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Spray-drying of trypsin — surface characterisation and activity preservation

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Abstract

In the present study trypsin mixed with various carbohydrates, i.e. lactose, sucrose, mannitol, α-cyclodextrin and dextrin, was spray-dried in order to investigate the effects of spray-drying on this enzyme, with particular emphasis on the effects of interactions between trypsin and the surface formed during spray-drying. The protein was strongly over-represented at the surface of the powder particles, the surface coverage ranging from 10 to 65%, depending on the amount of trypsin in the solids (0.2–5%). This indicates that the protein adsorbs at the air/liquid interface of the spray-droplets, and that this surface is also largely preserved after drying. The surface concentration of protein in the spray-dried powders could be controlled by adding a surfactant to the mixture before drying, since the surfactant adsorbs preferentially at the air/liquid interface of the spray droplets, thus expelling protein from the surface. In general, the residual activity of trypsin in these non-optimised formulations was 90% or higher, and in no case less than 82%. It was found that the loss of activity could partly be explained by inactivation of the protein adsorbed at the surface. For mannitol and sucrose, however, the level of inactivation was higher than could be explained by surface inactivation alone, and additional mechanisms must also be considered.

Keywords: Spray-drying; Trypsin; Surface analysis; Electron spectroscopy for chemical analysis (ESCA); Activity preservation

1. Introduction

Spray-drying is commonly used in the pharmaceutical industry for various substances, such as antibiotics, vitamins, vaccines, enzymes, plasma, plasma substitutes and excipients (Masters, 1991; Broadhead et al., 1992), as well as for preparation of microcapsules and slow-release formulations (Deasy, 1984; Bodmeier and Chen, 1988; Giunchedi and Conte, 1995). In a few cases spray-drying has been evaluated for drying of bioactive proteins with varying success (Werner et al., 1993; Broadhead et al., 1994; Mumenthaler et al., 1994; Maa et al., 1997; Chan and Gonda, 1998; Maa et al., 1998), while it has been used more as a routine

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drying method by other groups (Hampel et al., 1994; Lefranc-Millot et al., 1996).

Spray-drying is often regarded as a 'harsh' drying method, due to the high temperature of the drying gas, which potentially could be detrimental to sensitive biological materials. However, considering the drying process in more detail, it is evident that the spray droplets and the dried powder particles will maintain a temperature well below the inlet temperature of the drying gas throughout the process. As long as water evaporates form the droplets, a cooling effect will be achieved, which prevents the temperature of the drying material to rise above the wet bulb temperature of the drying air. Only in the last stages of drying, when the water activity of the drying material is low, can the temperature of the particles rise above this temperature, but will still remain below the temperature of the air leaving the spray-dryer (typically 15–20°C lower) (Masters, 1991). In a typical spray-drying procedure for pharmaceutical materials, the inlet temperature is 180°C and the outlet temperature is 55–60°C, indicating that the powder temperature is in the order of 40–45°C when leaving the spray-dryer (Masters, 1991). Furthermore, the drying time is in the order of milliseconds, and generally the total residence time in the spray-dryer is in the order of seconds (Masters, 1991; Linders et al., 1996). From these arguments, extensive thermal damage to the protein seems less likely.

