Recombinant Human Albumin: Applications as a Biopharmaceutical Excipient

In addition to batch-to-batch consistency, the use of recombinant human albumin in biopharmaceutical formulation provides many of the recognised benefits of using human serum albumin as an excipient, whilst avoiding the risks of transmitting viral and prion contaminants.

By David Mead, Dermot Pearson and Maree Devine at Novozymes Delta Ltd

Dr David Mead is Director of Intellectual Property and Business Development at Novozymes Delta Ltd, based in Nottingham (UK). His first degree was in Microbiology from the University of Kent, followed by a PhD from UMIST (Manchester, UK) in plasmid-host interactions in yeast. He initially worked as a Research Scientist in Glaxo's Biotechnology Group, followed by a post-doc back in academia (University of Manchester) managing a project between chemistry and molecular biology on superoxide dismutase. Dr Mead has had a number of roles within Novozymes Delta Ltd, including Manager of Fermentation with responsibility for the development of commercial and scaleable fermentation processes integrated with molecular biology and downstream purification, including technology transfer, both internally and externally. He was also responsible for setting up and managing the Technical Support function for Recombumin® manufacturing, before taking responsibility for the company's intellectual property and business development.

Dermot Pearson has worked for Novozymes Delta Ltd since 1987, following completion of a PhD in Fermentation Technology at Dublin City University (Dublin, Ireland). Prior to that, he was a graduate in Biochemistry from University College, Dublin. While with Novozymes Delta Ltd, he has performed a number of scientific and management roles, from R&D Process Development through QC and Operations Management to his current role as Director of Commercial Operations. In this role, he is responsible for Marketing and Business Development, promoting products and services and negotiating technology licence and product supply agreements with customers worldwide; in this capacity, he is also responsible for Novozymes Delta Ltd's Regulatory Affairs function.

Maree Devine is Commercial Operations Manager at Novozymes Delta Ltd. She has a degree in Parasitology from the University of Glasgow (Scotland) and, since completing her PhD at Nottingham University, has held positions in both research and sales and marketing. Prior to joining Novozymes Delta Ltd, she was a Technical Product Specialist with EMD Biosciences, and then joined ThermoFisher (UK) as a Product Merchandising Manager, Life Sciences, where she maintained, developed and marketed the product portfolio. In her current role as Commercial Operations Manager, Dr Devine is responsible for sales and marketing co-ordination, customer liaison, event management, market research and marketing campaign development.

Human serum albumin (HSA) is one of the most widely used proteins in the pharmaceutical industry. Synthesised in the liver, this non-glycosylated 66kD molecule is well characterised and occurs naturally in the body as a plasma protein at concentrations of 42-45mg/ml (1). HSA regulates the colloidal osmotic pressure of blood and buffers acid-base changes; it is also responsible for the transportation of a range of substances, which have the potential to be toxic in the unbound state, but are non-toxic when bound to albumin (1). Traditionally used as a therapeutic agent, HSA's primary function is the restoration and maintenance of blood volume in situations such as surgery and blood loss, traumatic shock, plasma exchange and the treatment of burns. Exhibiting a lack of toxicity and immunogenicity, HSA has also been used as a manufacturing excipient for numerous pharmaceutical and biological products – for example, as a stabiliser in vaccines and therapeutic protein drugs, in coatings for medical devices, and as a component in drug delivery systems and imaging reagents such as those used for X-rays (2).

HARNESSING HSA PROPERTIES

HSA's various *in vivo* functions and physical properties have been exploited in a number of biopharmaceutical applications – for example, as an excipient:

- Its amphiphilic properties make it suitable as an additive to inhibit adsorption of the active protein to the container via competitive adsorption mechanisms
- Its surface active character enables it to fulfil the role of a surfactant, thereby preventing protein aggregation
- In some instances, it stabilises the conformational structure of the active molecule to maintain its bioactivity throughout the product shelf-life

HSA also has a high glass transition temperature, which in combination with its amphiphilic nature makes it an ideal vehicle for cryoprotection.

