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A Biological Role for α-Lactalbumin as a Component of an Enzyme Requiring Two Proteins

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Milk is a unique product produced by mammals for the nourishment of the young. It contains a great variety of compounds including lipids, carbohydrates, vitamins, salts, proteins, and many miscellaneous compounds and, in a sense, it comes close to being a perfect food. The origin of all the components of milk is not clearly understood at this time. Part of them are true secretory products of the gland, some represent cellular constituents, and others are derived from the blood. It should be pointed out that the process of secretion appears to involve the partial destruction of the secretory cells.

The principal proteins of milk[2a] are the caseins which are a complex mixture of phosphoproteins and may be precipitated at pH 4.6. The proteins which are not precipitated at pH 4.6 are called the whey proteins and consist of two major components, β-lactoglobulin (3 mg/ml) and α-lactalbumin (0.7–1.5 mg/ml), and many minor proteins, including a great variety of enzymes.[2b]

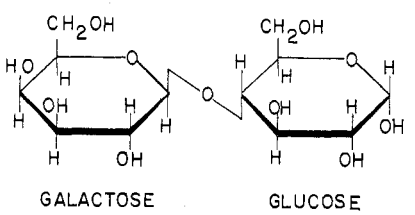
α-Lactalbumin

Wichmann[3] in 1899 obtained a crystalline material from milk whey which he called "lactalbumin." In 1936 Pederson[4] showed by ultracentrifugation studies that milk whey contained three main proteins, and the slowest moving component was designated as α (s_{20} = 1.9 S). The α component was subsequently isolated by Kekwich[4] and called α-lactalbumin by Svedberg[5] in 1937. Sorensen and Sorensen[6] isolated a crystalline, insoluble substance from whey which was the basis for the isolation method used by Gordon and Semmett,[7] who suggested in 1953 that the name α-lactalbumin be given to the same protein as the α component of whey originally described by Pederson.[4]

α-Lactalbumin is easy to crystallize, and in 1955 Gordon and Ziegler[8] determined the molecular weight to be 15,500 based on amino acid composition (129 residues). The physical properties of α-lactalbumin were characterized in 1961 by Wetlaufer[9] and in further detail by Kronman[10,11] and associates beginning in 1964. These data have been summarized in a comprehensive review by McKenzie.[2a]

In 1964, no biological function for α-lactalbumin was known. It was not too unreasonable to assume that α-lactalbumin was serving a nutritional function in milk even though the amino acid composition was not unique. Recently a biological role has been assigned to α-lactalbumin as a participant in lactose biosynthesis. The discovery of its function came about in an indirect manner during an investigation of the enzyme lactose synthetase.[12]

LACTOSE



Lactose Biosynthesis

Both the galactosyl and glucosyl moieties of lactose (4-*O*-β-D-galactosyl-α-D-glucose) are derived from blood glucose. Basically, three enzymes are involved in the

(1) Recipient of the 1969 American Chemical Society award in the Chemistry of Milk, sponsored by the Borden Company Foundation, Inc.

(2) (a) H. A. McKenzie, *Advan. Protein Chem.*, **22**, 55 (1967); (b) K. M. Shahani, *J. Dairy Sci.*, **49**, 907 (1966).

(3) A. Wichmann, *Z. Physiol. Chem.*, **27**, 575 (1899).

(4) K. O. Pederson, *Biochem. J.*, **30**, 948 (1936).

(5) T. Svedberg, *Nature*, **139**, 1051 (1937).

(6) M. Sorensen and S. P. L. Sorensen, *Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim.*, **25**, 55 (1939).

(7) W. G. Gordon and W. F. Semmett, *J. Am. Chem. Soc.*, **75**, 328 (1953).

(8) W. G. Gordon and J. Ziegler, *Arch. Biochem. Biophys.*, **57**, 80 (1955).

(9) D. B. Wetlaufer, *Compt. Rend. Trav. Lab. Calrsberg, Ser. Chim.*, **32**, 125 (1961).

(10) M. J. Kronman and R. E. Andreotti, *Biochemistry*, **3**, 1145 (1964).

(11) M. J. Kronman, R. Blum, and L. G. Holmes, *ibid.*, **5**, 1970 (1966).

(12) K. E. Ebner and U. Brodbeck, *J. Dairy Sci.*, **51**, 317 (1968).

biosynthesis of lactose, and they catalyze the following reactions.