Drying in general may also alter the secondary structure of the protein (Prestrelski et al., 1993; Griebenow and Klibanov, 1995), since the hydration of the protein is partly lost. By replacing water with excipients, e.g. sucrose or lactose, which are capable of forming hydrogen bonds with the protein, this effect can largely be compensated for (Carpenter and Crowe, 1989). Other sources of loss of protein structure, and thus activity, are the shear forces in the spray nozzle, as well as protein adsorption at the droplet surface (Maa et al., 1998). Relating to the latter of these, adsorbed proteins change their conformation to an extent that depends on numerous factors, including the protein structural stability, pH, electrolyte concentration, and notably, the protein-surface interaction. Frequently, but not always, this interfacial conformational change (e.g. loss of α-helix content) is accompanied by a reduction of the biological activity (Kondo et al., 1993; Kondo et al., 1996). Since the air/water interface is a distinct one, to which numerous proteins have been found to adsorb (Paulsson and Dejmek, 1992; Tripp et al., 1995), one could also expect spray-drying to be detrimental to the activity of spray-dried enzymes and other proteins. If this is the case, minimising the surface accumulation of the protein in the spray droplets should suppress the activity loss in the drying process. Indeed it was previously shown that the stability and integrity in spray-drying of a surface sensitive protein, recombinant human growth hormone (rhGH), could be improved by adding zinc (a known stabiliser of this protein) and a surfactant (Maa et al., 1998), the latter reducing the effects of the interactions between the protein and the air/liquid interface.

Considering the probable importance of protein adsorption on retained activity after spray-drying, the possibility of at least partial control this surface accumulation, e.g. by surfactant addition, as well as the potential of spray-drying as an alternative to other drying techniques in a range of biomedical applications, the objective of the present investigation was to study the surface accumulation of a model protein (trypsin) during spray-drying, to correlate this with findings of retained activity, and to see whether surfactants may be used to reduce the surface accumulation of the protein, and hence the activity loss after the spray-drying procedure.

2. Materials and methods

2.1. Materials

Crystalline porcine trypsin 4500 K (5300 and 5000 USP/mg, respectively) was obtained from Novo Nordisk A/S (Bagsværd, Denmark). Mannitol was obtained from Merck (Darmstadt, Germany), lactose from BDH (Poole, UK), and sucrose from Kebo (Stockholm, Sweden). Dextrin and α-cyclodextrin were purchased from Fluka (Buchs, Switzerland). Tween 80 was obtained

from ICI Surfactants (Cleveland, UK). *N*-α-benzoyl-L-arginine ethyl ester (BAEE) was purchased from Sigma (St. Louis, MO). Other chemicals were of the highest purity available. Doubly distilled water was used throughout.

2.2. Preparation of solutions

Solutions of lactose, sucrose, mannitol, α-cyclodextrin and dextrin were prepared to the desired concentration and pH was adjusted to 7.0 using NaOH. An appropriate amount of trypsin was dissolved in the carbohydrate solutions to give proportions of trypsin to excipient of 0.2/99.8, 1/99, and 5/95 (w/w), with a total solids content of 10% by weight. Tween 80 was added to one series of samples containing trypsin and lactose to give a final content of 0.1% Tween 80 in the solids, which corresponds to 75 times the cmc for Tween 80 in the solution at 25°C (Becher, 1966). Since trypsin is a self-digesting protein, the solutions were prepared immediately before drying. No effects of hydrolysis of trypsin were observed.

2.3. Spray-drying of the solutions

Trypsin/carbohydrate solutions were spray-dried in a laboratory spray-dryer built at the Institute for Surface Chemistry. The dimensions of the drying chamber are 0.5×0.15 m. The spray dryer operates concurrently and has a two-fluid spray-nozzle with an orifice 1 mm in diameter. In the present preparations the inlet gas temperature was 180°C while the outlet gas temperature was kept between 65 and 70°C. Liquid feed to the dryer was 4.6 ml/min, while the flow of drying air was 0.8 m^3/min. The dried powder was collected in a cyclone at the outlet, yielding approximately 6 g of powder per 100 ml of solution with 10% solids. The powders were stored dry at room temperature in closed containers.

2.4. Freeze-drying

Solutions containing 1/99 trypsin/carbohydrate (total solids 10% w/w) were freeze-dried to produce powders for a comparison of spray-drying and freeze-drying. In order to be able to perform controlled freeze-drying experiments, the sample tubes were fitted in an aluminium block acting as stable heat reservoir, with a low volatility vacuum oil between the block and the tubes to ensure close contact (Millqvist-Fureby et al., 1999). For fast freezing of samples, the block and tubes were pre-cooled to -28°C, and thereafter a small volume (300 μl) of the solution was added to each tube. The observable freezing during such a procedure was essentially instantaneous. The freeze-drying was started from -28°C at $6–8 \times 10^{-2}$ mbar, resulting in a large part of the primary drying (≈ 3 h) occurring at about -34°C.

