EFFECT OF NEURAMINIDASE ON κ-CASEIN

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Interest in sialic acid (N-Acetyl Neuraminic Acid, NANA) has extended into the field of milk protein chemistry. This carbohydrate has been identified as a constituent of human milk oligosaccharides and appears in dialysates of bovine colostrum (4). NANA appears in the glyco-macropeptide (GMP) which is released from whole casein by the action of rennin (2, 7, 9), and exists in several minor protein fractions of bovine milk (11). Dumas and Alais (3) recently isolated, crystallized, and characterized NANA obtained from bovine casein.

The presence of strongly hydrophilic NANA in κ-casein (1) has prompted us to determine the possible role of this carbohydrate in the stabilization of α_s-casein in the presence of Ca^{++}. This paper reports the effects of neuraminidase (NANAse) on the release of NANA from κ-casein and its effect on the stabilizing power of κ-casein.

κ-Casein was prepared by the improved method of McKenzie and Wake (8). The κ-casein obtained by this method was essentially free of contamination in starch-gel-urea electrophoresis (8), possessed an S_{20} of 14 at pH 6.98, $\Gamma/2 = 0.20$ in the phosphate buffer of Waugh and von Hippel (15), and was an excellent stabilizer of α_s-casein in the presence of Ca^{++}. The κ-casein contained 0.22% phosphorus and 2.5% NANA. Three hundred milligrams of κ-casein was dissolved at pH 8.5 in a volume of 58 ml and adjusted to pH 7.0. One and two-tenths milliliters of V. cholerae NANAse, supplied by Behringwerke A. G.,[1] Marburg-Lahn, Germany, with an activity of 200 units per milliliter, was added to the protein solution at 37 C. The final pH of the solution was adjusted to 6.0 with 0.1 N HCl. Aliquots in which NANA was to be determined were withdrawn after the reaction had proceeded for 0, 15, 30, 60, and 180 min, heated to 80 C for 2 min, cooled, and dialyzed at 4 C against several changes of distilled water. NANA concentrations were determined using the resorcinol method of Svennerholm (10), with α_1 acid glycoprotein (orosomucoid) as a secondary standard for NANA. After the above reaction times, aliquots were also withdrawn for determination of stabilizing power by the method of Zittle (16), and the tests run immediately.

Measurements of the concentration of NANA remaining after dialysis revealed that V. cholerae NANAse liberated NANA from κ-casein at a rapid rate (Figure 1). Thompson et al. (12) observed electrophoretic alterations in influenza virus receptor destroying enzyme (RDE) -treated (4) sodium caseinate which they attributed to a release of NANA. Although Thompson et al. did not demonstrate NANA release from whole casein, Jollés and Alais (1, 7) showed that V. cholerae (RDE) attacked the GMP obtained from whole or κ-casein and liberated 80 and 66% NANA, respectively, from the two peptides. Jackson (6) observed that NANAse releases NANA from a glycopeptide obtained from the interfacial protein of bovine milk (5). Thompson and Brunner (11) had discovered earlier that a similar protein contained about 2% NANA, but did not ascertain the effect of NANAse on its release.

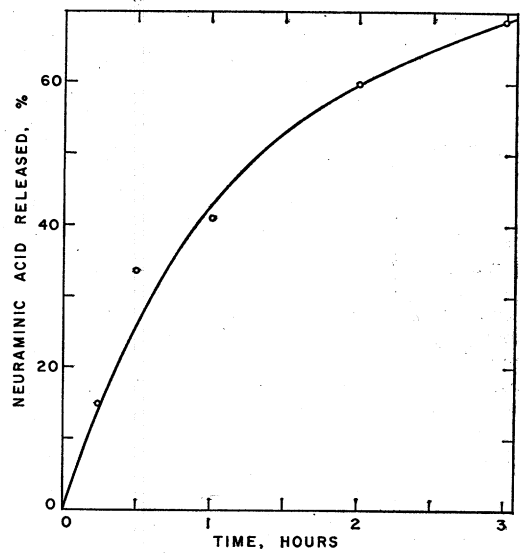

FIG. 1. The release of NANA from κ-casein by NANAse as a function of time.