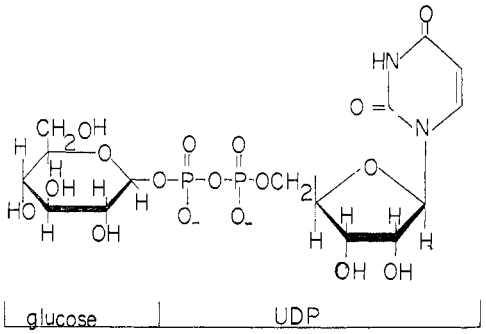
$$\text{UTP}^{13} + \text{glucose-1-P} \rightleftharpoons \text{UDP-glucose} + \text{PP} \quad (1)$$

$$\text{UDP-glucose} \rightleftharpoons \text{UDP-galactose} \quad (2)$$

$$\text{UDP-galactose} + \text{glucose} \longrightarrow \text{lactose} + \text{UDP} \quad (3)$$

URIDINE-5'-DIPHOSPHOGLUCOSE

(UDP-glucose)



Reaction 1 is catalyzed by UDP-glucose pyrophosphorylase (EC 2.7.7.9), reaction 2 by UDP-galactose-4-epimerase (EC 5.1.3.2), and reaction 3 by lactose synthetase (EC 2.4.1.22). It was the study of lactose synthetase that led to the discovery of a biological function of α-lactalbumin[12] as one of two proteins required in the lactose synthetase reaction.

Lactose synthetase was first described by Watkins and Hassid[14a] in 1962 as an enzyme which catalyzed the formation of lactose from particles prepared from guinea pig or bovine mammary glands. In 1964, Babad and Hassid[14b] showed that the enzyme existed in a soluble form in bovine milk, whereas their previous work[14a] had shown that the enzyme was not extractable in a soluble form from tissue. The enzyme was partially purified from bovine milk.[14c]

Studies were under way in this laboratory to purify lactose synthetase from bovine mammary microsomes,[15] and the efforts were generally unsuccessful except that slight activity was found in extracts obtained by sonic oscillation of microsomal particles. Efforts were made to purify the enzyme from bovine skim milk. Casein was removed and an ammonium sulfate fraction was passed through a Bio-Gel P-30 column to remove the ammonium sulfate from the protein. It was observed that the activity curve for lactose synthetase did not correspond to the protein concentration curve. These results suggested that there was a partial separation of proteins, and indeed rechromatography on another Bio-Gel P-30 column, under more carefully defined conditions, resulted in separation of two separate protein peaks. There was no enzymatic activity in either protein peak, named A for the higher molecular weight protein and B for the lower molecular weight protein. Lactose synthetase activity was obtained when fractions from each peak were combined.[16] Thus, lactose synthetase activity was dependent upon the presence of both the A and the B proteins for significant enzymatic activity (Figure 1).

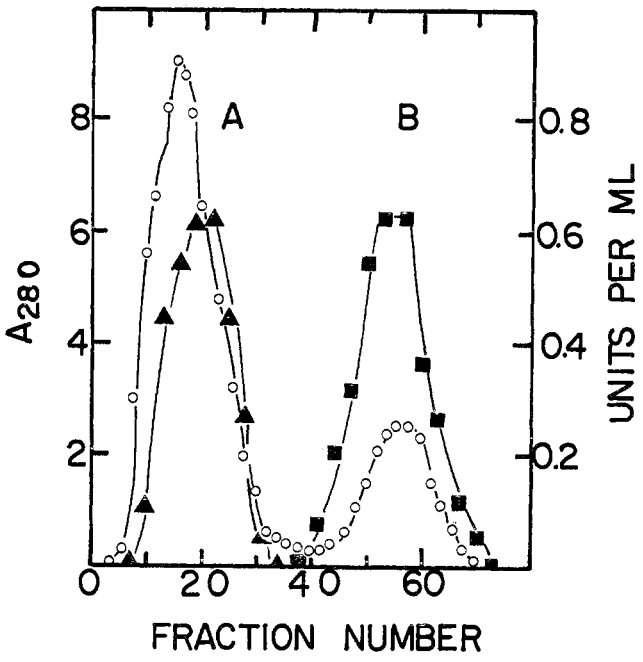


Figure 1. Gel filtration of partially purified lactose synthetase on Bio-Gel P-30 into two protein components. Details are presented elsewhere.[17] ○, protein concentration as A_{280}; ▲, lactose synthetase activity of fraction A in the presence of 0.2 ml (0.5 mg of protein) of fraction B from tubes 56 and 57; ■, lactose synthetase activity of fraction B assayed with 0.2 ml (1.1 mg of protein) of fraction A from tubes 22 and 23. Fraction B was later identified as α-lactalbumin.[20,21]

