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PII: S0144-8617(97)00098-2

Carbohydrate Polymers 34 (1997) 55–59

0144-8617/97/$17.00 + .00

Adsorption of α-amylase on dextrin immobilized on kieselguhr or chitin

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(Received 6 March 1996; revised version received 11 March 1997; accepted 4 April 1997)

Dextrin prepared from corn starch was immobilized on kieselguhr or chitin using a crosslinking reagent (epichlorohydrin), to produce a new adsorbent having a high affinity for α-amylase. The dextrins were immobilized at levels of 300–340 and 200–270 mg/g adsorbent on chitin and kieselguhr, respectively. There was an optimum crosslinking time for α-amylase adsorption because of a balance between the number of adsorption sites and the affinity of the immobilized dextrin for α-amylase. Adsorption of α-amylase on the crosslinked immobilized dextrin was as much as 50 times higher than the value on raw corn starch. When expressed in terms of the content the adsorption of α-amylase varied with the type of support (kieselguhr and chitin) because of the different state of the immobilized dextrin. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

In industrial separations of α-amylase from culture filtrates, various starches are used because of their specific affinity for α-amylase. Ethanol or sodium sulfate are added to aid adsorption but even so adsorption capacity is limited. For example, the adsorption of α-amylase on corn starch was about 44 000 DU/g with 30% v/v ethanol (Kurakake *et al.*, 1996). For industrial processes, an adsorbent with a high affinity for α-amylase without the requirement for an additional reagent such as ethanol is required. Additional requirements are ease of filtration and enzyme recovery from the adsorbent. Many studies have been undertaken to develop an adsorbent with a high affinity for α-amylase (Kasabou *et al.*, 1960; Komaki & Koizumi, 1964; Komaki, 1964; Komaki *et al.*, 1965; Rozie *et al.*, 1991). Recently, it has been reported that heat-moisture treated corn starch has a high affinity for α-amylase (Maruta *et al.*, 1994). In our study, using H-100, a commercial heat-moisture treated starch, high adsorption was found to depend on the residue that was resistant to α-amylase recovered after the addition of 30% v/v ethanol (Kurakake *et al.*, 1996). From this finding, dextrin may be considered to be a good adsorbent for α-amylase if its digestibility can be restricted.

In this study, we propose a new adsorbent with a high affinity for α-amylase by immobilization of dextrin on a support such as kieselguhr or chitin particles. Two types of dextrin (of different molecular size) were prepared by the partial enzymatic hydrolysis of corn starch. As shown in Fig. 1, it is proposed that the dissolved dextrin was precipitated and aggregated around the support by the gradual addition of ethanol, and then immobilized by crosslinking with epichlorohydrin. The preparation of

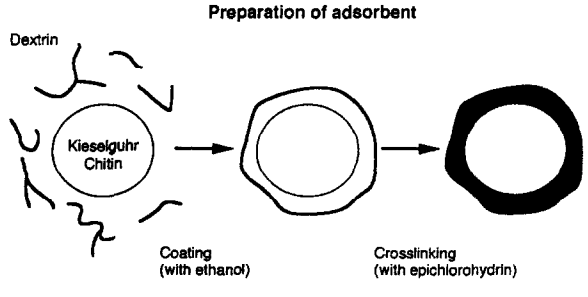

Fig. 1. Scheme for immobilization of dextrin on support.

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the new adsorbent and the adsorption of α-amylase are now described.

MATERIALS AND METHODS

Materials

Commercial α-amylase (Supitase HK, *Bacillus subtilis* origin) and glucoamylase (Supitase XL-4, *Aspergillus niger* origin) were supplied from Nagase Biochemicals Ltd. These were partially purified concentrates from culture filtrate. Sugar or sodium chloride (about 25% w/w) present in the enzyme preparation was removed by dialysis against 10 mM acetate buffer (pH 6) before the adsorption test. Chitin (crab origin) was purchased from Wako Chemicals Ltd.