The action of NANAse on κ-casein NANA discloses the position of this carbohydrate in the κ-casein molecule. First, the release of NANA is evidence that it occupies a position terminal to an adjacent sugar residue in a disaccharide, trisaccharide, or polysaccharide because the action of NANAse is contingent upon this condition (4). The studies of Alais and Jollés (1) have shown that NANA is terminal in the GMP. Secondly, NANA is probably joined to either D-galactose or D-galactosamine through an α-glycoside (α-ketoside) linkage (4). Both of these carbohydrates are constituents of κ-casein (1). Studies currently in progress in this laboratory on the GMP are designed to determine the partner of NANA and the carbohydrate–amino acid linkage between the polysaccharide moiety and polypeptide portion of the molecule.

[1] It is not implied that the U. S. Department of Agriculture recommends the above company or its product to the possible exclusion of others in the same business.

Figure 1 shows that within 1 hr of reaction approximately 40% of the NANA had been released from κ-casein by NANAse. After 3 hr, about 69% of the total NANA had been released, whereas after 4 hr (not seen in Figure 1) 82.5% of the total NANA had been released. After longer reaction times, a 100% release of the carbohydrate could not be realized. Examination of the enzyme-reacted κ-casein by starch-gel-urea electrophoresis (13) revealed no change in the pattern as a result of the enzyme treatment. The protein continued to smear, as did the untreated control. At pH 6.98, $\Gamma/2 = 0.10$, phosphate buffer, and pH 8.6, $\Gamma/2 = 0.10$, veronal buffer, in free boundary electrophoresis, the enzyme-reacted κ-casein migrated as one symmetric component. The enzyme did not appear to alter the protein, except for a slight decrease in electrophoretic mobility which resulted from the loss of negatively charged NANA.

When NANAse treated κ-casein was examined for its ability to stabilize α_s-casein (Figure 2), it was observed that a loss of stabilizing power of κ-casein occurred within the first hour of enzyme reaction where the greatest amount (40%) of NANA was released. However, the protein still stabilized approximately 80% (κ/α_s ratio of 0.12) of the α_s-casein even after removal of 69% of the NANA.

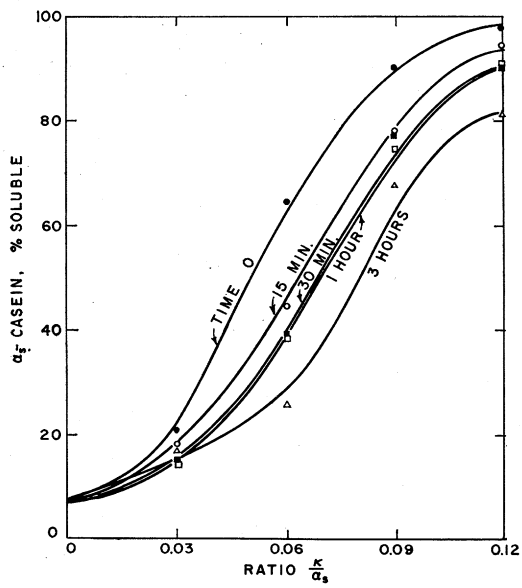

FIG. 2. Stabilization of α_s-casein by κ-caseins treated with NANAse for various periods of time.

The decrease in stabilizing power of κ-casein as a result of the release of NANA was not unexpected considering Waugh's (14) proposed structure of the casein micelle consisting, in part, of α_s-κ-casein. Waugh has depicted that a portion of the κ-casein monomer projects from the α_s-κ complex into the surrounding medium. Presumably, this portion of κ-casein is the hydrophilic or glyco-macropeptide portion of the molecule. The release of any hydrophilic groups from this portion of κ-casein, namely NANA, would result in a less soluble κ-casein which would lead to a decreased complex solubility.

In addition to NANA, we feel that other moieties of κ-casein, for example, the carbohydrate remaining after NANA removal, phosphate groups, and possibly –S–S groups contribute to the ability of κ-casein to form micelles with α_s-casein in the presence of Ca^{++}.

The observation that a loss of NANA alters a particular property of a protein is not peculiar to κ-casein. NANAse treatment of the follicle-stimulating hormone, for example, causes this mucoprotein to lose 97% of its biological activity (4).

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