Further work showed that the A protein was mainly associated with the "microsomal" fraction of the cell whereas the B protein was more evenly distributed between the "microsomal" and soluble portions of the cell.[17] However, recent studies by Coffey and Reithel[18,19] have suggested that the A protein is associated with the Golgi apparatus and particles similar in size to lysosome and secretory granules. It appears that the method used to break the tissue greatly influences the size distribution of the A protein of lactose synthetase in crude homogenates. The B protein of lactose synthetases was purified and crystallized from bovine milk, and it soon became evident that it had chemical and physical properties which were similar to those of α-lactalbumin. Indeed, five-times-crystallized bovine α-lactalbumin purified by a different procedure

(13) UTP, uridine 5'-triphosphate; UDP-glucose, uridine-5'-diphosphoglucose; PP, pyrophosphate; UDP-galactose, uridine-5'-diphosphogalactose; UDP, uridine 5'-diphosphate.

(14) (a) W. M. Watkins and W. Z. Hassid, *J. Biol. Chem.*, **237**, 1432 (1962); (b) H. Babad and W. Z. Hassid, *ibid.*, **239**, PC 946 (1964); (c) H. Babad and W. Z. Hassid, *ibid.*, **241**, 2672 (1966).

(15) The microsomes were prepared from a homogenate of mammary gland[16] by collecting the material which sedimented between 15,000*g* for 15 min and 50,000*g* for 90 min. Enzymes found in such preparations are insoluble and require solubilization for further purification.

(16) U. Brodbeck and K. E. Ebner, *J. Biol. Chem.*, **241**, 762 (1966).

(17) U. Brodbeck and K. E. Ebner, *ibid.*, **241**, 5526 (1966).

(18) R. G. Coffey and F. J. Reithel, *Biochem. J.*, **109**, 169 (1968).

(19) R. G. Coffey and F. J. Reithel, *ibid.*, **109**, 177 (1968).

substituted for the B protein in the enzymatic rate assays at identical protein concentrations.[20] Further chemical, physical, and immunological similarities led to the conclusion that the B protein of lactose synthetase was α-lactalbumin,[21] the common milk-whey protein. Thus, a biological activity was assigned to α-lactalbumin as one protein of an enzyme complex requiring two proteins for activity.

The discovery that α-lactalbumin was one of two proteins required for lactose synthetase activity has a unique biological significance in the sense that the majority of enzymes found in nature are single proteins and the catalytic activity is associated with the protein. Many enzymes are known to have subunit structure; that is, the protein may dissociate under strong perturbations into their component protein subunits. These protein subunits usually have no biological activity, and under certain conditions they may reassociate to again form active enzyme. This type of phenomenon is usually associated with "regulatory" enzymes whose catalytic activity may be controlled by small molecules.

Lactose synthetase is an unusual mammalian enzyme since in milk both proteins are readily separated. However, in mammary tissue the A protein is primarily associated with the particulate material of the cell whereas the B protein or α-lactalbumin is more evenly distributed between the soluble and the particulate fraction. This may be an artifact of the distribution studies since it was found that α-lactalbumin was easy to dissociate from microsomal preparations.[17] To date, lactose synthetase is the only mammalian enzyme which requires two proteins, although several other examples are known in bacterial systems, and it is quite conceivable that other examples will be found in mammalian systems. Indeed, Ritter and Dempsey[22] have discovered a heat-stable protein activator in the conversion of Δ^7-cholestenol to cholesterol in rat liver.

Originally, Babad and Hassid[14c] showed that glucose was the principal galactosyl acceptor (reaction 3), although N-acetylglucosamine was 25% as effective as glucose. Hill and his coworkers[23,24] have made the very important observation that the A protein by itself catalyzes reaction 4. Furthermore, α-lactalbumin

$$\text{UDP-galactose} + \text{N-acetylglucosamine} \longrightarrow \text{N-acetyllactosamine} + \text{UDP} \quad (4)$$

inhibits reaction 4 and allows lactose synthesis to proceed in the presence of glucose. The observations of Hill and his coworkers[23,24] suggest that α-lactalbumin changes the acceptor specificity of the A protein (galactosyl transferase) from N-acetylglucosamine to glucose. It would appear that α-lactalbumin acts in a manner similar to other enzymes which have regulatory subunits. In these enzymes the binding of ligands influences the activity of the catalytic subunit, presumably by some induced change in the structure of the protein.

