OPPORTUNITIES FOR SPACE BIOPROCESSING

by

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ABSTRACT

Space bioprocessing has been thrust into an unexpected limelight as a result of the pioneering McDonnell Douglas/Johnson & Johnson experiments in continuous flow electrophoresis. Thus, there is an urgent need to reassess the opportunities and pitfalls in this area of space commercialization.

NASA's program of space bioprocessing may become the unwitting beneficiary of equally revolutionary recent ground-based developments in genetic engineering and other allied areas of applied biology. As a result of these advances, a number of important new biologicals have become available in unlimited quantities, subject only to the proviso that adequate purification methods can be devised. It is not an exaggeration to state that the need for ultimate purity is a major bottleneck in this emerging industry. NASA's space bioprocessing program may become a key factor, as mandated by ever increasing needs for sophistication in purification technologies.

Our process of recycling isoelectric focusing is characterized by very high purity of final products. A key question is whether space provides sufficient advantages over our ground-based process. A first pilot experiment is planned for STS 11, scheduled for January 29th. While primarily an experiment in basic science, it was designed to provide crucial information on the likely performance of focusing in a microgravity environment.

INTRODUCTION

It is a pleasure to participate in this Symposium on Space Industrialization. The opportunity to do so is particularly appreciated in view of the successful McDonnell Douglas/Johnson & Johnson space electrophoresis experiments. They have suddenly thrust us into a radically new era of industrialization of space. With it, they have imposed on us, the other space-concerned biotechnologists, the need to reassess the opportunities and pitfalls of operations in space.

In this context, I will address the following points: (1) The need for improved purification technologies; (2) the role of electrophoresis; and (3) isoelectric focusing.

PURIFICATION TECHNOLOGY

We are in the midst of a revolution in applied biology due to advances in genetic engineering and the allied fields of monoclonal antibodies, synthesis or isolation of individual

genes and solid phase synthesis of peptide hormones. The social and economic impacts of this new biotechnology have been amply covered in the scientific as well as lay press, including an authoritative review by the Office of Technology Assessment of the U.S. Congress (1).

These technologies have rendered possible the production of virtually unlimited quantities of important new biologics, which were previously available only in minute quantities. Interferon, human growth hormone, a foot and mouth disease vaccine, are but a few of such pharmaceuticals which can be cited as examples.

It should be emphasized that these biologics are often first obtained in the form of crude extracts, heavily contaminated by extraneous matter. For example, in recombinant DNA, a gene, coding for the desired pharmaceutical, is implanted into bacteria or yeast, thereby "engineering" the microorganism to produce a protein which it normally does not synthesize. The new protein has to be separated from the myriad of other proteins native to the microorganism. Similarly, monoclonal antibodies are produced by hybrid cells, obtained through the fusion of an antibody-producing lymphocyte and a cancerous cell. It is the cancerous moiety which confers to the hybrid its ability to rapidly reproduce. Again, the monoclonal antibodies need to be separated from the other proteins of the hybrid cell. The magnitude of the problem can best be visualized if one realizes that the host cells, the modified microorganism or the hybrid, may contain well over 5,000 different proteins, only one of which is the desired active principle.

The utmost in purity is essential for any of these products to be administered to a patient. Present purification technology is as much an art as a science, and is often a major bottleneck in production. It mostly involves a series of purification steps, artfully sequenced until the required purity is obtained. Chromatography is the main workhorse, and the whole process is costly and inefficient – at times only a minute fraction of total material synthesized is obtained in purified form.

We are only at the beginning of this revolution in biology. Modified plants and animals are on the horizon. It is indisputable that these advances will require ever increasing sophistication in the art of separation and purification. Thus, NASA's program is the unwitting beneficiary of these new developments and may become of crucial importance for future biotechnology.

Many of the biologics are in a price range which could justify space processing. Their unit value may exceed millions or tens of millions of dollars per kilogram. On the other hand, total demand may be only a few kilograms annually. Any new separation process has to have high resolution and high yield, that is, isolate the desired product in high purity and with minimal losses.

ELECTROPHORESIS

Electrophoresis is a most elegant technique for the separation of complex mixtures of biological origin. Because of its unique usefulness, a great variety of instruments have been developed and these are used in literally thousands of laboratories the world over. Most of this usage, however, is confined to analytical applications only, and is of little relevance to the task at hand, namely the purification of pharmaceuticals on an industrial scale.

At present, a paradoxical situation prevails. Analytical electrophoresis, in particular the so-called two-dimensional electrophoresis, offers the highest resolution of any separative technique and is routinely utilized for quality control in the production of biologicals. Nevertheless, no form of electrophoresis is utilized for the actual production of these compounds. In the past the requisite instruments may not have been available, but this is no longer true. It should be emphasized, however, that no single technique, chromatographic or electrophoretic, can be a panacea for all separation problems. Instead, they all should be considered as components of an increasingly more complex armamentarium of purification methods, to be integrated in an overall production scheme.

Three electrophoretic instruments are presently available for large scale processing:

1. The McDonnell Douglas continuous flow electrophoresis apparatus. This type of instrument was pioneered by Prof. Hannig of Germany. In ground-based operation, Hannig's instruments were characterized by rather limited resolution and throughput. Thus, it was rarely used for separation of proteins, its main application being in research with living cells.

The McDonnell Douglas apparatus was superbly engineered and performed well in the microgravity environment. This was certainly no mean achievement. Most important is their claim of greatly increased throughput, rather than high purity.

