



Biotherapeutics

Recombinant Human Albumin for Use as Pharmaceutical Excipient

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Summary

With the ongoing challenge of reducing the risk of infectious disease transmitted by human blood or plasma components, the pharmaceutical industry is adopting recombinant forms of production for these products. We describe recombinant human albumin (rhA), fully purified from transgenic bovine milk. rhA purity exceeds commercial-grade albumin for injection derived from human plasma. Source herd quality is achieved and maintained by well-controlled herd management and veterinary practices, herd surveillance, qualified processes and analytics. High purity research-grade rhA has demonstrated comparability in structure and peptide map to plasma-derived human albumin. Recombinant human albumin from a reliable source for excipient use can improve product quality, solve regulatory sourcing issues, and simplify purchasing and documentation requirements.

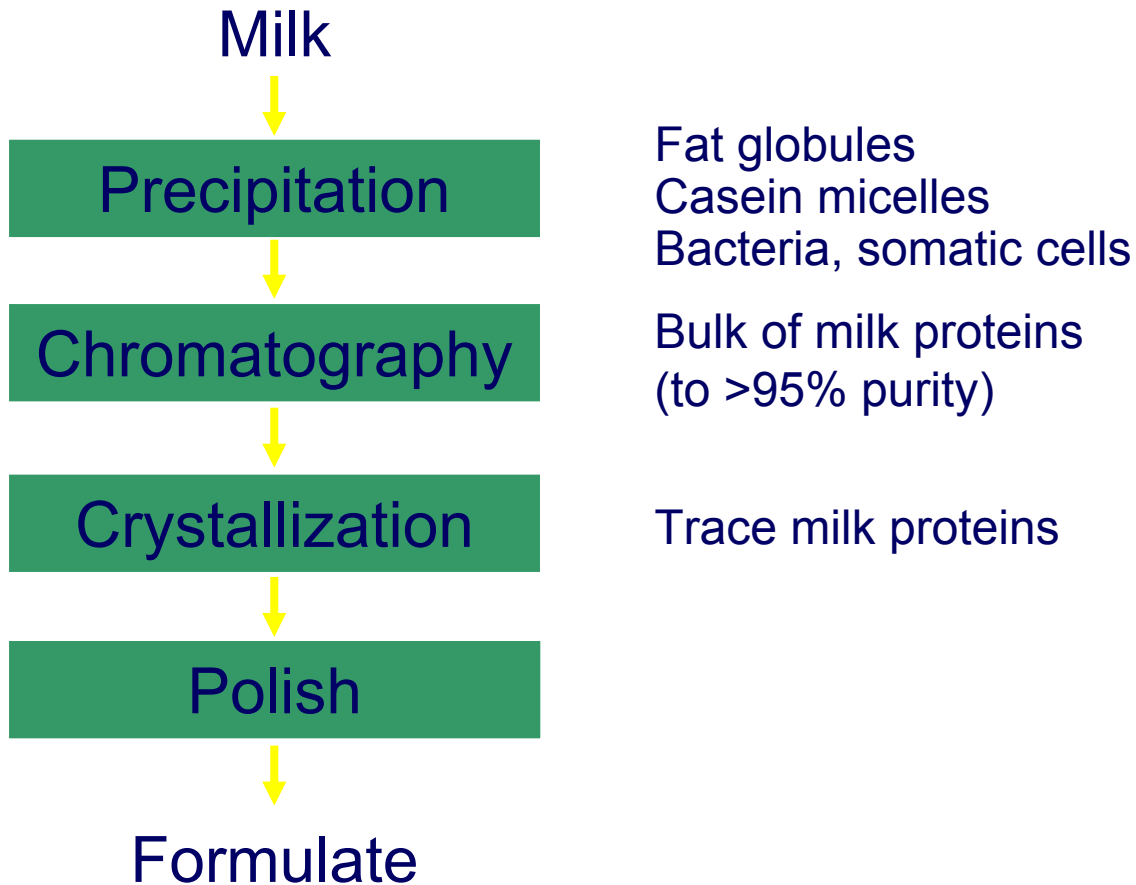
Materials and Methods

Transgenic cows producing human albumin in their milk were developed by nuclear transfer (Echelard *et al.*, *Theriogenology* 57: 779; 2002).