Hill[23] has suggested that α-lactalbumin be considered as a "specifier protein" which has the ability to change the activity of an existing protein. That is, α-lactalbumin changes the acceptor specificity of a general galactosyl transferase. In the case of reaction 3 the acceptor is glucose, but in the case of reaction 4 it is N-acetylglucosamine. In a sense, this represents a different type of control of biological reactions where the presence of the "specifier protein" directs a given protein to catalyze one reaction in preference to a slightly different but related reaction which occurs in the absence of the "specifier protein." There are many glucosyl and galactosyl transferring enzymes, and it is quite possible that a "specifier protein" may modify their donor or acceptor specificity. Such modification reactions would represent a conservation of an active site since the "specifier protein" could alter the donor or acceptor specificity by inducing appropriate conformational changes. Indeed, other examples of this type of control are appearing in the literature. Grimes, *et al.*,[25] have observed with sucrose synthetase from *Phaseola aureas* that the nucleotide base (XDPG, where X may be uridine, cytidine, adenosine, guanine, or thymine) of the glucosyl donor to fructose may be varied by the addition of a protein fraction to the enzymatic system.

α-Lactalbumin and Lysozyme

Hill and his colleagues[26] have essentially completed the amino acid sequence of bovine α-lactalbumin and have noted a striking similarity to the amino acid sequence of hen egg-white lysozyme where some 40 residues were identical with corresponding residues in lysozyme (Figure 2). The structural similarities between hen egg-white lysozyme and bovine α-lactalbumin have been recently summarized.[24] Results from model building experiments have shown a high degree of similarity between a proposed structure for α-lactalbumin and the known structure of lysozyme previously determined by X-ray crystallographic analysis. The X-ray analysis of goat α-lactalbumin is under progress in Dr. Phillips' laboratory at Oxford, and these data will allow a direct comparison of the tertiary structure of these two proteins.

It should be pointed out the α-lactalbumin does not have lysozyme activity nor does lysozyme interfere with the lactose synthetase reaction, even though both proteins are involved in similar reactions. Lysozyme

(20) K. E. Ebner, W. L. Denton, and U. Brodbeck, *Biochem. Biophys. Res. Commun.*, **24**, 232 (1966).
(21) U. Brodbeck, W. L. Denton, N. Tanahashi, and K. E. Ebner, *J. Biol. Chem.*, **242**, 1391 (1967).
(22) M. C. Ritter and M. E. Dempsey, personal communication.
(23) K. Brew, T. C. Vanaman, and R. L. Hill, *Proc. Natl. Acad. Sci. U. S.*, **59**, 491 (1968).
(24) R. L. Hill, K. Brew, T. C. Vanaman, I. P. Trayer, and P. Mattock, *Brookhaven Symp. Biol.*, **21**, 139 (1969).
(25) W. J. Grimes, B. L. Jones, D. P. Delmer, and P. Albersheim, *Fed. Proc.*, **28**, 341 (1969).
(26) K. Brew, T. C. Vanaman, and R. L. Hill, *J. Biol. Chem.*, **242**, 3747 (1967).

α-LA H_2N-Glu-Gln-Leu-Thr-Lys-*Cys*-*Glu*-Val-Phe-Arg-Glu-Leu-Lys- -Asp-*Leu*-Lys-Gly-*Tyr*-Gly-

Lysoz H_2N-Lys-Val-Phe-Gly-Arg-*Cys*-*Glu*-Leu-Ala-Ala-Ala-Met-*Lys*-Arg-His-Gly-*Leu*-Asp-Ans-*Tyr*-Arg-

α-LA *Gly*-Val-*Ser*-*Leu*-Pro-Glu-*Trp*-*Val*-*Cys*-Thr-Thr- -*Phe*-His-Thr-*Ser*-Gly-Tyr-*Asx*-*Thr*-*Glx*-*Ala*-

Lysoz *Gly*-Tyr-*Ser*-*Leu*-Gly-Asn-*Trp*-*Val*-*Cys*-Ala-Ala-Lys-*Phe*-Glu- -*Ser*-Asn-Phe-*Asn*-*Thr*-*Gln*-*Ala*-

α-LA Ile-Val-Gix-*Asx*- -*Asx*(Glx, *Ser*, *Thr*)*Asx*-*Tyr*-*Gly*-Leu-Phe(*Glx*, *Ile*, *Asx*, Asx)Lys-Ile-*Trp*-Cys-

