THE SUBSTITUTION OF α-LACTALBUMIN FOR THE B PROTEIN OF LACTOSE SYNTHETASE *

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Received June 16, 1966

The presence of a soluble form of lactose synthetase (UDPgalactose: D-glucose 1-galactosyl transferase EC 2.4.1c) in bovine milk was first demonstrated by Babad and Hassid (1964). Recently, Brodbeck and Ebner (1966a, 1966b) have resolved the soluble lactose synthetase into two protein components, called A and B, which individually did not exhibit catalytic activity. However, recombination of fractions A and B restored catalytic activity and the suggestion was made that the A and B proteins represent subunits of lactose synthetase. Recently, several other enzymes have been shown to exhibit similar behavior: tryptophan synthetase from E. coli (Creighton and Yanofsky, 1966); glutamate mutase from C. tetanomorphum (Suzuki and Barker, 1966); (+) citramalate hydro-lyase from C. tetanomorphum (Blair and Barker, 1966) and glycine decarboxylase from Peptococcus glycinophilus (Klein and Sager, 1966). In all cases, maximum activity is dependent upon the presence of both protein fractions.

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* Supported in part by United States Public Health Service Grant No. AM 06889.

Studies in this laboratory have been concerned with the purification of the A and B protein fractions of lactose synthetase. The B protein has been crystallized and was estimated to have a molecular weight of about 15,000 from the elution pattern off Bio Gel P-30 (Brodbeck, 1966). It was stable to boiling for 20 minutes at pH 7.4 and to acid precipitation with 10% trichloroacetic acid. The ultraviolet spectrum had a pronounced shoulder at 290 mμ indicating exposed tryptophyl residues. The properties of the B protein appeared to be very similar to the properties of α-lactalbumin and therefore, α-lactalbumin was tested for its ability to substitute for the B protein in the lactose synthetase reaction. The results presented show that α-lactalbumin does substitute for the B protein of lactose synthetase and appears to be identical with α-lactalbumin.

Methods. Lactose synthetase activity was determined either by assaying spectrophotometrically for the amount of UDP formed or by measuring the incorporation of UDP-D-galactose-1-^{14}C into ^{14}C-lactose as previously described (Brodbeck and Ebner, 1966a). Immunological assays were carried out by Dr. Bruce Larson at the University of Illinois by the Oudin technique as previously described for the determination of α-lactalbumin in complex mixtures (Larson and Hageman, 1963). Antibody was prepared from 5 times crystallized α-lactalbumin isolated from bovine milk. Two times, three times and five times recrystallized α-lactalbumin were gifts from Dr. Bruce Larson. The A protein was a 200 fold purified preparation from bovine skim milk and the B protein was a crystalline preparation which was homogenous in the ultracentrifuge (Brodbeck, 1966). An $\epsilon_{280}^{1\%} = 20.9$ was used for determining protein concentration of α-lactalbumin and the B protein

(Wetlaufer, 1961). α-lactalbumin and the B protein were dissolved in 20 mM Tris, pH 7.4 and adjusted to identical protein concentration as determined by absorbance at 280 mμ.

Results and Discussion. The substitution of α-lactalbumin for the B protein was determined in both the spectrophotometric and incorporation assay. Fig. 1 shows the formation of UDP when a constant concentration of either B protein or α-lactalbumin was titrated by varying amounts of A protein. In a converse experiment, similar results were

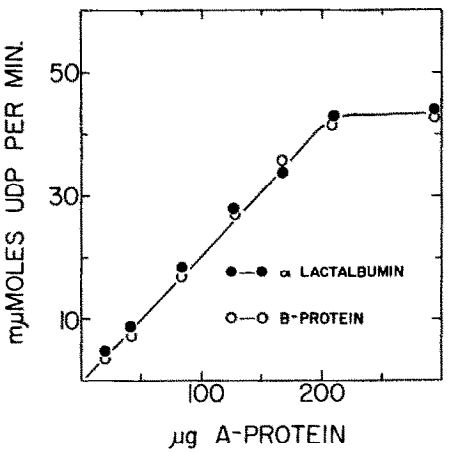

Fig. 1. The rate of UDP formation when a constant amount of 3X crystallized α-lactalbumin (18.0 μg) or B protein (17.8 μg) was titrated with varying amounts of A protein. Lactose synthetase activity was determined spectrophotometrically on a Cary Model 14 recording spectrophotometer.

obtained when a constant amount of A protein was titrated with varying but identical amounts of B protein or α-lactalbumin. Fig. 2 shows the relationship between lactose-^{14}C formation and varying amounts of a mixture of B and A protein or α-lactalbumin and A protein. In both cases, the B protein or α-lactalbumin were saturated by the A protein. The specific activities of the B protein, 2X, 3X and 5X crystallized α-lactalbumin were determined at saturating levels of A protein and were

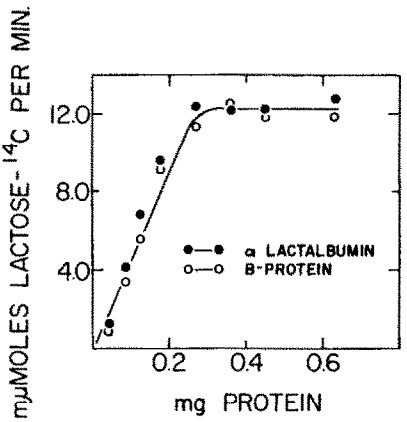

Fig. 2. The rate of ^{14}C-lactose formation when the B protein or α-lactalbumin was saturated with A protein. A mixture was formed from one volume of B protein or α-lactalbumin (each at 0.88 mg per ml) and one volume of A protein (17 mg per ml). Both solutions were prepared in 20 mM Tris, 5 mM $MgCl_2$, pH 7.4. Under these conditions the B protein and α-lactalbumin were saturated by the A protein. The mixture containing B and A proteins is designated as O—O and the mixture containing α-lactalbumin and A protein is ●—●. The mg protein refers to the protein content of the mixtures.

respectively 79.6, 81.0, 80.5 and 81.6 units/mg. By immunological analyses, the B protein assayed as a minimum as 91% α-lactalbumin which was comparable in purity to 3X crystallized α-lactalbumin. Other data also strongly indicate that the B protein of lactose synthetase is α-lactalbumin. The ultraviolet spectra are identical and the ratio of A_{280}/A_{290} is 1.31 for the B protein which is in good agreement to 1.32 for α-lactalbumin (Wetlaufer, 1961). The crystal forms of the B protein are similar to those reported by Aschaffenburg and Drewry (1957) for α-lactalbumin.

α-lactalbumin can substitute for the B protein of lactose synthetase at identical protein concentrations in both the spectrophotometric and incorporation rate assays. The immunological data showed that the B protein is primarily α-lactalbumin. The specific activities of the B pro-

tein and 2X, 3X and 5X recrystallized α-lactalbumin are essentially the same.

α-lactalbumin, a major constituent of the whey proteins of milk, has been recognized for some twenty years though no biological function other than a nutritional one could be ascribed to this protein. The present results suggest an enzymatic role for α-lactalbumin as one of the subunits of lactose synthetase.

ACKNOWLEDGMENTS

The authors wish to thank Dr. B. L. Larson of the University of Illinois for his gifts of α-lactalbumin, for performing the immunological assays for his timely discussions.

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