2.5. Electron spectroscopy for chemical analysis

Electron spectroscopy for chemical analysis (ESCA) was used to probe the elemental composition of the powder surfaces with an analysis depth of less than 100 Å. Using this method, the elemental composition of the powder surface can be analysed (Fäldt et al., 1993). Previous studies have shown that the surface of spray-dried protein/carbohydrate powders to a large extent is covered by protein, even when small protein concentrations are used (Fäldt and Bergenståhl, 1994; Landström et al., 1999). Relating these results to the surface tension of the corresponding protein solutions, it was inferred that the powder surface reflects the composition of the air/liquid interface of the spray droplets (Fäldt and Bergenståhl, 1994).

The ESCA measurements were performed with an AXIS HS photoelectron spectrometer (Kratos Analytical, UK). The instrument uses a monochromatic Al Kα X-ray source. The pressure in the vacuum chamber during analysis was less than 10^{-7} Torr. In the present investigation, a take-off angle of the photoelectrons perpendicular to the sample holder was used throughout. The area analysed consisted of a circular region of an approximate diameter of 1.3 mm, and three measurements were made for each sample. The standard deviation in the measurements was 1.8% units. The surface coverage of trypsin was calculated from the nitrogen content of pure trypsin, as measured by ESCA, and the nitrogen content of the spray-dried and freeze-dried samples.

2.6. Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed with a Mettler Toledo DSC821 instrument. Throughout, helium was used as the purge gas at 40 ml/min. The lid of the aluminium pans was pierced just before analysis, allowing desorbed water to leave the pan. Typically, a heating rate of 20°C/min was used. Sample weights were 1–5 mg. The crystallinity of the carbohydrates was estimated as the measured enthalpy of fusion divided by the enthalpy of fusion for complete crystallisation (taken from the literature), taking the fraction of the carbohydrate in the total solids into account.

2.7. Measurement of surface tension

The surface tension at the air/water interface of protein solutions was determined according to the Wilhelmy plate method using a Sigma 70 tensiometer (KSV Instruments, Finland). The equilibrium time for surface tension measurements was 12–15 min. The measurements were reproducible within 0.5 mN/m.

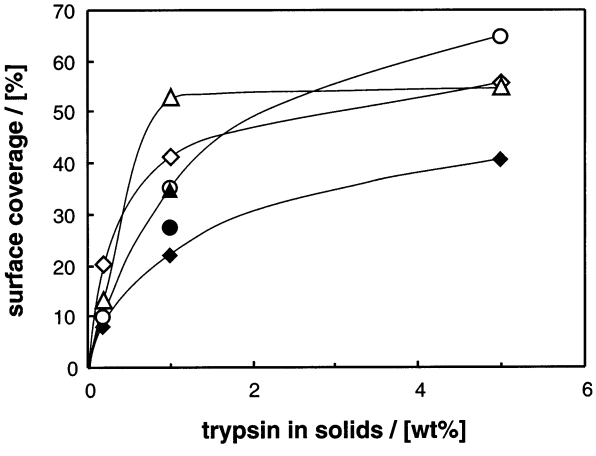

Fig. 1. ESCA estimate of the surface coverage of trypsin on powders prepared with different carbohydrate excipients as a function of trypsin concentration of the spray-dried solution. (◇) Lactose, (△) sucrose, (○) dextrin, (▲) mannitol, (●) α-cyclodextrin and (◆) lactose/Tween 80.