Immobilization of dextrin on support using epichlorohydrin

Corn starch was liquefied by α-amylase (Supitase HK) at 90°C after passing through a jet-cooker at 105°C. The corn starch was hydrolyzed to DE 3 or DE 19% (DE = dextrose equivalent), and filtered and spray dried. The dextrins produced were termed DE 3 or DE 19, respectively. 10% w/v dextrin solution (6 ml) was boiled with 1 g of kieselguhr or chitin. After cooling to room temperature, ethanol (20 ml) was gradually added into the mixture with stirring when the support was coated with a layer of precipitated dextrin. The crosslinking reaction of dextrin was carried out with 5 M NaOH (3 ml) and epichlorohydrin (1.8 ml) at 40°C. At a given time, the alkaline mixture was neutralized by 2 M HCl (7.5 ml) to stop the crosslinking reaction. The dextrin immobilized on the support was washed with 40 ml water (four times), 40 ml acetone (twice) and 30 ml ether (once) and vacuum-dried. The prepared adsorbent was termed DE 3-C or DE 3-K for DE 3 dextrin immobilized on chitin or kieselguhr, respectively.

Adsorption of α-amylase

200 mg adsorbent (4% w/v) and 8240–10 500 DU/ml α-amylase solution (total volume 5 ml) were suspended at 4°C and pH 6 for 30 min. One dextrinogenic unit (DU) was defined as the amount of α-amylase that decreased the blue value by 1% in 1 min. After centrifuging at 1500 g for 10 min, α-amylase activity in the supernatant solution was measured. The amount of adsorbed enzyme was calculated from the activities in the liquid phase before and after adsorption. The amount of dextrin digested in the adsorption process was calculated from the concentration of the dissolved dextrin in the supernatant, as measured by the phenol-sulfuric acid method (Dubois *et al.*, 1956).

Assay of enzyme activities

α-amylase

The enzyme sample (1 ml) was incubated with 1% gelatinized potato starch solution (10 ml) at pH 6 and 40°C for 10 min. 1 ml 2 M HCl was added to the mixture (total volume, 12 ml) to stop the reaction. An aliquot (0.1 ml) was mixed with 10 ml I_2/KI solution and the absorbance at 660 nm (blue value) was measured after 2 min.

Glucoamylase

The enzyme sample (0.1 ml) was incubated with 1% soluble starch solution at pH 5 and 40°C for 10 min (total volume 1 ml). 1 M sodium carbonate (0.5 ml) was added to the mixture to stop the reaction. The glucose produced was determined by a glucose oxidase/peroxidase method (Glucostat kit, Eiken Chemical Ltd). One unit was defined as the amount of glucoamylase that produced 1 μmol of glucose in 1 min.

RESULTS AND DISCUSSION

Immobilization of dextrin on kieselguhr or chitin

As shown in Fig. 2, the immobilization of dextrin was almost completed after crosslinking for more than 3 h. 300–340 mg DE 3 and 200–270 mg DE 19 were immobilized per gram of support. The higher the molecular size of dextrin (DE 3 > DE 19), the more dextrin was immobilized. The immobilization varied with the nature of the support (kieselguhr or chitin). The amount of dextrin immobilized on the support was

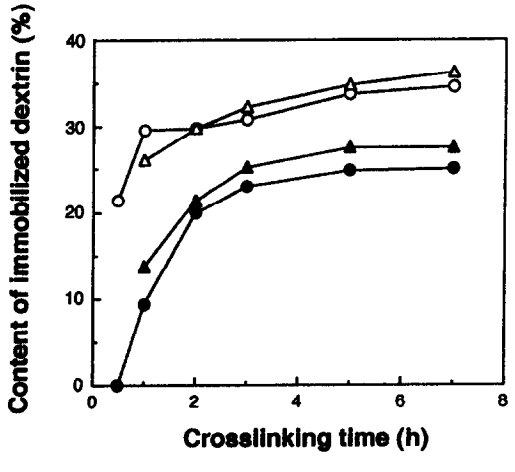

Fig. 2. Relationships between the amount of the immobilized dextrin on the support and the crosslinking time: ○, DE 3-K; ●, DE 19-K; △, DE 3-C; ▲, DE 19-C. The crosslinking reaction was carried out at 40°C with 3 ml epichlorohydrin/g dextrin.

estimated from dry weight (support and immobilized dextrin) after immobilization.

