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Adsorption behaviour of lipase from *Staphylococcus carnosus* on a hydrophobic adsorbent

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ABSTRACT

The adsorption of proteins on a solid surface with a subsequent desorption is a well known final purification step in downstream processing. Here the adsorption behaviour of a microbial lipase on the hydrophobic Fractogel TSK butyl 650 in a crude fermentation supernatant is investigated. The measured equilibrium curves differ from fermentation to fermentation by up to ±65%. The adsorption capacity increases with decreasing particle diameter of the adsorbent and is influenced by the method of contacting the supernatant with the adsorbent. The rate of desorption depends largely on the adsorption conditions, which is an indication of different orientations of the adsorbed enzyme.

INTRODUCTION

Lipases (EC 3.1.1.3) are enzymes, that hydrolyse triglycerides to diglycerides, monoglycerides, glycerol and fatty acids or, depending on the reaction conditions, synthesize certain ester compounds. They are used in the food industry, as admixtures in washing materials and, because of their substrate-, stereo- and regiospecificity, they are employed in the production of pharmaceutical substances. Another possible use in medicines is to aid the digestion of fat. Therefore industrial interest in microbial lipases has increased greatly in recent years.

Apart from the production and optimization of the substrate and controlling and supervising the fermentation, great attention has to be paid to the downstream processing to obtain high yields of the biotechnological products [1]. Owing to the sensitivity of biological substances and their low concentration in production by fermentation, the total number of downstream processes has to be reduced and each step has to be optimized. This is especially true for industrial large-scale production to minimize expenditure.

One important step in the purification of the fermentation broth is the adsorption of the desired enzyme on a suitable adsorbent and the subsequent desorption. Although this method is usually the final step in the purification, it can also be one of the first procedures. For the design of any separation process a knowledge of the equilibrium state is a prerequisite. In contrast to most publications, which deal with the adsorption of pure one-component enzyme solutions, in this paper the influence of several parameters on the adsorption of complex fermentation supernatants on a hydrophobic adsorbent is examined.

EXPERIMENTAL

The lipase used in the experiments is an extracellular enzyme from the strain *Staphylococcus*

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carnosus (pLipMut 2) [2], fermented in a bubble column or in a centrifugal field reactor as described by Voit *et al.* [3]. The pH value at the end of the fermentation runs ranged from 8.6 to 8.8. After separating the cells by centrifugation and concentrating from 10 to 2 l by ultrafiltration (cut-off of the membrane: 20 kDa) the supernatant served as starting material for the adsorption process. In all fermentations the conductivity of the concentrated solution was below 0.1 mS cm^{-1}. The lipase activity was measured according to Winkler and Stuckmann [4] with *p*-nitrophenyl caprylate as substrate and was measured in all cases at room temperature. Fractogel TSK butyl 650 from Merck was found to be an appropriate adsorbent [5]. All chemicals were of reagent grade.

The adsorption equilibrium was determined in batch experiments. The adsorption took place in a beaker, shaken at 350 min^{-1}, in which different quantities of lipase were brought into contact with a known amount of adsorbent. Equilibrium was reached after about 6 h, indicated by a constant value of the activity in the supernatant. The difference between the activity before and after the adsorption is proportional to the amount of adsorbed enzyme, q.

After adsorption the adsorbent was allowed to settle and the supernatant was replaced by bidistilled water or a supernatant with a lower content of lipase. The desorption conditions were the same as for adsorption.

The column experiments were carried out in a glass column with a diameter of 1 cm and bed heights between 2 and 20 cm.

RESULTS

Although all fermentations were carried out under the same conditions and with the same ingredients, the lipase activity varied from 60 to 160 U ml^{-1} and the dry weight of the supernatant ranged from 30 to 120 mg ml^{-1}. Thus the equilibrium curves differ with each fermentation (Fig. 1). The differences of up to ± 65% can be explained by the fact that the metabolic products and the substrate, which was not consumed, were

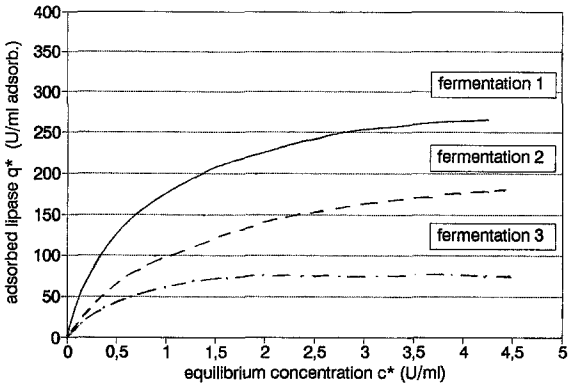

Fig. 1. Equilibrium curves from different fermentations.

different from fermentation to fermentation and competed for the same adsorption sites.