Native HSA demonstrates remarkable stability, with an *in vivo* half-life of 15-20 days; this is attributable in part to the presence of 17 disulphide linkages in the protein. *In vitro*, the molecule's stability is increased and it remains in solution at room temperature helping to sustain the shelf-life of the final biopharmaceutical product. During manufacture, HSA can withstand heating to 60°C for 10 hours to facilitate viral inactivation.

TRADITIONAL HSA MANUFACTURE

HSA is currently used in greater volumes than any other biopharmaceutical solution, with worldwide manufacture in the order of hundreds of tonnes annually (3). Since 1940, it has been produced by fractionation of plasma obtained from donors (4). While the safety profile of HSA with respect to viral transmission has been excellent, the theoretical risk of the transmission of new and known infectious agents (such as variant Creutzfeld-Jacob disease, HIV, hepatitis and West Nile virus) via the continued use of blood- and plasma-derived products is ever-present and unlikely to be completely eliminated. This has resulted in regulatory authorities worldwide creating a myriad of regulations to limit the use of plasma-derived materials with the aim of minimising transmission risks and necessitating a dedicated drive from within the industry to develop substitute products and ever-more sophisticated tools for the detection, clearance and removal of adventitious agents from serum-derived products (5).

Such safety concerns provide the strongest motivations to develop recombinant human albumin (rHA) as a suitable alternative to HSA, for use as an excipient in biotherapeutics. As well as avoiding the transmission of serum-derived disease agents, other key advantages of using rHA over HSA include increased batch-to-batch consistency (which for industrial applications could mean the difference between performing several timely and costly batch verifications per year or not) and breaking a heavy reliance on an increasingly unpredictable supply chain.

DEVELOPING AN rHA

A number of microbial host/vector systems – including *K. lactis* (6), *P. pastoris* (7), *H. polymorpha* (8) and *S. cerevisiae* (baker's yeast) (9) – have been looked at for the production of rHA. However, over the past few years particular advances in yeast-based protein expression and scale-up have led to the development of an industrial-scale manufacturing process that can produce a high purity, high quality rHA that is animal-free and suitable for use as an excipient in biotherapeutics.

The molecular engineering of a series of proprietary *S. cerevisiae* strains to select for various traits, such as genetic stability and high copy number, has been pioneered by Novozymes Delta Ltd (previously Delta Biotechnology Ltd) for the production of rHA (Recombumin®, the company's lead product). Based on a proprietary 2-micron plasmid construct, their yeast-based expression system is optimised for the production of recombinant proteins where glycosylation does not naturally occur or can be engineered without loss of performance of the active molecule. This proprietary 2-micron plasmid construct is in an otherwise plasmid-free background, and the high copy number plasmids are very stable with an expression cassette consisting of only yeast DNA and the cDNA for HSA, removing concerns about the use of antibiotic resistance genes of bacterial origin. The Novozymes Delta *S. cerevisae* strains have been engineered to be protease-deficient, and can generate yields of up to 5g/L, avoiding the use of hazardous solvents in the process. Furthermore, like the majority of molecules expressed in the system, the Recombumin® molecule is secreted which significantly aids down-stream processing. The Recombumin® production process has been successfully scaled up from 10L to 8,000L at the company's cGMP-compliant rHA manufacturing facility at Nottingham, UK.

Two physiological phenomena related to the many accumulative genetic changes in *S.cerevisae* had to be overcome during the development of a robust industrial-scale process for the production of such a high grade rHA. The first was a reduction in the critical growth rate, μ_{crit}, which is the highest rate at which growth is fully aerobic without production of ethanol or acetate. Values above μ_{crit} will result in the build-up of unwanted by-products. Although a lower μ_{crit} value theoretically results in a reduction in bioreactor productivity, this is of little economic significance since – at large scale – factors such as mass and heat transfer limit the maximum growth rate. The decreased μ_{crit} is accommodated by lowering the parameter used in the automatic feed control algorithm that determines the effective growth rate in the process.