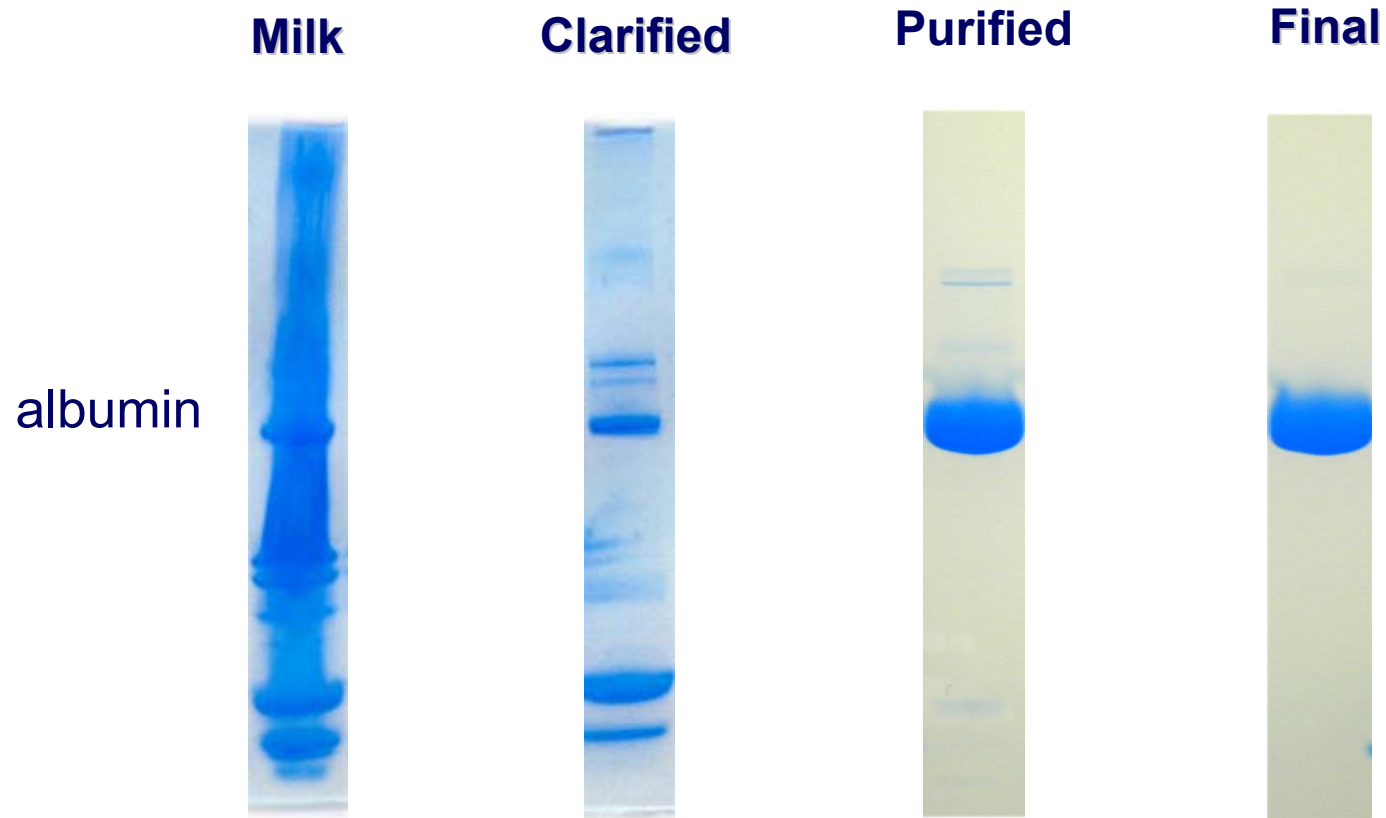
Purification of rhA from milk was achieved by the following process: casein micelles and lipids were removed by a series of precipitation and centrifugation steps, majority of soluble proteins were removed by affinity chromatography, and additional purity was achieved by crystallization. Purified material was aseptically filtered and filled.

Sequence identity to a commercially available plasma derived albumin was confirmed by tryptic peptide mapping. Endotoxin, mycoplasma, and viruses were assayed by standard methods. Final vial product is an aqueous 25-27% solution stable at 4°C.