2.8. Trypsin activity assay

The enzymatic activity of resolubilized trypsin was determined according to a modified version of a method developed by Schwert and Takenaka (1955). Powders containing trypsin were dissolved in 67 mM sodium phosphate buffer pH 8.0 (10 μg trypsin/ml) and the samples were kept on ice in order to reduce trypsin self-digestion. A total of 0.25 mM *N*-α-benzoyl arginine ethyl ester (BAEE) in 67 mM sodium phosphate buffer pH 8.0 (1 ml) was hydrolysed with trypsin at 25°C (100 μl ≈ 1 μg trypsin), and the progress of the reaction was followed spectrophotometrically at 235 nm, using a Perkin-Elmer UV-VIS Spectrometer. The specific activity was calculated in BAEE-units/mg trypsin, 1 BAEE-unit corresponding to 0.001 ΔA/min, and 270 BAEE-units being equivalent to 1 U (1 U = 1 μmol BAEE/min). The activity of untreated trypsin was measured using the same procedure, resulting in a specific activity of 27×10^3 BAEE-units/mg trypsin, and this value was used as a reference value for 100% retained activity. The values reported are the averages of at least two measurements and the standard deviation was 2% units.

3. Results and discussion

3.1. Surface coverage of trypsin in spray-dried powders

The results from the ESCA analysis of the surface coverage of trypsin on the spray-dried powders are presented in Fig. 1. It can be seen that the surface accumulation of trypsin is very high, over 100 times for the lowest trypsin content. However, proteins in general have a high tendency to accumulate at the air/water interface whereas this is generally not the case for carbohydrates. This is shown for trypsin in water and 10% lactose solution by the decreasing surface tension at the air/water interface with increasing trypsin concentration (Fig. 2). Surface enrichment of protein is thus expected for the spray droplets, even though the rapid drying kinetics in spray-drying provide a different situation than the equi-

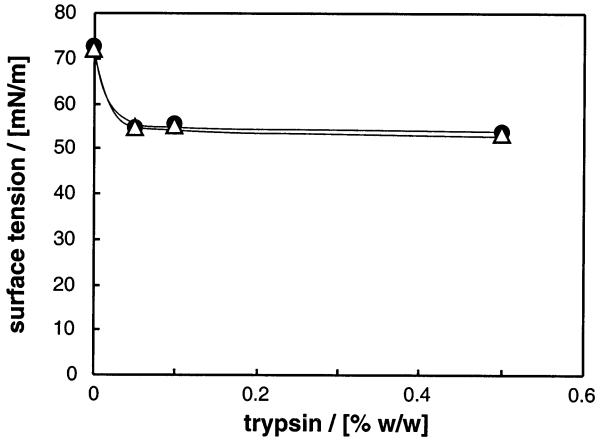

Fig. 2. Surface tension of trypsin solutions as a function of the trypsin concentration. (●) Trypsin dissolved in water, and (△) trypsin dissolved in 10% w/w lactose solution.

librium conditions during, e.g. surface tension measurements. Apparently, the interfacial accumulation at the droplets is preserved also after the solvent evaporation. These results agree with previous findings of the surface accumulation of other spray-dried protein/carbohydrate mixtures (Fäldt and Bergenståhl, 1994, 1996; Landström et al., 1999). It therefore seems plausible that the protein surface accumulation is a general phenomenon, and not particular to specific proteins. The degree of surface accumulation of trypsin varied with the different carbohydrates (Fig. 1). These differences may be due to specific interactions between the protein and the different sugars. The viscosity of the drying liquid could, in principle, be expected to constitute an important factor in determining the surface coverage of protein due to the effects on the transport rate in the spray-droplets. It has been shown that viscosity differences become significant for the surface composition when there is a large difference in viscosity in the spray-drying solutions, but not at moderate viscosity differences (Fäldt et al., 1998). In the present cases there are only small differences in viscosity between the various carbohydrates, which thus can be neglected. Note that at 5% trypsin in the powder, dextrin as carrier material, shows the highest trypsin coverage, despite the fact that this carbohydrate would yield the highest viscosity. The high surface coverage of the sucrose powder at 1% trypsin (56%) may possibly be due to sucrose crystallising during drying, but further investigations are required to clarify this matter.