Lysoz Thr-Asn-Arg-*Asn*-Thr-*Asp*-Gly-*Ser*-*Thr*-*Asp*-*Tyr*-*Gly*-Ile-Leu-*Gin*-*Ile*-*Asn*-Ser-Arg-Trp-*Trp*-*Cys*-

α-LA Lys-*Asx*-Asx-Glx-Asx-*Pro*-His-*Ser*-Ser-Asx-Ile-*Cys*-*Asn*-*Ile*-Ser-*Cys*-Asp-Lys-Phe-*Leu*-Asx-Asx-

Lysoz Asn-*Asp*-Gly-Arg-Thr-*Pro*-Gly-*Ser*-Arg-Asn-Leu-*Cys*-*Asn*-*Ile*-Pro-*Cys*-Ser-Ala-Leu-*Leu*-Ser-Ser-

α-LA Asx-Leu-*Thr*-Asx-Asx-Ile-Met-*Cys*-Val-*Lys*-*Lys*-*Ile*-Leu- -*Asp*-Lys-Val-*Gly*-Ile-*Asn*-Tyr-*Trp*-

Lysoz Asp-Ile-*Thr*-Ala-Ser-Val-Asn-*Cys*-Ala-*Lys*-*Lys*-*Ile*-Val-Ser-*Asp*-Cly-Asp-*Gly*-Met-*Asn*-Ala-*Trp*-

α-LA Leu-*Ala*-His-Lys-Ala-Leu-*Cys*-Ser-Glu-Lys-Leu-Asp-*Gln*- -*Trp*-Leu- -*Cys*-Glu-Cys-*Leu*-COOH

Lysoz Val-*Ala*-Trp-Arg-Asn-Arg-*Cys*-Lys-Gly-Thr-Asp-Val-*Gln*-Ala-*Trp*-Ile-Arg-Gly-*Cys*- -Arg-*Leu*-COOH

Figure 2. Comparison of the amino acid sequence of bovine α-lactalbumin and hen egg-white lysozyme.[26] The residues occupying identical positions in both sequences are in italics.

catalyzes the hydrolysis of a β-(1–4) linkage whereas this linkage is synthesized in the lactose synthetase reaction. On the basis of amino acid composition data, Green[27] has suggested that avidin, found in egg white as a protein inhibitor of biotin-catalyzed reactions, may be structurally related to α-lactalbumin and hen egg-white lysozyme. However, DeLange[28] has concluded, on the basis of amino acid sequence studies, that there is no similarity in the amino acid sequence of avidin to lysozyme or α-lactalbumin.

Hill[24] has also speculated on the evolutionary origins of α-lactalbumin and hen egg-white lysozyme. The high degree of homology in the primary structure suggests that both proteins arose from a common primitive ancestral gene which later, through the process of gene duplication and then divergence by mutation, gave rise to two genes, one of which controlled the sequence of lysozyme and the other controlled the sequence of α-lactalbumin. Since α-lactalbumin acts as a "specifier protein," it may be probable that lysozyme has this function, and indeed Hill[24] has made this suggestion.

α-Lactalbumin and Lactose Synthetase

Work in this laboratory has shown that lactose synthetase from the milk of the bovine, sheep, goat, and human could be separated into two protein fractions by molecular sieve chromatography.[21] Both fractions, designated the A and B proteins, were required for enzymatic activity, and it was also observed that the A protein from a given species could react enzymatically with the B protein (α-lactalbumin) obtained from the other species. The reverse situation was also true, and these data suggest that there was a fair degree of structural similarity in the active sites of α-lactalbumin proteins obtained from different species. However, it was also observed that there were immunological differences between various sources of α-lactalbumin.[29] Antisera specific to bovine α-lactalbumin would cross-react with α-lactalbumin obtained from other ruminants but would not cross-react with α-lactalbumin obtained from nonruminant animals. A recent experiment with antiserum to pig α-lactalbumin showed that this antiserum would not cross-react with the ruminant or other nonruminants tested.[30] It would appear that the groups determining antigenicity in the various α-lactalbumins are structurally different, more so than the structural requirements for enzymatic activity, since all the α-lactalbumins tested to date will react enzymatically with bovine A protein.

