Self-Release of Lipase from Compacts

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INTRODUCTION

The possible delivery of physiologically active proteins prepared by genetic engineering techniques using subdermal compacted implants (1,2) has heightened interest in the properties of proteins under compactional pressure and release of these proteins from compacts (3–6). Many of these earlier studies utilized delivery systems containing formulation adjuvants such as lubricants and fillers and were concerned mainly with digestive enzymes for oral delivery (4–6). Nevertheless, fundamental studies or protein compaction are required in order to provide information that may be generalized and applied to the more active, and therefore sensitive, materials currently being produced. These materials are also costly, which precludes their investigation on a realistic scale. The search for a model protein with measurable biological activity has been extended to a commercial lipase preparation having some of the required hallmarks of reproducible activity, stability, and availability.

Wheat germ lipase, a protein with esterase activity, is involved in hydrolyzing insoluble triacylglycerols to the more soluble glycerol, free fatty acids, and mono- and diacylglycerols for membrane transport and, later, resynthesis of the initial fatty acid esters (7). The enzyme is believed to be adsorbed onto the surface of the emulsified substrate, activity being determined by the number of enzyme molecules adsorbed per unit area of the surface (8). Various proteins from a number of biologically different sources, with widely differing molecular weights, are known to have lipase activity (9,10).

Previous studies (11) indicated that a lipase preparation was sensitive to thermal energy produced during compaction. However, studies on a urease preparation in this laboratory suggested (12) that thermal degradation may be only part of the inactivation process, with volume reduction probably accounting for the major part of activity loss. Since other studies (13) had demonstrated the importance of moisture on the compaction process of an otherwise inert soy albumin, these investigations were extended to a commercially available lipase preparation obtained from wheat germ.

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EXPERIMENTAL

Materials

Materials used were as follows: wheat germ lipase (Cat. No. L-3001, Lot No. 37F8025, lyophilized powder; Sigma Chemical Company, St. Louis, MO)—the preparation contains 97% (w/w) protein by Lowry measurement (14); Lowry reagent (L-1013 Kit. No. 5656, Sigma); Folin and Ciocaleus phenol reagent (F9252, Sigma); lithium chloride (Fisher); calcium chloride, USP fine granulated (Fisher); calcium nitrate tetrahydrate (Sigma); and ammonium chloride, ACS certified (Fisher). All were used as received. Water was double-distilled.

Equipment

Equipment used was as follows: Carver laboratory press, Model 2702; Perkin Elmer Lambda 3B UV/VIS Spectrophotometer; and USP dissolution equipment, six-spindle Type II method (Vanderkamp, Model 600, VanKel Industries Inc).

Experimental Methods

Lipase Compaction. Wheat germ lipase powder was spread on petri dishes to a depth of about 1 mm and exposed for 7 days in closed desiccators in the dark to atmospheres over saturated aqueous solutions of lithium chloride, calcium chloride, calcium nitrate, or ammonium chloride, providing, at ambient room temperature, relative humidities of 15, 31, 51, and 80%, respectively (15). Aliquots, equivalent to 100 mg anhydrous protein, were compressed at a rate of 18.65 cm/min in the Carver press at various pressures over the range of 60–470 MPa immediately after removal from the desiccator. A 7-mm flat-faced polished stainless-steel punch-and-die set was employed in all cases. Tablet weight, thickness, and diameter were determined immediately after compaction. Compacts were stored prior to dissolution measurements in closed containers in the dark.

Dissolution. The dissolution procedure outlined in the *USP XXI* (16), Method II, was followed, using 1 liter of water at 37°C and stirring at 80 ± 1 rpm. Six tests were run simultaneously, 1-ml filtered samples being removed for analysis at 15, 30, and 60 min and 1-hr intervals thereafter for a total of 8 hr.

Protein Measurement. Protein released from the compacted matrices was determined nonspecifically using the Lowry procedure (17). Results are the means of three determinations.

RESULTS AND DISCUSSION

The effect of pressure on the density of the resulting compact is shown for lipase preparations equilibrated at various humidities in Fig. 1. The density is clearly affected by the protein moisture; higher moisture levels result in more compact matrices. The influence of pressure, on the other hand, is higher on proteins equilibrated at lower relative humidities. Ganderton *et al.* (18), also found a decrease in sucrose tablet porosity with rising water content. The decrease

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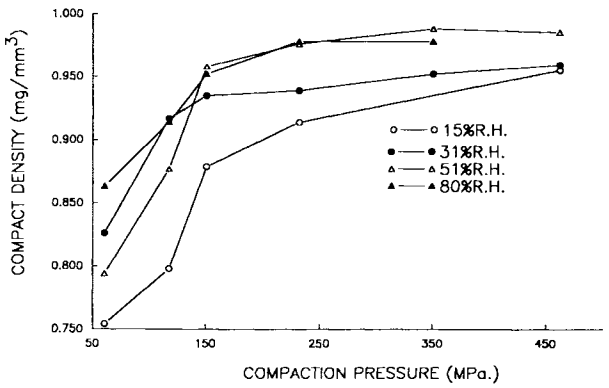

Fig. 1. Effect of compactional pressure on the density of the resulting compact of wheat germ lipase initially stored at different equilibrium relative humidities (RH).

in interparticle distances and improvement of compressibility with increased humidity have also been reported elsewhere (13,19,20). Typically linear self-dissolution profiles for these compacts could be approximated by percentage release/root time plots (Fig. 2) similar to those proposed by Higuchi for diffusion-controlled matrix models (21,22). Other dissolution models were evaluated, but in general, the diffusion-controlled process appeared to be the optimum model. This model was valid up to the point where the matrix disintegrated, 60–70% by weight of the protein compact. The self-solution of the protein compact is, therefore, apparently occurring in stages so that it appears to be diffusion controlled.