In the presence of a surfactant, the surface coverage of trypsin is reduced by 30–75%, depending on the overall trypsin concentration (Fig. 1). This indicates a competitive adsorption process at the air/water interface in the spray-droplets, where the surfactant is more efficient in adsorbing at the interface. Since the time scale in spray-drying is very short (order of milliseconds) (Masters, 1991; Linders et al., 1996), it is likely that an equilibrium situation will not be reached. Nevertheless, it is interesting to compare these findings with those on competitive protein-surfactant adsorption at other surfaces. In numerous studies it has been found that not only are a wide range of surfactants able to reduce or eliminate protein adsorption in a competitive adsorption situation, but also numerous surfactants are able to desorb pre-adsorbed proteins (Arnebrant and Wahlgren, 1995; Karlsson et al., 1996; Rinella et al., 1998). Indeed, this is an important mechanism in everyday detergency applications. Given also that the adsorption kinetics of surfactants typically are much faster than for proteins (Wahlgren and Arnebrant, 1991; Tiberg et al., 1994; Brinck et al., 1998; Malmsten, 1998), a (partial) blocking of the protein adsorption by the surfactants is expected (see also discussion below).

3.2. Activity of trypsin after spray-drying

In order to evaluate the effects of the drying procedure on the protein, the catalytic activity was assayed after redissolution, and the results are presented in Fig. 3. For the spray-dried trypsin preparations, the residual activity reached a plateau when the trypsin content was 1% or higher. Sucrose and mannitol were less efficient than the other carbohydrates in preserving the trypsin activity, however, even in the worst case (1/99 trypsin/mannitol) the residual activity was above 80%. Considering the well-known sensitivity of trypsin in immobilisation, in particular involving adsorption at surfaces (Kondo et al.,

1993), this is a surprisingly high level of residual activity. Interestingly, the addition of Tween 80 to the drying liquid improved the residual activity of trypsin at all levels of trypsin in the powder.

As discussed above, one mechanism for the loss of activity observed could be interaction of the protein with air/liquid interface of the spray droplet. This is indicated by the positive effect on the residual activity of adding a surfactant, since this reduced the surface coverage of trypsin, and hence the possible surface-induced conformational changes in the protein. Such interactions have been put forward as the main reason for loss of activity for recombinant human growth hormone (Maa et al., 1998), and the problem was alleviated by adding a surfactant (Polysorbate 20) and a protein stabilising agent (zinc). However, care should be taken when using surfactants in protein solutions, since these may also denature proteins (cf. SDS–PAGE). Particularly anionic, but also cationic, surfactants are efficient in causing protein denaturation through binding to the protein and formation of protein-surfactant complexes (Ananthapadmanabhan, 1992; Creagh et al., 1993). However, since non-ionic surfactants have been shown to display only very weak tendency for such complex formation, they could be expected to interact with the protein in the spray-drying process largely through competitive adsorption, in analogy to the behaviour of protein-non-ionic surfactant mixtures (Ananthapadmanabhan, 1992; Wahlgren et al., 1998). Non-ionic surfactants therefore seem suitable for activity preservation of spray-dried proteins, although some care has to be taken, e.g. the reversed temperature dependent solvency typically displayed by some of these surfactants (Mitchell et al., 1983; Schick, 1987; Lindman et al., 1990).