α-Lactalbumin inhibits the formation of N-acetyllactosamine (N-acetylglucosamine as substrate, reaction 4), and this inhibition has apparent biological significance. Turkington, *et al.*,[31] have shown that the activity of the galactosyl transferase (reaction 4) is high during pregnancy whereas lactose synthetase activity is practically nonexistent. However, at

(27) N. M. Green, *Nature*, **217**, 254 (1968).
(28) R. J. DeLange, *Fed. Proc.*, **28**, 343 (1969).
(29) N. Tanahashi, U. Brodbeck, and K. E. Ebner, *Biochim. Biophys. Acta*, **154**, 247 (1968).
(30) B. Colvin and K. E. Ebner, unpublished results.
(31) R. W. Turkington, K. Brew, T. C. Vanaman, and R. L. Hill, *J. Biol. Chem.*, **243**, 3382 (1968).

parturition α-lactalbumin is formed; this inhibits reaction 4 and allows lactose synthesis to procede. During pregnancy and just prior to parturition, the mammary gland synthesizes glycoproteins that are important in the formation of antibodies which appear in the colostrum. The data in Figure 3 show that the N-acetyllactosamine reaction is inhibited 50% with 5 μg of α-lactalbumin/ml and 100% by 100 μg/ml. The concentration of α-lactalbumin in milk is about 1 mg/ml, and a conservative estimate of its concentration in bovine mammary tissue is 30–40 μg/g of wet tissue, which is sufficient to inhibit the formation of N-acetyllactosamine and allow lactose synthesis to procede. Thus, a rather high concentration of α-lactalbumin is required to completely inhibit the N-acetyllactosamine reaction. These results may partially explain the high concentration of α-lactalbumin found in milk and tissue.

The whey portion of milk also contains another protein, β-lactoglobulin, and its concentration is about twice that of α-lactalbumin. To date no biological function is known for β-lactoglobulin, and it is highly likely that it has a biological function similar to that of α-lactalbumin.

It is possible to assay for either of the protein components in the lactose synthetase reaction, and several assays have been described. Basically, a limiting amount of one of the protein components is assayed for enzymatically by the lactose synthetase reaction in the presence of an excess amount of the other protein component. The development of such assays is important since both the A protein and α-lactalbumin may be used as biological indicators of mammary gland function, and indeed such studies have been initiated.[31] Also, a reliable assay is required for α-lactalbumin, especially for chemical modification studies where the effect of a chemical modification of an amino acid residue is measured as a function of activity in the lactose synthetase reaction.

Two observations prompted a thorough investigation of the assay. First, the specific activity of α-lactalbumin increased as the A protein became more purified; second, under certain conditions there was a nonlinear response of enzymatic activity to protein concentration. Also it was observed that, in the assay for the A protein, high levels of α-lactalbumin were inhibitory in a linear manner resulting in an optimum level of α-lactalbumin required for maximum activity of a limiting amount of the A protein. These studies are completed, and satisfactory assays are available for both the A protein[32] and α-lactalbumin[33] provided certain precautions are taken. In developing the assay for the A protein, it was observed that there was an inverse relationship between the concentration of glucose and α-lactalbumin required to give optimum activity in the assay.[32] This was not the case with UDPGal where the K_m was essentially independent of α-lactalbumin concentration.

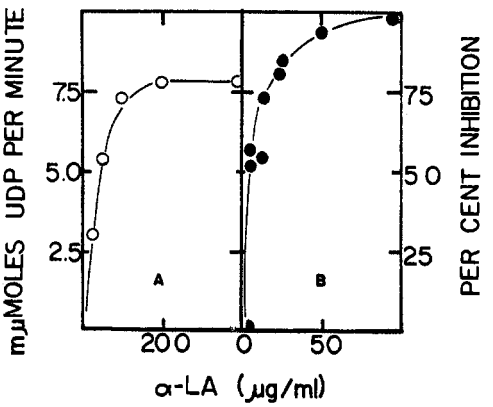


Figure 3. The role of α-lactalbumin (α-LA) in the lactose synthetase (A protein limiting, LS_A) and N-acetyllactosamine (NAL) assays.[32] Part A: LS_A activity *vs.* α-LA concentration in the standard assay. Part B: per cent inhibition of the NAL reaction by α-lactalbumin (see reaction 4 in the text). The concentration of the A protein was 12 units/ml.

