The Assay of α -Amylase

By M. J. CLANCY and C. E. O'HARA. (*Department of Plant Chemistry and Biochemistry, The Agricultural Institute, Oak Park, Carlow, Irish Republic*)

Many methods are described in the literature for estimating α -amylase activity in wheat and wheaten flour, but most of them do not distinguish between the action patterns of wheat α - and β -amylase, which are quite distinct. No one has described the action pattern resulting from the joint action of α - and β -amylase on starch. Our studies have shown that α -amylase from wheat cannot be reliably assayed by measurement of reducing power (product formation) irrespective of whether a soluble starch or amylopectin β -limit-dextrin is used as a substrate when β -amylase is present. In a study of the separate and joint actions of α - and β -amylases on starch as measured by iodine staining (substrate disappearance) it was found that the presence of β -amylase markedly affected the rate of starch degradation.

We have investigated the selective assay of α -amylase by (a) the attempted specific inhibition of β -amylase (Clancy, O'Hara, Risseuw & Ryan, 1968) and (b) the use of a specific substrate for α -amylase. In the first approach (a) we have established that the inhibition with various thiol reagents is not enzyme-specific: both enzymes (amylases) are partially inhibited when an amount of inhibitor sufficient for the complete inhibition of β -amylase is used. We have also observed that the inhibition of β -amylase is not independent of starch concentration. The overall protein concentration in the assay mixture is also important since thiol-blocking reagents react with the thiol groups of this protein. In the second approach (b) we have found pure amylopectin β -limit-dextrin to be a reasonably satisfactory substrate for α -amylase assay when the amounts of β -amylase are not too high.

The arguments for these conclusions will be outlined.

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Dissociation of β -Casein from Casein Micelles and Complexes

By W. K. DOWNEY, R. F. MURPHY and S. A. AHERNE. (*The Agricultural Institute, Moorepark, Fermoy, Co. Cork, Irish Republic*)

The endothermic self-association of both α_s - and β -casein has been well documented (Hippel & Waugh, 1955; Payens & van Markwijk, 1963;

Payens & Schmidt, 1965). In this study the effect of temperature on the dissociation of β -casein from the casein micelles of milk and from the soluble casein complexes of colloidal-phosphate-free milk (Pyne & McGann, 1960) was investigated by high-speed centrifugation and by gel filtration at various temperatures (0–25°) on Sephadex G-200 and Sepharose 2B (Pharmacia, Uppsala, Sweden) columns equilibrated with a buffer similar in composition to milk ultrafiltrate.

The removal of most (approx. 65%) of the β -casein from casein micelles of milk by gel filtration at 5° on Sepharose 2B columns was not attended by a marked decrease in the size of the casein micelles, nor was there any indication of micellar disintegration. Thus, although β -casein may constitute the structural lattice of the subunits of casein micelles (Rose, 1969), less than 40% of the total β -casein appears to be required for this function. Further, the elution from Sepharose 2B columns at 25° of all the β -casein of colloidal-phosphate-free milk, together with the α_s - and κ -caseins, apparently as complexes of molecular size approx. 2×10^6 (Murphy, Downey & Kearney, 1969), indicates that colloidal phosphate is not essential for the association of β -casein with the structural subunits of casein micelles.

The decrease in free β -casein obtained by gel filtration on Sepharose 2B, from 65% to approx. 20%, when the fractionation temperature is decreased from 5° to 0°, appears to be due to a decreased dissociation of β -casein from casein micelles or complexes, rather than to a polymerization of free β -casein.

The large amount of non-micellar β -casein (approx. 65% of the total) obtained on gel filtration at 5° on Sepharose 2B, relative to that (approx. 10% of total) obtained by comparable fractionations on Sephadex G-200, or in high-speed supernatants (34000g at 5° for 2hr.) is difficult to explain. However, it appears that, on cooling milk to 5°, approx. 10% of the β -casein dissociates from the micelles as aggregates (mol.wt. approx. 200000) that are in equilibrium with the micellar β -casein. During gel filtration on Sepharose 2B, the β -casein enters the gel pores and is rapidly separated from the micellar casein, which is excluded from the gel. Additional β -casein is consequently released from the micelles to re-establish equilibrium. In contrast, on Sephadex G-200, the liberated soluble β -casein aggregates are not completely separated from the casein micelles, and the equilibrium is not sufficiently disturbed to result in appreciable dissociation of β -casein from the micelles.

Hippel, P. H. von & Waugh, D. F. (1955). *J. Amer. chem. Soc.* **77**, 4311.

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