Observation by light microscopy after staining with Direct Sky Blue showed that DE 3 was almost homogeneously immobilized on kieselguhr particles, doubling the diameter from the initial value of 10–20 μm. The larger chitin particles (200–300 μm diameter) were also uniformly coated by DE 3, giving a thin layer.

Adsorption of α-amylase on the immobilized dextrin

Figure 3 shows the relationship between the adsorption of α-amylase on 1 g of the prepared adsorbent and crosslinking time. Adsorption of α-amylase on DE 3- and DE 19-coated kieselguhr was maximal after 3 and 5 h crosslinking time, respectively. The adsorption of α-amylase was very large in both cases. When chitin was used as a support, α-amylase adsorption was optimal at a crosslinking time of 5 h for both DE 3 and DE 19.

Figure 4(A) presents changes in the α-amylase digestibility of the immobilized dextrin and the amount of the residual dextrin after the adsorption process as a function of the crosslinking time (0–5 h), where DE 3-C was used. The α-amylase digestibility of the immobilized dextrin decreased with increasing crosslinking time and the amount of residual dextrin increased. This phenomenon was also observed for dextrins immobilized on kieselguhr (data not shown).

The apparent amounts of α-amylase adsorbed on the dextrin region of the adsorbent were calculated from the data in Fig. 4(A). The amount of dextrin in the adsorbent was measured by enzymatic digestion. As shown in Fig. 4(B), the adsorption was very large (more than 0.4×10^6 DU/g dextrin) and greatly decreased with increasing crosslinking time.

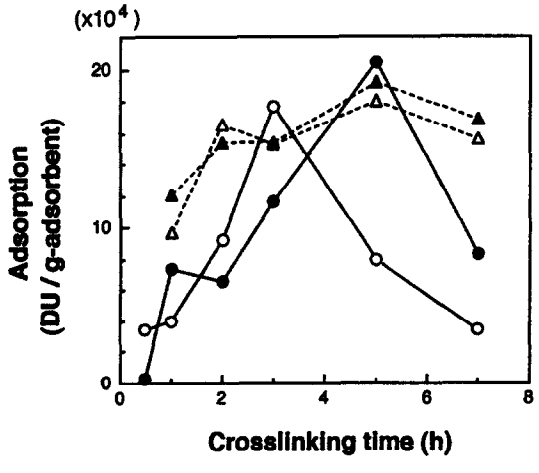

Fig. 3. Relationships between the amount of the adsorbed α-amylase per 1 g adsorbent and the crosslinking time: ○, DE 3-K immobilized on kieselguhr; ●, DE 19-K immobilized on kieselguhr; △, DE 3-C immobilized on chitin; ▲, DE 19-C immobilized on chitin. 4% w/v adsorbent was suspended with 8240 (for DE 3-K and DE 19-K) or 10 500 (for DE 3-C and DE 19-C) DU/ml α-amylase at 4°C for 30 min.