2. For the past ten to fifteen years, Dr. Thompson at the Harwell Atomic Energy Establishment in England has been developing a centrifugal electrophoresis apparatus of a radically new design, first proposed by Prof. Philpot, some 40 years ago. It is an impressive machine, for which large throughputs are claimed. It is marketed under the tradename BIOSTREAM by John Brown Limited of Portsmouth, U.K., but has become available only quite recently. As a result, its actual performance in daily usage is still largely undocumented.

3. The Recycling Isoelectric Focusing Apparatus (RIEF), developed in my laboratory under NASA's sponsorship. The RIEF was first proposed for operation in microgravity, but it proved to be quite effective in its ground-based operation. Schering Corporation and Ely Lilly Laboratories, two leading companies in the genetic engineering field, as well as Ionics, Inc., an engineering company, have already acquired the apparatus. We anticipate that its resolution may be further improved in microgravity.

A brief comparison of these instruments may be in order. The Harwell and the McDonnell Douglas instruments separate materials on the basis of differences in electrophoretic mobilities of components, this separating principle is technically known as zone electrophoresis. The RIEF apparatus separates components on the basis of differences in their isoelectric point. Moreover, the first two instruments are both adapted to continuous flow, the RIEF being a batch apparatus.

The Harwell apparatus has a very high throughput, but I suspect it has the lowest resolution of the three instruments. The RIEF apparatus is of modular design and, at least in principle, it could be scaled to any desired capacity. The McDonnell Douglas apparatus takes advantage of microgravity to achieve the desired throughput. Even so, a battery of such instruments will be required for actual commercial production.

Unfortunately, there was as yet no opportunity to compare directly the resolving power of the three instruments, which is a crucial factor. In analytical applications, isoelectric

focusing is characterized by very high resolution, and I expect that we have some advantages in this regard also in our RIEF. On the other hand, focusing imposes more drastic conditions on treated materials than continuous flow electrophoresis. Thus, the McDonnell Douglas apparatus is gentler and is applicable to a wider range of products. Of specific importance, it can separate living cells, which cannot be treated in the RIEF. In general, I expect that each of the three instruments will find its own niche, and that they should be viewed as complementary rather than competitive.

ISOELECTRIC FOCUSING

Isoelectric focusing is a particularly powerful variant of electrophoresis in which separation is carried out in a pH gradient. The proteins migrate, i.e., "focus" to the pH region corresponding to their isoelectric point, where they become immobilized due to zero net charge. The isoelectric point of a protein is a rather characteristic parameter, accounting for the exquisite resolution obtainable. The pH gradient is established by the actual effect of the electric current on the appropriately chosen buffer.

The RIEF apparatus was designed to adapt isoelectric focusing to large scale industrial capacities. Due to the fact that proteins focus to a stationary point, a novel recycling principle could be adopted. Fluid stabilization is accomplished by a parallel array of filter elements, which assure laminarity of flow. Monofilament nylon screens of fine mesh porosity are used for this purpose. Several reports describe the details of instrument design (2) and performance (3).

We expect a substantial improvement in the resolution of the RIEF when operated in a microgravity environment. The absence of terrestrial gravity could permit the elimination of the screen elements, essential for the RIEF ground-based operation. In principle, such an apparatus would combine some of the advantages of the McDonnell Douglas apparatus with the sharpness of resolution achievable by recycling in a pH gradient.

The shuttle is potentially a vehicle for the industrialization of space. At the present time it is even more important to consider it as a unique national research facility, capable of providing specific data not obtainable otherwise. In electrophoretic separations, fluid disturbances can arise from two causes: gravity-dependent convection and gravity-independent electroosmosis. Electroosmosis is the streaming of fluid induced by the electric field itself and it is caused by the residual electric charge of the walls of the vessels. In the case of focusing, it is impossible to distinguish between these two effects in ground-based operation. In space, gravity effects will be abolished, permitting a clear assessment of the effects of electroosmosis alone.

We are presently preparing an experimental package comprising eight focusing columns integrated in a middeck locker of the shuttle orbiter. These columns, of varying internal configuration, will be filled with a mixture of two colored proteins, the red hemoglobin and the blue-stained human serum albumin. The separation of the two proteins will be recorded photographically, with pictures taken every 3 minutes over a 90 minute interval. In ground-based operation, only one of these columns permits clear focusing of the proteins, both gravity and electroosmosis being simultaneously abolished by screen elements. All the other cells fail to focus. Operation in space should provide a clear-cut answer to the cause of failure, whether gravity-dependent or gravity-independent. The results will greatly influence our design efforts for a space RIEF and we are most anxious to see the results of the experiment, scheduled to fly January 29th.

REFERENCES

1. Office of Technology Assessment, U.S. Congress: "Impacts of Applied Genetics – Micro-organisms, Plants, and Animals".

2. M. Bier and N. B. Egen in "Electrofocus '78", (Haglund, Westerfeld and Ball, Eds.), Elsevier North Holland, New York, 1979; M. Bier, N. B. Egen, T. T. Allgyer, G. E. Twitty and R. A. Mosher in "Peptides: Structure and Biological Function", (Gross and Meienhofer, Eds.), Pierce Chemical Co., 1979; M. Bier, N. B. Egen, R. A. Mosher and G. E. Twitty in "Materials Processing in the Reduced Gravity Environment of Space", (Rindone, Ed.), Elsevier, 1982.

3. S. B. Binion, L. S. Rodkey, N. B. Egen and M. Bier, Electrophoresis 3, 284, 1982; M. Bier, O. A. Palusinski, R. A. Mosher and D. A. Saville, Science 219, 1281, 1983.