In the development of the assay for α-lactalbumin, two important observations were made. The first was that the A protein which has a very low ability to synthesize lactose in the absence of α-lactalbumin[23] can be stimulated to synthesize appreciable amounts of lactose in the presence of high concentrations of glucose, and the apparent K_m for glucose in this reaction is about 1.4 *M*.[33] The stimulation of the endogenous lactose synthetase activity of the A protein was specific for glucose since mannose, glactose, and sucrose had no effect. It appears that high concentrations of glucose and α-lactalbumin may be acting in a similar manner since there is a reciprocal relationship between the concentration of α-lactalbumin and glucose required for maximum activity in the assay for the A protein. It is probable that both α-lactalbumin and high concentrations of glucose may be causing similar conformational changes in the A protein which allow the A protein to preferentially synthesize lactose. Thus, the catalytic site of lactose synthetase resides in the A protein since it does have a very low endogenous activity for lactose synthesis which may be stimulated by high concentrations of glucose.

Other experiments have been concerned with the possibility of detecting a stable complex between the A protein and α-lactalbumin. Such complexes have been demonstrated in the tryptophan synthetase system[34] where one of the substrates is required for complex formation and in the ribonucleotide reductase system where Mg^{2+} is required for complex formation.[35] Sev-

(32) D. K. Fitzgerald, U. Brodbeck, I. Kiyosawa, and K. E. Ebner, in preparation.

(33) D. K. Fitzgerald, B. Colvin, R. Mawal, and K. E. Ebner, in preparation.

(34) T. E. Creighton and C. Yanofsky, *J. Biol. Chem.*, **241**, 980 (1966); M. E. Goldberg, T. E. Creighton, R. L. Baldwin, and C. Yanofsky, *J. Mol. Biol.*, **21**, 71 (1966).

(35) N. C. Brown, A. Larsson, and P. Reichard, *J. Biol. Chem.*, **242**, 4272 (1967).

Table I

Enzymes in Which Two Proteins Participate

Enzyme	Source	No. of proteins	Major reaction catalyzed	Comments
1. Tryptophan synthetase (EC 4.2.1.20)	*E. coli*[34]	2 (α, β)	Indoleglycerol phosphate → L-tryptophan + glyceraldehyde 3-phosphate	Complex ($\alpha_2\beta_2$) requires serine and pyridoxal phosphate. α subunit modifies activity of β subunit[a]
	Neurospora crassa[b]	1		
	Nicotiana tabacum[c]	2 (A, B)		No evidence for complex. B component reacts with α of *E. coli* to give enzymatic reaction
2. Lipoic acid activating	*S. faecalis*[d]	2 (PS-2A, PS-2B)	Participates in pyruvate dehydrogenation; pyruvate + $NAD^+ \rightleftarrows$ acetyl-CoA + CO_2 + NADH + H^+	PS-2A forms lipoly adenylate; PS-2B functions as a lipoyl carrier
3. Glutamate mutase (EC 5.4.99.1)	*Cl. tetanomorphum*[e,f]	2 (S, E)	Glutamate $\leftrightarrows$ β-methyaspartate	E binds coenzyme and binding is increased by S; no evidence for complex
4. (L)-Citramalate hydrolase (EC 4.2.1)	*Cl. tetanomorphum*[g]	2 (I, II)	Mesaconate $\rightleftarrows$ citramalate	No evidence for complex. Component II dissociates[p] and has two conformational states[q]
5. Glycine decarboxylase	*Peptococcus glycinophilus*[h]	2 (P_1, P_2)	Bicarbonate exchange with glycine	
6. Acetyl-CoA carboxylase (EC 6.4.1.2)	*E. coli*[i]	2 (E_a, E_b)	ATP + HCO_3^- + acetyl-CoA $\rightleftarrows$ ADP + P_i + malonyl CoA	$E_a + HCO_3^- + ATP \xrightleftharpoons{Mn^{2+}} E_aCO_2 + ADP + P_i$; $E_aCO_2 + \text{acetyl-CoA} \rightleftarrows E_a + \text{malonyl-CoA}$; E_a contains biotin and in the presence of E_b it is more sensitive to avidin
7. Ribonucleoside reductase	*E. coli*[j]	2 (B_1, B_2)	$CDP \xrightleftharpoons[B_1, B_2]{ATP, Mg^{2+}}$ deoxy-CDP	Complex[35] requires Mg^{2+}; B_1 binds allosteric effectors
8. PEP-dependent formation of Fru-1-P	*A. aerogenes*[k]	Enzyme II has two components	PEP + HPr $\xrightarrow{\text{enz I}}$ Pry + Phospho-HPr; sugar + Phospho-HPr $\xrightarrow[II]{enz}$ sugar-P + HPr (HPr = heat-stable protein)	Heavy component of enzyme II is constitutive, light component is inducible. Light component (fructose specifier protein reduces K_m for fructose in the presence of heavy component)
9. QB-RNA polymerase	QB phage infected *E. coli*[l,m]	2 (heavy, light)	RNA-dependent, RNA polymerase	Light component found in uninfected *E. coli*. Heavy component has poly C dependent poly G activity
10. Δ^7-Sterol Δ^5-dehydrogenase	Rat liver[22]	2	Δ^5-Cholestenol → $\Delta^{5,7}$-cholestadienol	Heat-stable protein which activates both reactions
$\Delta^{5,7}$-Sterol Δ^7-reductase		2	$\Delta^{5,7}$-Cholestadienol → cholesterol	
11. Sucrose synthetase	*Phaseolus aureus*[25]	2	XDPG + Fru → sucrose + XDP	Modifier protein inhibits synthesis with UDPG and stimulates synthesis with ADPG, CDPG, and GDPG
12. Lactose synthetase	Mammary gland[16,17,20,21]	2 (A, α-lactalbumin)	UDPGal + Glu → Lac + UDP	A protein catalyzes[23] UDPGal + NAG → NAL + UDP; α-lactalbumin inhibits NAL reaction. See text.
13. RNA polymerase	*E. coli*[n,o]	2 (PC, σ)	DNA-dependent, RNA polymerase	Required for transcription[n] of T_4 DNA and acts in a cyclic manner[o]