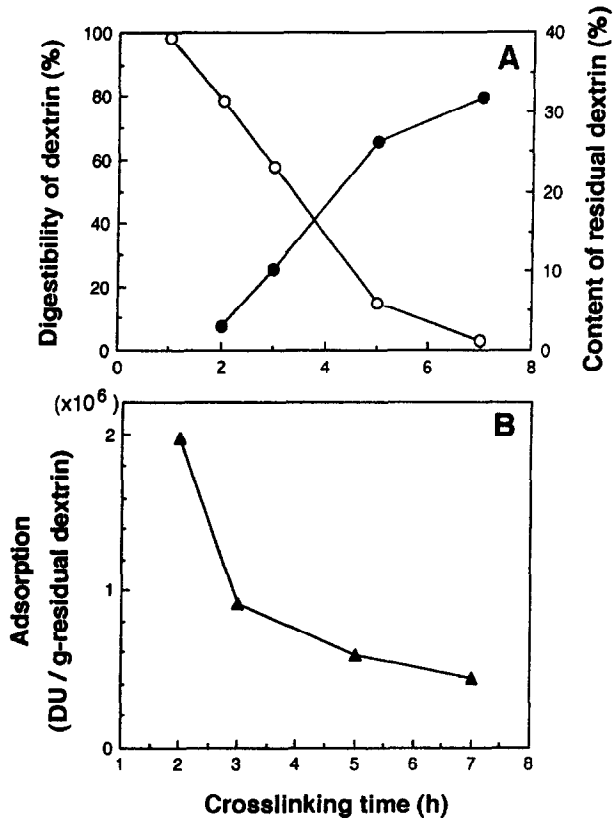

Fig. 4. Digestibility, residual amount and adsorption of the immobilized dextrins (DE 3-C) prepared at several crosslinking times. A: ○, digestibility; ●, residual amount during hydrolysis. B: ▲, adsorption. 4% w/v adsorbent was suspended with 10 500 DU/ml α-amylase at 4°C for 30 min.

If the degree of the crosslinking is smaller, the adsorption on the crosslinked dextrin is greater but many adsorption sites are digested. On the other hand, if the degree of crosslinking is larger, the digestion of dextrin by α-amylase is depressed and its affinity for α-amylase is reduced. The optimal crosslinking time for α-amylase adsorption can be understood in terms of a balance between the number of adsorption sites and the affinity for α-amylase.

The optimal crosslinking time of DE 19 was longer than that of DE 3 for immobilization on kieselguhr because of its smaller molecular size. In the case of chitin, the adsorption was largely independent of the crosslinking time over the range from 1 to 5 h. This difference between kieselguhr and chitin seems to be due to the state of the immobilized dextrin. In the case of a kieselguhr particle, the dextrin is immobilized around it, forming a spherical shape so that the inside portion is not involved in the adsorption of α-amylase. However, with a chitin particle, the dextrin adheres to its wider surface, forming a thin layer, which leads to a greater affinity for α-amylase. The immobilized dextrin

Table 1. Adsorption of α-amylase on the prepared adsorbents[a]

Adsorbents	Crosslinking time (h)	Carbohydrate content (%)	E_{ads}/E_0 (%)[b]	Digestibility (%)	Adsorption A[c] (1×10^4 DU/g)	Adsorption B[d] (1×10^4 DU/g)
Raw corn starch	—	100	8.44	1.80	1.80	1.83
DE 3-K (kieselguhr)	3	30.9	60.4	35.6	12.9	62.0
	7	34.6	13.0	3.38	3.40	6.92
DE 19-K (kieselguhr)	5	24.8	70.2	23.4	15.0	75.8
	7	25.1	31.0	11.0	8.31	30.9
DE 3-C (chitin)	5	34.8	85.2	10.1	18.2	58.1
	7	36.3	74.8	3.07	15.6	43.8
DE 19-C (chitin)	5	27.5	88.4	22.3	18.9	88.2
	7	27.6	80.5	5.23	16.8	63.5

[a]Conditions: 4% w/v adsorbent, 8240 (for DE 3-K and 19-K, 7 h), 10 500 (for DE 3-C and 19-C, 7 h) or 8530 (for others) DU/ml α-amylase, pH 6, 4°C, 30 min.
[b]E_{ads} = concentration of adsorbed enzyme; E_0 = initial concentration of enzyme.
[c]Adsorption on 1 g of adsorbent used.
[d]Adsorption on 1 g of dextrin in the adsorbent (the amount of dextrin was corrected by enzymatic digestion).

