Resistance of Extracellular Yeast Invertase and other Glycoproteins to Denaturation by Tannins

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Although micro-organisms and enzymes (Benoit & Starkey, 1968; Goldstein & Swain, 1965; Lewis & Starkey, 1969) alike succumb to the toxic effects of both the condensed and hydrolysable tannins, the condensed tannins have been shown repeatedly to be the more potent antagonists (Gustavson, 1956; Pridham, 1963). For example, Lewis & Starkey (1969) observed virtual elimination of microbial growth when a condensed-tannin fraction was added to soil. In contrast, they found that hydrolysable tannins supported the growth of various filamentous soil fungi. Similarly, Goldstein & Swain (1965) measured the interaction of several enzymes with a condensed-tannin fraction from wattle bark and with several hydrolysable tannins. Although both types of tannins reacted with the enzymes to form complexes possessing markedly decreased activity, only the condensed tannins produced stable enzyme complexes that could not be reactivated even partially. Also, Strumeyer & Malin (1969) have reported on the properties of some polymeric procyanidin tannins that were isolated from seeds of *Leoti sorghum*. When these condensed tannins were incubated with an equivalent or slightly lower weight of enzyme, activity was either abolished or markedly diminished. Included in that survey were pectinesterase, polygalacturonate lyase and ribulose diphosphate carboxylase as well as three crystalline α -amylases. The findings of these experiments are compatible with the classical concepts of protein and tannin interaction (Gustavson, 1956).

In this communication we report on an enzyme, the extracellular invertase of yeast, that is unlike the typical protein because it is able to tolerate the denaturing action of a condensed tannin. To our knowledge, yeast invertase is the first enzyme that has been recognized to resist this type of condensed-tannin inactivation.

The isolation and properties of the sorghum tannin that was used in these experiments have been described previously (Strumeyer & Malin, 1969). Invertase, isolated from *Saccharomyces* FH4C as described by Neumann & Lampen (1968), was prepared and assayed by Dr N. Neumann of the Rutgers Medical School. The effect of condensed tannin on this enzyme was examined by incubating 10 μ g of invertase with the sorghum tannin in

0.20 ml of 0.05 M-sodium acetate buffer, pH 5.0, for 5 min. To assay for enzyme activity, 0.1 ml of a 100-fold dilution of the reaction mixture was added to 0.3 ml of acetate buffer containing 0.175 M-sucrose, and after 15 min at 30°C the glucose formed was measured by an enzymic procedure (Gascon & Lampen, 1968). In these experiments even 100 times more condensed tannin than invertase was unable to produce a detectable decrease in the activity of this enzyme. The insusceptibility of invertase to inhibition by a condensed tannin is in marked contrast with what has been observed with other enzymes.

On the other hand, some enzymes have been reported to demonstrate resistance towards the hydrolysable tannins. For example, the fungal tannases not only escape the inhibitory effects of a hydrolysable tannin such as tannic acid, but they also render the tannin innocuous by cleaving it to the inert products, glucose and gallic acid. The tannase from *Aspergillus flavus* has been purified and characterized in order to learn more about this unusual property (Adachi, Watanabe & Yamada, 1968). Moreover, in several instances, the complex formed with tannic acid has been exploited for the isolation and purification of specific enzymes and proteins. Subsequent treatment of the tannin-precipitated protein with aqueous acetone or caffeine regenerated the purified active enzyme or unaltered native protein from the complex. The glucose oxidase from *Penicillium notatum* (Coulthard *et al.* 1945), orosomucoid, gonadotrophins, thyrotrophin, haptoglobulin, lysozyme and ovomucin (Mejbaum-Katzenellenbogen, Dobryszcka & Morawiecka, 1961) are representative of the proteins prepared in this manner.

Interestingly, the resistant enzymes, i.e. yeast invertase (Neumann & Lampen, 1968) and *A. flavus* tannase (Adachi *et al.* 1968), are glycoproteins and contain 50% and 25.4% of carbohydrate respectively. Also, in the above lists of proteins isolated as readily reversible complexes with tannic acid, except for lysozyme (Elyar, 1965), all contain significant amounts of carbohydrate, e.g. from 16% to 40%. Although this correlation is by no means exhaustive, it appears attractive to propose that resistance of enzymes and other proteins to de-

naturation by tannins is a consequence of their glycoprotein nature.

It is generally accepted that tannins associate strongly with polyamides, polypeptides and proteins by hydrogen-bonding with the polypeptide structure and susceptible amino acid side chains (Goldstein & Swain, 1965). These interactions markedly distort the native conformation of the protein, and decrease or eliminate enzymic activity. Extensive binding of the tannin and intermolecular cross-linking ultimately lead to precipitation of the protein-tannin complex. In glycoproteins, a carbohydrate coating most probably protects the polypeptide backbone, which would then be less accessible to tannin molecules. Consequently, binding of tannin by glycoprotein probably occurs with the carbohydrate rather than with the protein, perhaps creating a weaker and more readily reversible complex. In this manner invertase may complex substantial amounts of tannin without precipitation or even noticeable effect on activity. The carbohydrate coating on tannase may protect it similarly, and also may direct the substrate to the limited but accessible active-site region where cleavage to non-tannin products can be accomplished.

On the basis of the frequently observed association of bound carbohydrate with extracellular proteins and the absence of a functional role for the carbohydrate portion of active glycoproteins, Elyar (1965) proposed that the carbohydrate acts as a chemical label that promotes the passage of the newly synthesized glycoprotein across cell membranes. In view of the ordinarily conservative design of biological mechanism, we would extend Elyar's (1965) hypothesis to include a second role for the carbohydrate portion. Thus, in addition to facilitating transport from the cell, it is proposed that the sugar coating of the extracellular protein serves as a structural modification to provide a protective barrier against inactivation by the variety of denaturants, such as tannins, found in the natural environment.

Related to this proposal is the well-confirmed observation that glycoproteins are less susceptible to heat denaturation than are other proteins (Bettelheim-Jevons, 1958). Yeast invertase appears

to be a typical glycoprotein in this regard. In a study by Arnold (1969) a commercial preparation of yeast invertase was reported to be a polydisperse system in which the invertase fractions differed only in the proportion of bound carbohydrates. The resistance of these fractions to heat inactivation was found to increase with increasing carbohydrate content, conforming to the idea that the carbohydrate coating contributes to the protection and stabilization of the enzyme.

Like the secreted enzymes, proteins found in delicate membranous structures, such as the mucosal linings, contain significant amounts of bound carbohydrate that may serve as a protective barrier. On the tongue, for example, the carbohydrate portion of the glycoproteins may (a) guard against irreversible heat denaturation of taste receptors and (b) minimize the binding of such potential chemical denaturants as the tannins.

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