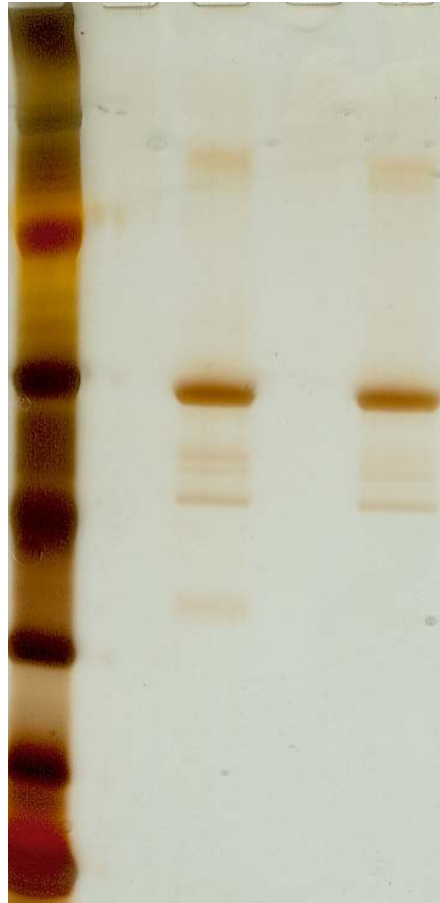
rhA Purification Process



Removal of rhA Impurities: SDS PAGE



Comparison of Purified rhA and Plasma-Derived Human Albumin by Silver Stained Gel



albumin

Results

Fifty-seven transgenic cows producing rhA have been developed. rhA was purified from the milk using precipitation, affinity chromatography and crystallization resulting in a highly purified final product. Purified rhA was analyzed and compared to commercially available plasma-derived serum albumin. Results showed comparability by tryptic peptide map and dichroism, and the rhA had a higher purity by SEC and silver stained SDS-PAGE analysis.

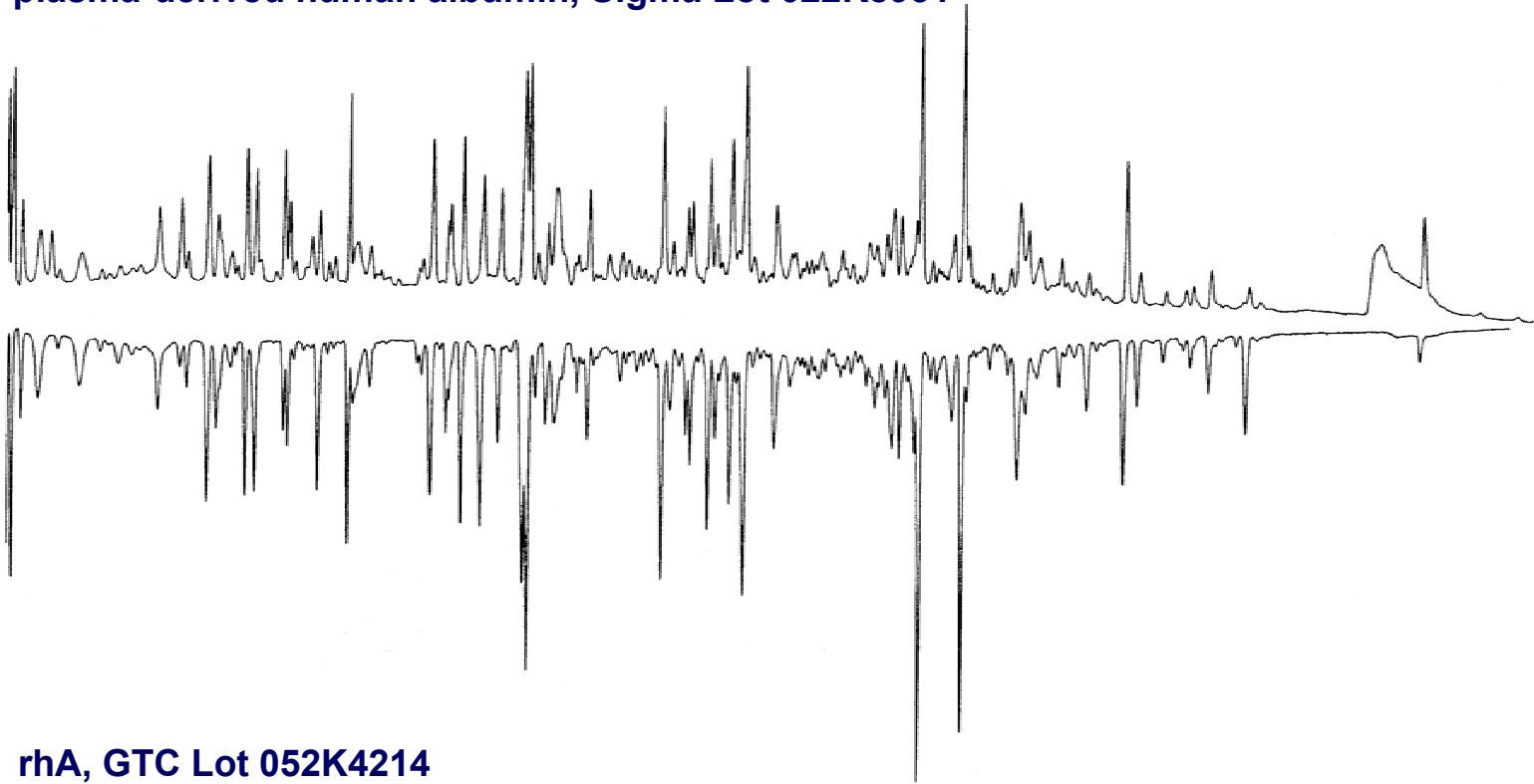
The product was tested for endotoxin, mycoplasma, bovine host proteins, adventitious (viral) agents and DNA. Endotoxin was 0.6EU/mg, bovine host proteins <25 ppm, no mycoplasma or bovine viruses (9 CFR) were detected and residual DNA 0.12 pg/mg.