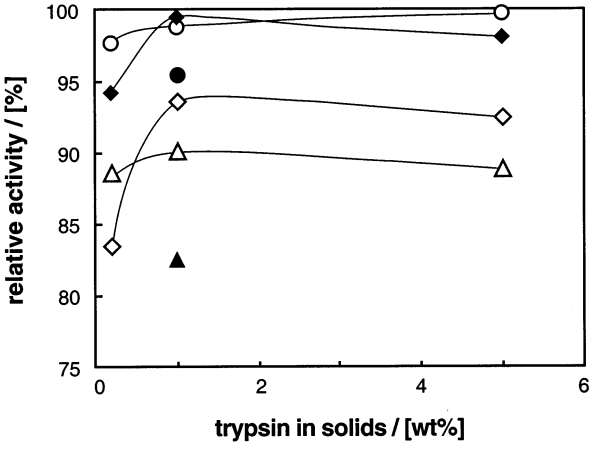

Fig. 3. The relative activity of trypsin after spray-drying with various excipients. The activity of the native trypsin corresponds to 100%. (◇) Lactose, (△) sucrose, (○) dextrin, (▲) mannitol, (●) α-cyclodextrin, and (◆) lactose/Tween 80.

The amount of the total trypsin that is present in the surface layer can be estimated from the ESCA analysis and particle size analysis. A first-order estimate yields that the analysed surface layer in ESCA for powder particles is in the order of 5 nm, considering the escape depth for photoelectrons in organic polymers and the spherical geometry of the particle surface (Fäldt et al., 1993). The particle diameter of the particles is estimated from SEM micrographs to be 6–9 μm (not shown). Under these assumptions about 4% of the total trypsin is present in the surface layer for the highest trypsin content, while the corresponding value for the lowest trypsin content is ~28%. These estimates show that an essentially negligible amount of the total protein is present at the surface at the highest bulk concentration, while a significant amount is present at the surface for the lowest bulk concentration. These results compare favourably with other measurements of protein content in the surface layer of spray-dried protein/carbohydrate particles (Landström et al., 1999).

Thus, once more, there is a clear correlation between the degree of protein surface accumulation on one hand, and the retained activity on the other. Accordingly, with an increasing protein concentration present in the drying solution, a decreasing fraction of protein will be present at the surface (assuming a reasonable and typically observed shape of the adsorption isotherm (Fäldt and Bergenståhl, 1994; Landström et al., 1999)). If the protein molecules in the proximity of the surface retain roughly the same activity, an increasing protein concentration in the drying solution will therefore result in an effectively increased retained activity of the dissolved powder, since a smaller fraction of the total protein is present in

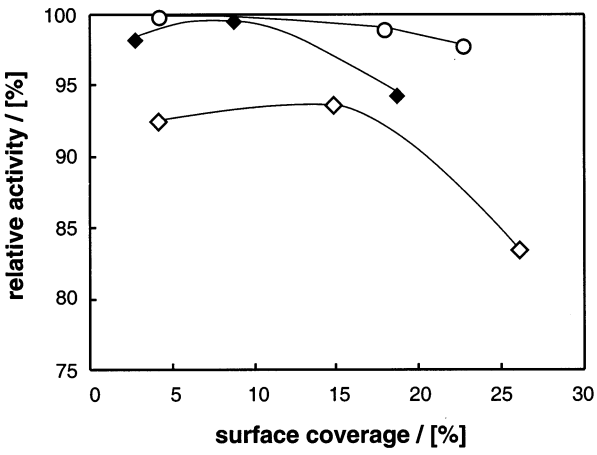

Fig. 4. Relative activity of trypsin after spray-drying as a function of the fraction of the estimated total trypsin present in the surface layer of the powder. (◇) Lactose, (○) dextrin, and (◆) lactose/Tween 80.

the surface layer. A clear correlation of the activity with the fraction of total trypsin present in the surface layer is shown in Fig. 4. The results suggest that the interactions with the surface may account for at least part of the activity loss during spray-drying. However, considering the fraction of total trypsin present in the surface layer, a significant portion of the 'surface protein' is still active in most cases. Thus, the effect cannot only be understood from surface accumulation related effects. In particular, there are considerable variations between different carbohydrates. This is most pronounced in the case of mannitol, for which total inactivation of the protein in the surface layer (corresponding to an activity loss of ~9%) does not correspond to the measured level of loss of activity (19%); hence additional mechanisms must also be at play reducing the activity of trypsin.

