

## Enzymatic Conversion of Common Aldoses into Valuable Aldonic Acids Used in Pharmaceutical and Industrial Products.

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A mission of our research group is to convert low-valued agricultural by-products into value-added food and industrial products. As part of that mission, we previously developed new methods to convert inexpensive, agriculturally-derived reducing sugars (aldoses) such as lactose (a by-product of cheese manufacture) into commercially valuable derivatives (Fig. 1) such as lactulose [1-3] and tagatose [2,4]. In spite of these efforts, several million tons of lactose still go un- or under-utilized annually in the U.S.

We have recently begun studying an aldonic acid derived from lactose, lactobionic acid (4-O- $\beta$ -D-galactopyranosyl-D-gluconic acid) (Fig. 2). While current world consumption of this derivative is small, it has several unique and promising applications. First, lactobionic acid is a critical component of perfusion solutions that are used to sustain the viability of human organs during emergency transport to organ recipients [5]. In addition, lactobionic acid is covalently linked to commercial antibiotics, such as erythromycin, to enhance solubility and stability [6]. Finally, lactobionic acid was recently shown to be an exceptional chelator of metal ions, and thus it can serve as an environmentally safe substitute for phosphates in laundry detergents [7]. This latter application has potential to utilize great quantities of lactobionic acid *if* economical methods for its synthesis from lactose can be devised.

Currently, lactobionic acid is produced by either bromine [8] or electrochemical [9] oxidation of lactose. It has also been reported [10] that lactose can be oxidized to lactobionic acid by fermentative microorganisms. None of these methods, however, are practical for economical, large scale production. We are therefore studying the use of enzymes for this purpose. Since there are no commercially available enzymes capable of catalyzing the conversion of lactose to lactobionic acid, we conducted a search for an enzyme with this capability. Our objective was to find an enzyme with many of the properties of glucose oxidase (GO), e.g. high stability, no requirement for external co-factor, and the ability to oxidize C-1 of an aldose to a carboxylate group, but with the additional ability to oxidize lactose and other aldoses (GO is very specific for glucose and will not oxidize other substrates).

We have now isolated such an enzyme from a proprietary source. The enzyme has now been partially purified (Fig. 3) yielding a preparation that converted lactose quantitatively into lactobionic acid (11). Approximately 50 units of the partially purified enzyme was immobilized on a commercial, glutaraldehyde-type enzyme support. An oxygenated, buffered solution containing 12.5 grams of lactose was re-circulated through the reactor for 72 hours. The original (zero time) and 72 hour solution was analyzed (Fig. 4) by HPLC, using a  $\beta$ -cyclodextrin bonded stationary phase, as previously described (12). The top panel shows minor buffer and solvent peaks (3 and 8 minutes) and a major peak for lactose at 6.7 minutes in the zero time sample. The bottom panel (72 hr) shows a small

trace of lactose, buffer peaks, and one new peak at 9.5 minutes, which was identified as lactobionic acid. Quantitative analysis of the chromatograms showed that greater than 90% of the lactose was converted into lactobionic acid by the enzyme during the 72 hour period. Analysis of the 72 hour reaction mixture by carbon and proton NMR spectroscopy showed that no other enzyme products were present, indicating that the partially purified enzyme was "functionally" pure for our synthetic purposes. The reactor was re-usable with approximately 20% loss of activity following each use.

In addition to oxidation of lactose, the new enzyme oxidized glucose and other mono- and oligosaccharides. This provides a new opportunity to produce novel aldonic acids for which there has been limited availability.

In summary, we have:

1. Identified and isolated an enzyme that converts lactose into lactobionic acid.
2. Prepared a functionally-pure form of the enzyme.
3. Developed a simple immobilized enzyme system capable of converting lactose to lactobionic acid on the multi-gram scale.

In the future we plan to:

1. Optimize the stability of the immobilized enzyme.
2. Work with industrial partners to determine commercial applicability.
3. Use the enzyme to produce other novel or rare aldonic acids from common aldoses.

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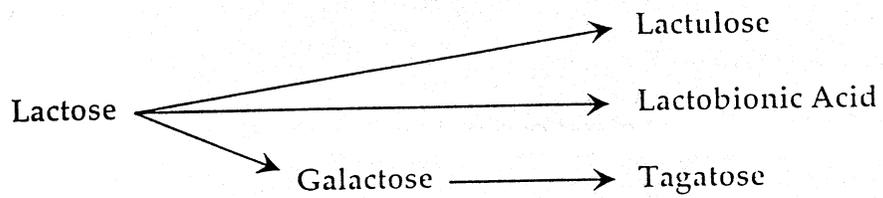


Figure 1. Program on conversion of lactose (milk sugar) into value-added products.

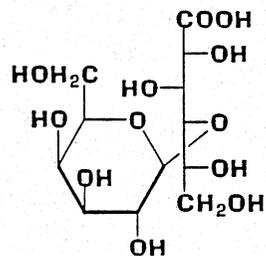


Figure 2. Lactobionic acid (4-O- $\beta$ -D-galactopyranosyl-D-gluconic acid) [96-82-2].

