The Assay of α -Amylase

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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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Clancy, M. J., O'Hara, C. E., Risseuw, P. S. & Ryan, E. (1968). *Biochem. J.* **110**, 7P.

Dissociation of β -Casein from Casein Micelles and Complexes

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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.

Murphy, R. F., Downey, W. K. & Kearney, R. D. (1969). *Biochem. J.* **115**, 22P.

- Payens, T. A. J. & Schmidt, D. G. (1965). *Biochim. biophys. Acta*, **109**, 214.
- Payens, T. A. J. & van Markwijk, B. W. (1963). *Biochim. biophys. Acta*, **71**, 517.
- Pyne, T. G. & McGann, T. C. A. (1960). *J. Dairy Res.* **27**, 9.
- Rose, D. (1969). *Dairy Sci. Abstr.* **31**, 171.

Dissociation of Casein Micelles to Soluble Casein Complexes

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The internal cohesion of the various protein components within the intact casein micelle was investigated by removal of the colloidal calcium phosphate and the soluble salts of milk under mild conditions to cause dissociation of the casein micelles to soluble casein complexes.

On fractionation at 5° of skim milk on Sepharose 2B (Pharmacia, Uppsala, Sweden) columns, the casein micelles were eluted at the void volume. As with Sephadex G-200, the molecular-weight exclusion limit of Sepharose 2B for proteins is likely to be at least four times the value (2.5×10^7) quoted for dextrans by the manufacturers. Consequently the molecular size of casein micelles appears to exceed 10^8 , in agreement with results obtained by electron microscopy (Nitschmann, 1949; Carroll, Thompson & Nutting, 1968). To prevent micellar disintegration during gel filtration, similar to that encountered by Yaguchi & Tarassuk (1967), columns were equilibrated with a buffer identical in composition with milk ultrafiltrate.

On removal of the colloidal calcium phosphate from milk by the method described by Pyne & McGann (1960), the casein micelles are dissociated to smaller complexes, which were eluted from Sepharose 2B columns in the expected position for a globular protein of mol.wt. approx. 2×10^6 .

These soluble complexes contain α_s -, β - and κ -casein apparently in aggregated form, and it is suggested that they may constitute the subunits (Rose, 1969) of casein micelles in which they are bonded together by colloidal calcium phosphate.

When the soluble salts of milk are removed together with colloidal calcium phosphate by dialysis in the cold against 0.2–1 M-NaCl, the casein micelles are solubilized. As indicated by gel filtration on Sepharose 4B columns equilibrated with various concentrations of NaCl, the molecular weights of the casein aggregates present in these preparations vary with the concentration of NaCl used for dialysis. The smallest aggregates were obtained by dialysis against 0.5 M-NaCl.

The relationship of these various submicellar complexes to the intact casein micelle and its subunits will be discussed.

- Carroll, R. J., Thompson, M. P. & Nutting, G. C. (1968). *J. Dairy Sci.* **51**, 1903.
- Nitschmann, Hs. von (1949). *Helv. chim. Acta*, **32**, 1258.
- Pyne, G. T. & McGann, T. C. A. (1960). *J. Dairy Res.* **27**, 9.
- Rose, D. (1969). *Dairy Sci. Abstr.* **31**, 171.
- Yaguchi, M. & Tarassuk, N. P. (1967). *J. Dairy Sci.* **50**, 1985.

DEMONSTRATION

Clinical Biochemical Analysis: The Baird and Tatlock Analmatic Instrument

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This is an automatic analyser of the so-called 'discrete' type, processing serum analyses in batches of 100. Methods are available for a variety of tests and some of these will be demonstrated.