For comparison, trypsin was freeze-dried in combination with the same carbohydrates as used for the spray-drying experiments in 1/99 trypsin/carbohydrate ratio. The residual activity of trypsin after freeze-drying was analysed together with the surface composition of the powders, and the results are collected in Table 1. A comparison with the residual activity of the spray-dried samples shows that the activity was better preserved by spray-drying than by freeze-drying, a somewhat unexpected result. Although spray-drying and freeze-drying are two entirely different techniques, these findings, particularly regarding the effect of the different carbohydrates, strongly suggest that the enrichment of trypsin at the powder surface of the spray-dried preparations is not the only mechanism of activity loss. Freeze-drying of trypsin is more extensively described elsewhere (Millqvist-Fureby et al., 1999).

3.3. Effects of carbohydrates

The crystallinity of the spray-dried powders was analysed by differential scanning calorimetry (DSC). The results showed that in the spray-dried powders lactose, α-cyclodextrin and dextrin were virtually amorphous in all samples, while sucrose showed varying degrees of crystallinity and mannitol was highly crystalline (Table 2). Correlation of these results with the results on residual activity suggests that the crystallinity of the carbohydrate

Table 1
Surface coverage of trypsin and residual activity of trypsin in freeze-dried trypsin/carbohydrate (1/99 w/w) powders

Excipient	Freeze-dried powders		Spray-dried powders	
	Trypsin coverage (%)	Residual activity (%)	Trypsin coverage (%)	Residual activity (%)
Lactose	<2	81	41	94
Lactose/Tween 80	2	65	22	99
Sucrose	2	63	53	90
Mannitol	10	73	35	82
α-Cyclodextrin	3	92	27	95
Dextrin, 15 DE	6	74	35	99

Table 2
Differential scanning calorimetry analysis of the spray-dried trypsin/carbohydrate powders[a]

Excipient	Crystallinity (%)
Lactose, 0.2%, 1%, 5% trypsin	Amorphous
Lactose/Tween 80, 0.2%, 1%, 5% trypsin	Amorphous
Sucrose, 0.2% trypsin	41
Sucrose, 1% trypsin	86
Sucrose, 5% trypsin	41
Mannitol, 1% trypsin	85
α-Cyclodextrin, 1% trypsin	Amorphous
Dextrin, 0.2%, 1%, 5% trypsin	Amorphous

[a] The crystallinity is calculated form the heat of fusion for the respective carbohydrates. ΔH_{melt}(sucrose) = 132 J/g (Te Booy et al., 1992), ΔH_{melt}(mannitol) = 300 J/g (Barone et al., 1990).

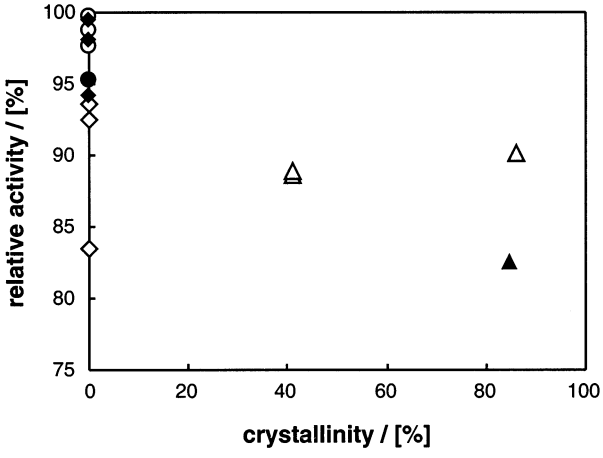

Fig. 5. The relative activity of trypsin after spray-drying as a function of the crystallinity of the carbohydrate excipient. (◇) Lactose, (△) sucrose, (○) dextrin, (▲) mannitol, (●) α-cyclodextrin, and (◆) lactose/Tween 80.