As expected, the adsorption capacity is highly influenced by the amount and the type of added salts. Up to a concentration of about 0.5 *M* ammonium sulphate no effect on the adsorption capacity is observed for different temperatures and pH values. The adsorption is increased by a factor of about 2.5–3 (depending on the pH value and the temperature) at salt concentrations of 1.3 *M*. In this context it should be mentioned that precipitation of this lipase begins at salt contents in the range of 1.8 *M* [6].

The adsorption capacity increases with increasing temperature, which indicates the hydrophobic character of the interaction between the protein and the adsorbent [7–10]. As can be seen from Fig. 2, for all ammonium sulphate concentrations the adsorption capacity reaches a maxi-

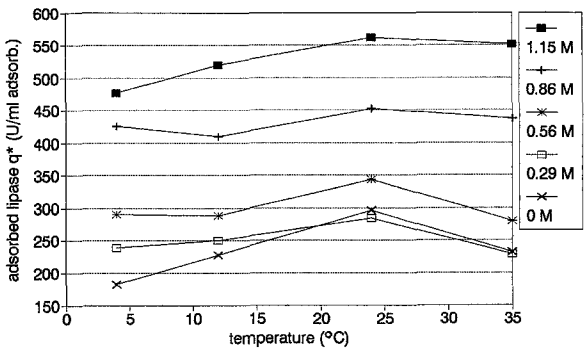

Fig. 2. Influence of temperature and ammonium sulphate concentration on the adsorption capacity.

mum and diminishes at higher temperatures.

The amount of lipase adsorbed increases when the pH value is reduced and approximates the p*I* value 5.6. At low pH values the effect of temperature is pronounced, whereas at higher pH values this influence is negligible. The supernatant should not be too acidic because of denaturation, precipitation and more difficult desorption.

It is not surprising that the kinetics of adsorption is influenced by the particle size of the adsorbent because the volume-based surface increases with decreasing diameter. The measured relationship between the maximum capacity and the particle diameter as shown in Fig. 3 is quite unexpected. This dependence is valid for different fermentation charges as well as for different temperatures. The reason is probably that the lipase does not penetrate the whole adsorbent particle but is only adsorbed in an outer shell. It is well known that the adsorption front in a particle is very steep when the equilibrium curves are favourable for adsorption [11]. This is the case for the equilibrium curves shown in Fig. 1. If one assumes that the lipase adsorbs only in an outer shell of the adsorbent particle (Fig. 4) and that because of the steep adsorption front the volume-based capacity, $q(r)$, has a constant value q_r in this shell (but could be a function of the adsorption conditions and the fermentation) the penetration depth Δr is determined for all fermentations and temperatures to be about 10 μm. This

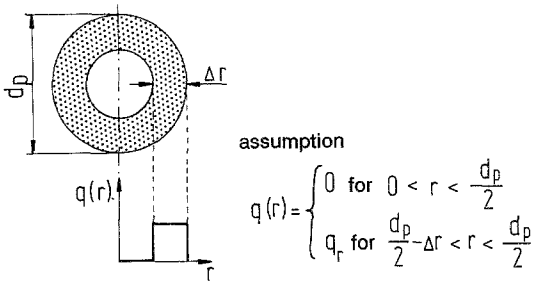

Fig. 4. Assumption to determine the penetration depth of lipase in Fractogel TSK butyl 650.

effect is the result of steric hindrance, because the ratio of the pore diameter of the adsorbent to the lipase diameter is less than 10.

Another observation, which is in accordance with the above-mentioned feature, is depicted for one experiment in Fig. 5. A total volume of 8 ml of fermentation broth is brought into contact with a known amount of adsorbent in aliquots of different volumes, 8 × 1 ml, 4 × 2 ml, 2 × 4 ml or 1 × 8 ml. After adsorption each aliquot is replaced by fresh fermentation medium. The total adsorption time in all experiments is 8 h. As expected, the total amount of adsorbed enzyme is different, but also the equilibrium curves do not coincide (especially for larger volume aliquots). The addition of larger volumes of fermentation broth again prevents penetration of lipase into the inner part of the adsorbent because of steric hindrance, based on a kinetic effect. With smaller volumes of supernatant the concentration in the fluid phase decreases more rapidly and the lipase

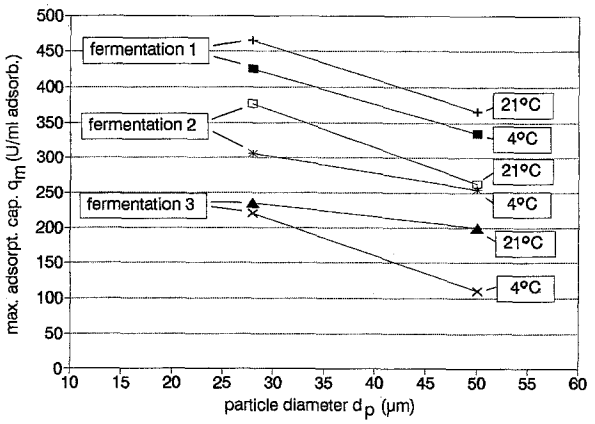

Fig. 3. Influence of the adsorbent particle size for different temperatures and fermentations on the adsorption capacity.

