20, 1133–1142.
Renkema, G.H., Boot, R.G., Muijsers, A.O., Donker-Koopman, W.E., Aerts, J.M.F.G., 1995. J. Biol. Chem. 270 (5), 2198–2202.
Tsigos, I., Martinou, A., Kafetzopoulos, D., Bouriotis, V., 2000. TibTech. 18, 305–312.

doi:10.1016/j.jbiotec.2007.07.461