has a detrimental effect on the trypsin activity, while the amorphous carbohydrates preserve the protein structure and activity better (Fig. 5). Studies have shown that activity of freeze-dried enzymes is lost when the carbohydrate excipient crystallises (Izutsu et al., 1994; Mazzobre et al., 1997), and that the activity retention is improved by delaying the crystallisation of the excipient by adding suitable non-crystallising carbohydrates (Mazzobre et al., 1997). The crystalline state of sucrose may also explain the high surface coverage of trypsin on the trypsin/sucrose 1/99 powders: the crystallisation of sucrose will presumably result in a phase-separation (Sun and Davidson, 1998), which is expected to expel trypsin from the carbohydrate matrix, which may result in an increased surface accumulation of trypsin. On the other hand, mannitol is present as a highly crystalline material, and yet, the trypsin coverage in this case is lower than for sucrose.

From the results collected in this investigation it can be inferred that the nature of the carbohydrate excipient has a profound effect on the preservation of the enzyme during drying. It is known that proteins may alter their three-dimensional structure upon drying, in particular the α-helix content typically decreases while the content of β-sheet increases (Prestrelski et al., 1993; Griebenow and Klibanov, 1995). Such changes in structure may or may not be reversible upon rehydration. Two major mechanisms have been put forward that can (at least in part) explain the stabilising effects of different carbohydrates: (i) carbohydrates replacing water in interactions with the protein and (ii) carbohydrates providing a glassy matrix.

The 'water replacement' hypothesis states that in order to preserve the native structure of a protein, the hydrogen bonds formed between the protein and water molecules in an aqueous solution need to be replaced by new hydrogen bonds in the dry state. Carbohydrates have indeed been shown to form hydrogen bonds with proteins in the solid state (Carpenter and Crowe, 1989; Prestrelski et al., 1993). The 'vitrification' hypothesis states that it is essential to maintain the excipient in an amorphous, or vitreous, state, which will prevent the protein from changing its shape due to the rigidity of the matrix (Franks et al., 1991). Since protein denaturation generally involves conformational changes, this can be avoided when the protein is maintained fully immobilised in the matrix. An additional effect of adding an excipient is the 'dilution' effect, which reduces the possibility for protein molecules to interact with one another and form aggregates, which may occur in the solid state (Constantino et al., 1994). However, it is not straightforward to point out which of these three effects is the more important, or if

all effects are significant. Moreover, these theories do not take any surface effects into account, which were shown in the present study to account for at least part of the denaturation of the protein. Clearly, the role of both the carbohydrate identity and crystallisation propensity in spray-drying is complicated and at present somewhat unclear. Therefore, further investigations on these subjects are required.

4. Conclusions

It was found that the surface of powders produced by spray-drying protein/carbohydrate mixtures show a strong protein accumulation at the surface. This is most likely due to protein adsorbing to the air/water interface of the spray droplets prior to solvent evaporation, and this surface being preserved also in the dried material. The main, and somewhat unexpected, finding of the present investigation was that the activity of the model protein trypsin was well preserved with all the carbohydrates and protein concentrations tested. The level of protein accumulation at the powder surface could be controlled by adding a non-ionic surfactant. By addition of the surfactant the surface accumulation of protein was reduced and the residual activity after drying was increased. From this and the concentration dependence, the loss of activity was (partly) related to the surface accumulation of trypsin. However, the identity of the carbohydrate also plays a significant role. In particular, it seems that crystallising carbohydrates are less suitable for preservation of the active enzyme. Overall, our studies show that at least for some systems, spray-drying is an interesting technique for drying sensitive biological material. Given the larger capacity and typically better economy of spray-drying compared to freeze-drying, this suggests that the former technique has the potential for drying of protein-containing systems.

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