Table 2. Adsorption of glucoamylase on the prepared adsorbents[a]

Adsorbents	Crosslinking time (h)	Carbohydrate content (%)	E_{ads}/E_0 (%)[b]	Digestibility (%)	Adsorption A[c] (1×10^4 U/g)	Adsorption B[d] (1×10^4 U/g)
DE 19-K (kieselguhr)	5	24.8	24.3	3.21	1.34	5.52
DE 19-C (chitin)	5	27.5	6.88	2.19	0.38	1.39

[a]Conditions: 4% w/v adsorbent, 2180 U/ml glucoamylase, pH 6, 4°C, 30 min.
[b]E_{ads} = concentration of adsorbed enzyme; E_0 = initial concentration of enzyme.
[c]Adsorption on 1 g of adsorbent used.
[d]Adsorption on 1 g of dextrin in the adsorbent (the amount of dextrin was corrected by enzymatic digestion).

was effectively used for the adsorption of α-amylase. A covalent bond between chitin and dextrin through the crosslinking reagent can be also considered as one of factors in forming such a structure.

Evaluation of the crosslinked dextrin on adsorption of α-amylase

Table 1 presents a summary of adsorption on some adsorbents prepared at their optimal crosslinking times (3 or 5 h) and on raw corn starch. The adsorbents were much more effective for α-amylase than for raw corn starch; $12.9–18.9\times10^4$ DU/g adsorbent and $62.0–88.2\times10^4$ DU α-amylase/g dextrin on the support. Their adsorptions were so large that an adsorption aid like ethanol was not required. The adsorbed enzyme was completely released by incubating in 0.1 M acetate buffer (pH 6) at 60°C for 10 min (data is not shown). These properties of the adsorbents could be very significant for industrial use. Incidentally, when the enzyme adsorbed on the dextrin is dried without release from support, it can be used immediately as an enzyme preparation for liquefaction of starch. The support is recovered from the liquefied starch solution by filtration.

Thus the immobilized dextrin has a high affinity for α-amylase but the digestibility in adsorption was larger than that of raw corn starch. In the adsorption on the dextrin immobilized by crosslinking for 7 h (Table 1), DE 3-C and DE 19-C show small digestibility and large adsorption for α-amylase, although the adsorption was relatively small compared to those prepared at optimal crosslinking time (5 h). These adsorbents could be used also for recycling or affinity chromatography.

Table 2 presents the adsorption of glucoamylase using DE 19-C or DE 19-K. Raw corn starch, DE 3-C and DE 3-K could not adsorb glucoamylase. Kieselguhr is a better support for the adsorption of glucoamylase than chitin, contrary to the adsorption of α-amylase. DE 19-K seems to have more flexible non-reducing terminal groups, which glucoamylase can bind to in the manner of exo-amylase. However, the adsorption for glucoamylase was much smaller than that for α-amylase.

The overall results show that excellent adsorbents for α-amylase could be prepared by immobilizing dextrin on kieselguhr and chitin supports. The adsorbent has a higher affinity for α-amylase and does not need adsorption aids such as ethanol.

REFERENCES

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analyst. Chem.* **28,** 350–356.

Kasabou, T., Odaka, T. & Ohno, M. (1960) Production of a high affinity starch for amylase. Japanese Patent KOHO S35-15437.

Komaki, T. (1964) Adsorption method of α-amylase using modified starch. Japanese Patent 685948.

Komaki, T. & Koizumi, K. (1964) Adsorption method of α-amylase by modified starch. Japanese Patent 654842.

Komaki, T., Koizumi, K., Tsubouchi, H. & Matsuba, Y. (1965) Method of adsorption of α-amylase. Japanese Patent 685949.

Kurakake, M., Tachibana, Y., Masaki, K. & Komaki, T. (1996) Adsorption of α-amylase on heat-moisture treated starch. *Journal of Cereal Chemistry* **23,** 163–168.

Maruta, I., Kurahashi, Y., Takano, R., Hayasi, K., Yoshino, Z., Komaki, T. and Hara, S. (1994) Reduced-pressurized heat-moisture treatment: a new method for heat-moisture treatment of starch. *Starch/Stärke* **46**(5), 177–181.

Rozie, H., Somers, W., Riet, K.V., Rombouts, F.M. and Visser, J. (1991) Crosslinked potato starch as an affinity adsorbent for bacterial α-amylase. *Carbohydr. Polym.* **15,** 349–365.