Proteins are largely composed of 20 amino acids, of which 25–30% are hydrophilic and 45–60% are ionic or contain uncharged hydrophilic side chains (23). The forces that contribute to the stability of protein structures are noncovalent in nature, namely, hydrogen bonding, hydrophobic interactions, and ionic pairing. In effect, water promotes hydrophobic interactions. However, according to nucleation theory, water will not condense onto an insoluble particle less than about 10 nm in diameter unless the pressure is higher than the equilibrium vapor pressure of water. Under normal laboratory conditions a dry globular protein would not hydrate if its surface consisted only of an amide backbone and other nonionizable residues. During hydration the

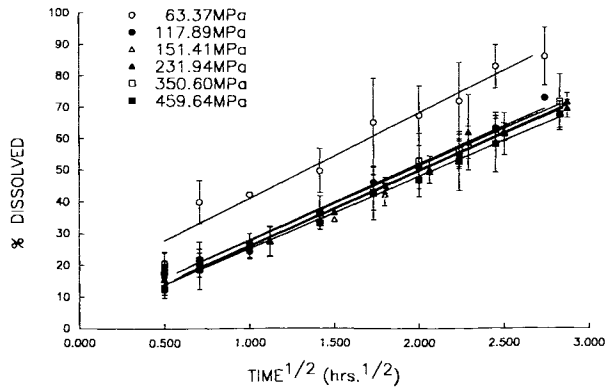

Fig. 2. Release of protein at 37°C from compacts of wheat germ lipase equilibrated at 15% relative humidity prepared over a range of pressures.

first stage would be ionization of side chains, followed by clusters of water forming around strong hydration sites which grow and envelop the surface backbone and nonionizable side chains (24). Water will be strongly adsorbed onto many surfaces, especially if surface functional groups are present that are capable of hydrogen bonding, electrostatic interactions or chemisorption. The whole process will be enhanced by capillary condensation into micro- and mesopores that might be present (25). The dry protein molecules that make up the tablet matrix will become progressively more hydrated, interacting with the water molecules and eventually acquiring enough vibrational energy to go into solution. The subject of water absorption has been reviewed for conventional tablet materials (26) but this concept does account for protein compacts apparently releasing their contents substantially in accordance with a diffusion-controlled model. Scanning electron micrographs published elsewhere (27,28) and the data in Fig. 1 suggest that protein compacts behave in much the same way as other tabletted materials in that macropores between particles are evident in tablets made at low compaction pressures. As the pressures are increased, these macropores become smaller but persist, suggesting that a porous tablet matrix exists throughout the tablet, with hydration and solution occurring only at the surface of the pores. As the solution process proceeds the composition of the matrix begins to disappear and the matrix breaks up. There is no reason to suggest, however, that the initial part of the dissolution process for a protein dissolving from its own compact is any different from that previously observed with a conventional drug dispersed in another protein matrix (13,28). Indeed Higuchi (21) noted that this relationship does not usually apply to situations in excess of 50% drug release. Benita *et al.* (29) confirm linearity up to 75% of drug content.

The equation of Higuchi (21,22) describing kinetics of release can be summarized in linear form by the equation

$$Q = k\, t^{1/2}$$

where Q is the amount released in time $t^{1/2}$, and k is a release rate constant, $Q/t^{1/2}$ (29). Thus, Q/t, the true rate, cannot be obtained directly from plots such as that shown in Fig. 2. However, extrapolation of this plot to the point where all the drug has been released yields an intercept $t_{100}{}^{1/2}$ which can be squared to provide a t_{max}, the time for maximum release. The value of t_{max} has the dimensions of a reciprocal rate, $t/100\%$, assuming no initial delay in the dissolution process, a valid assumption for a self-release process. Using the value of t_{max} as a measure of the dissolution process and plotting this as a function of the logarithm of the applied compaction pressure gave data shown in Fig. 3. This relationship, in the form

$$t_{max} = A \ln P \pm B$$

where A and B are constants and $\ln P$ is the natural logarithm of the compactional pressure, applied approximately only to compacts of protein equilibrated at relative humidities of 15, 31, and 80%, and not to the system equilibrated at 51% RH. This may be related to the manner in which water has been shown to adsorb onto this and other proteins and has been discussed elsewhere (14).

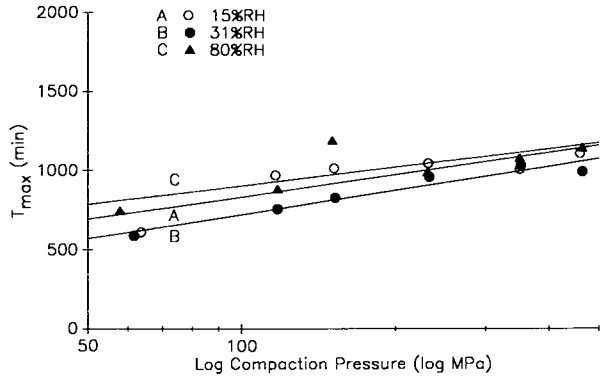

Fig. 3. The relationship between the estimated time for maximum release (T_{max}) of wheat germ lipase compacts and the logarithm of the compactional pressure.

Nevertheless, self-release of a protein from a compact (without any tablet adjuvant) will be influenced by three factors:

(i) the nature of the protein itself,
(ii) the relative humidity of the environment in which the protein is stored prior to compaction—itself related to the water content of the protein during compaction; and
(iii) the compactional pressure used to make the compact.

The interrelationship of these factors is evidently complex but it would appear to be feasible to design a protein pellet or compact which has a predetermined release behavior.

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