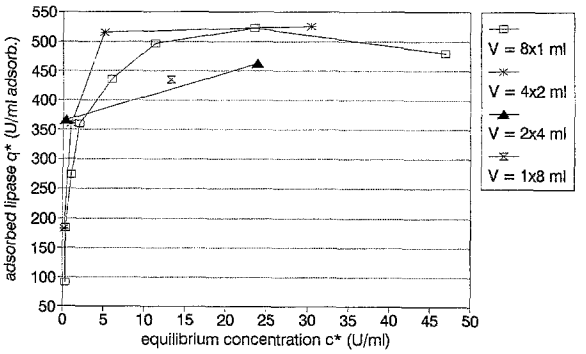

Fig. 5. Influence of the volume of supernatant in contact with the adsorbent on the equilibrium curves (see text).

molecules are allowed to get deeper into the particles. The same effect is observed when the adsorption is carried out in a column. A comparison of theoretical considerations [12] with measurements of breakthrough curves in a column at different bed heights showed that with increasing superficial fluid velocity (which corresponds to a larger fermentation broth volume in the above-specified experiment) the adsorbent in the column behaves more and more like a non-porous particle. This observation is identical to a diminution of the penetration depth, Δr.

The equilibrium in the lower concentration field in Fig. 5 is shifted in favour of adsorbed lipase molecules with increasing fermentation broth aliquots. The proportion of end-on adsorbed lipase molecules, which have a disc-like shape [13] increases slightly because of the initially increased supply of lipase.

As reported elsewhere [14–17], in many cases the adsorption of proteins on solid surfaces is not totally reversible. The same is true for the adsorption of lipase on Fractogel TSK butyl 650. To determine the reversibility of the adsorption, the fermentation broth, which is in equilibrium with the adsorbent, is replaced by the same fermentation broth but with a lower content of lipase (Fig. 6). After reaching equilibrium again, this procedure is repeated several times. As can be seen from Fig. 6 the equilibrium curves for desorption show an often reported [18] hysteresis, even though the adsorption equilibrium curve is of the reversible type. Moreover, the desorption is highly influenced by the amount of originally adsorbed lipase. Whereas in the lower concentration range the adsorption seems to be nearly irreversible, in the upper part of the equilibrium curves the desorption is expected to be easier. This effect is the result of different orientations of the molecules in the adsorbed state. In this context it should be mentioned that the adsorption conditions, *e.g.* salt concentration or pH value, also have an influence on the desorption (experimental results are not shown). This is also due to different orientations. The often reported conformational changes of adsorbed macromolecules [19,20] are unlikely to occur with this lipase, because measurements showed that the length of time for which the enzymes are adsorbed has no significant influence on the desorption [21].

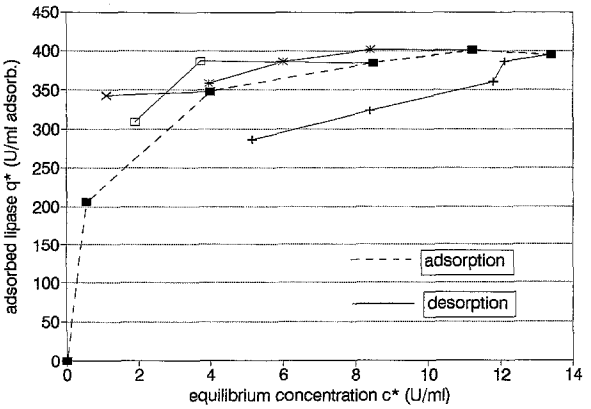

Fig. 6. Hysteresis effect of adsorption and desorption. The desorption begins at different points on the equilibrium curve for adsorption.

CONCLUSION

In contrast to analytical chromatography, in which only small samples are applied to a column and almost no interaction between the molecules occurs, in preparative chromatography (using large amounts of enzymes) during the course of the downstream processing the adsorption equilibrium is highly influenced by the compound of the fermentation broth. This may be because of competition for the adsorption sites (especially in the case of no affinity adsorption) and the varying extents of the often observed replacement of bound molecules [14].

The maximum in the temperature dependence of the adsorption as shown in Fig. 2 is the result of two opposing effects. At first the amount of adsorbed lipase increases with temperature because of the hydrophobic character of the interaction. At higher temperatures the thermal motion of the macromolecules increases and gives rise to a higher desorption rate, similar to the adsorption of gas [11]. Nevertheless, desorption should not be carried out a higher temperatures because of denaturation of the enzyme.

For a maximum exploitation of the adsorbent,

the particle radius has to be in the range of the penetration depth, Δr. However, this measure often coincides with a higher pressure drop when the adsorption takes place in a column.

The results show that the lipase could adsorb in different orientations, end-on and side-on, which is the reason for different desorption behaviours [14]. No conformational changes in the adsorbed lipase were discovered. This is a prerequisite in the purification of a fermentation broth if a high recovery is to be achieved.

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