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eral types of experiments have been performed with the bovine A protein and α-lactalbumin. These include density gradient experiments with various combinations of substrates, binding experiments with α-lactalbumin labeled with iodine-125, and fluorescence quenching with α-lactalbumin modified with dansyl chloride. To date all of the results have been negative, suggesting that under the experimental conditions used no stable intermediate exists. Thus, the complex between the two proteins appears to have a very high dissociation constant and is not detectable by present techniques. Switzer and Barker[36] were unable to demonstrate complex formation between the two protein components of the glutamate mutase reaction. The tryptophan synthetase[37] from *Nicotiana tabacum* has been resolved into two proteins and has many characteristics similar to the *E. coli* enzyme, but no evidence for a complex between the two proteins was found, as was the case with the *E. coli* enzyme.[34] It is of interest to note that the B protein of *N. tabacum* will react with the A protein of the *E. coli* enzyme to produce an active enzyme. Although the information is still somewhat limited, it would appear that in the enzymes where two proteins are required the ability to find chemical evidence for a distinct complex will be variable.

Concluding Remarks

The enzymes in which two proteins participate are an interesting group and provide systems for studying protein–protein interactions as related to catalysis. Some of these enzymes are listed in Table I, and detailed knowledge of their function and mechanism of action are at varying stages of development. It is highly likely that there will be differences in mechanism of action within the group, and indeed this becomes apparent when Table I is examined in detail.

Lactose synthetase is unusual in that one of the proteins, α-lactalbumin, is able to change the acceptor specificity of an apparent general galactosyl transferase. This also appears to be the case with sucrose synthetase[25] where the base of the glucosyl donor (to fructose) is modified. A similar situation exists with the PEP-dependent system forming fructose 1-phosphate[38] where a light component of enzyme II reduces the K_m for fructose.

Lactose synthetase has become an attractive experimental system for a variety of investigations. The enzyme may be used to study mammary gland differentiation, development, and mode of action of hormones. There is a high degree of structural homology between α-lactalbumin and hen egg-white lysozyme, thus allowing for studies on the structural evolution of proteins. α-Lactalbumin is an ideal system for chemical modification studies, especially since the X-ray analysis of the α-lactalbumin is under way, and it may be assayed enzymatically or immunologically. Palmiter[39] has recently evoked a third component in crude preparations of mouse mammary tissue, and further work is required to evaluate the significance of this observation. The protein of lactose synthetase appears to be a rather general galactosyl transferase and is found in many tissues.[24,40] Lactose is synthesized in the presence of A protein from these various sources and bovine α-lactalbumin, and thus it would appear that the mammary gland uniquely synthesizes α-lactalbumin and allows lactose synthesis to procede. The possibility also exists that α-lactalbumin may have a broader function in the mammary gland and be involved in other regulatory phenomena.

The A protein can transfer galactose[24] to other proteins such as orosomucoid, and thus the A protein may also be involved in the synthesis of various glycosyl proteins, including the blood group substances. It also is anticipated that other examples of this type of control where one protein influences the activity of another protein will be found in biological systems. Studies with lactose synthetase should give a deeper insight into this type of control mechanism.

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