The second phenomenon is a tendency of the organism to produce acetate under conditions where there is a slight excess in nutrient supply. Ethanol production is readily detected by a rise in respiratory quotient (RQ) determined by exit gas analysis. Hence, the control algorithm is designed to adjust the feed rate automatically. Acetic acid cannot be detected by a change in RQ but can

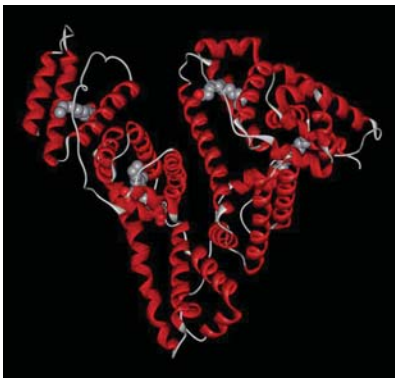

Figure 1: Structure of rHA with five molecules of myristate bound

be identified by changes in conductivity. This principle was used to develop a sub-routine in the automatic control procedure to adjust the feed rate appropriately.

HSA VERSUS rHA: SAFETY AND TOLERABILITY

X-Ray crystallography and mass spectrometry studies revealed that Recombumin® rHA is structurally identical to HSA (see Figure 1) and significantly more homogeneous (10). A Phase I study has been conducted comparing the safety, tolerability, and pharmacokinetics/pharmacodynamics of rHA with HSA (11). Two double-blind, randomised trials were performed in healthy volunteers using intravenous (IV) and intramuscular (IM) administration. Thirty volunteers participated in the IV trial, each receiving increasing doses (10g, 20g and 50g) of either rHA or HSA. The IM trial comprised 500 volunteers, each receiving 5 repeat doses of 5mg (100 subjects), 15mg (100 subjects) or 65mg (300 subjects) of rHA or HSA. Both trials recorded all adverse events and were conventionally classified; potential allergic responses were also monitored. Blood samples were taken in both studies to test for IgG or IgE antibodies against the test human albumin products and potential impurities.

For the IV study, pharmacokinetic/pharmacodynamic assessments were carried out to include measurement of serum albumin, colloid osmotic pressure and haematocrit pre- and post-infusion. No serious or potentially allergic events were noted with either product in the IV study. Furthermore, there was no immunological response to either product, and dose level did not influence the study outcomes. Serum albumin, colloid osmotic pressure changes and haematocrit ratio were as expected, with no differences between rHA and HSA. The study concluded that rHA and HSA exhibited similar safety, tolerability and pharmacokinetic/ pharmacodynamic profiles, with no evidence of any immunological response.

Another study found Recombumin® rHA to be equivalent to native HSA in its capacity to protect immunological, biological and biochemical properties in preparations of thyroid stimulating hormone (TSH), interleukin 15 (IL-15) and granulocyte colony-stimulating factor (G-CSF). The study recommended the use of rHA in the preparation of lyophilised products and reference agents (12).

COMMERCIAL VALIDATION AND REGULATORY STATUS

The first and only commercially available recombinant human albumin whose use has been approved by the FDA and EMEA in the manufacture of biotherapeutics, Recombumin® is used in the production of childhood vaccines for measles, mumps and rubella {M-M-R® II (Merck & Co) and M-M-RVAXPRO® (Sanofi Pasteur MSD)} and is supported by a Type V Biologics Master File (BMF) with the US FDA.

CONCLUSION

HSA is a well-characterised protein that is known to have an important therapeutic role and has been used previously as an excipient for biotherapeutics. Most recently, its use as a drug stabiliser has been met with increasing regulatory resistance due to the perceived risk of disease transmission. To address these concerns and enable the biotherapeutic industry to rediscover the benefits of albumin as an excipient, recombinant albumin (rHA) has been developed. At Novozymes Delta, we have successfully developed a robust industrial-scale manufacturing process using a proprietary *S. cerevisae* based expression system that produces Recombumin®, a highly consistent and pure animal-, virus- and prion-free recombinant human albumin product. Being structurally identical to HSA and with a similar safety, tolerability and pharmacokinetic/ pharmacodynamic profile, Recombumin® is now supplied worldwide for use in the manufacture of better biotherapeutics.

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