This preliminary work is encouraging for development of a replacement for other sources of albumin, as an excipient in formulations, media and other applications.

rhA Peptide Map

Comparison of Tryptic Maps

plasma-derived human albumin, Sigma Lot 022K8931



Results: Safety Tests

Mycoplasma	Cultivable and non-cultivable	Negative
Endotoxin	LAL	0.6 EU/mg

Bovine Residual Impurities

Bovine virus	9 CFR	Negative
Residual DNA	Hybridization	0.12 pg/mg
Residual BSA	ELISA	7 ppm
Residual IgG	ELISA	< 0.25 ppm
Residual α -lactalbumin	ELISA	< 0.4 ppm
Residual β -lactoglobulin	ELISA	6 ppm

Results: Product Characterization

Purity by SEC

98% monomer

Identity by peptide map

comparable to
plasma albumin

Structure by circular dichroism

comparable to
plasma albumin

Fatty acid

2.4 moles/molecule
albumin

Supply Benefits of rhA

- ◆ Single worldwide product formulation with single supplier
- ◆ From an identified, controlled source
- ◆ Uniform, well characterized, high purity product
- ◆ Large volume supply: each cow can produce 20-25L of milk containing 1-2 g/L rhA per day.

Regulatory and Quality Benefits

Sourcing and Regulatory Documentation

- ◆ Uniform product specifications world-wide
- ◆ Material from defined and managed source
- ◆ Reliable, predictable and traceable

Product Consistency

- ◆ Cloned genetic material assures absence of human albumin variants that could have charge or solubility effects on formulated product
- ◆ Well characterized final product

Discussion

The first challenge in sourcing is to ensure a safe supply. Although plasma-derived human albumin purified by ethanol fractionation processing has demonstrated acceptable safety from adventitious agents, there is always the potential risk of infection from new or undetected agents when using material derived from an uncontrolled source. Products containing plasma-derived human albumin must be labeled with a warning about the human derived material and the potential for infection.

A second challenge is to achieve consistent albumin sourcing. Plasma-derived human albumin, collected and processed in various locations are governed by local regulations. There is a need for a single world-wide standard for albumin.

The use of highly purified, recombinant albumin derived from a controlled and well-managed source can provide both a safe and consistent supply for many formulation uses.

rhA Project Status

GTC Biotherapeutics manages production and marketing of rhA on behalf of *Taurus LLC*.

Research grade samples of rhA are available for evaluation upon request at

www.gtc-bio.com/albumin