



Benchtop Detection of Proteins

This process is relatively rapid and simple.

John H. Glenn Research Center, Cleveland, Ohio

A process, and a benchtop-scale apparatus for implementing the process, have been developed to detect proteins associated with specific microbes in water. The process and apparatus may also be useful for detection of proteins in other, more complex liquids. There may be numerous potential applications, including monitoring lakes and streams for contamination, testing of blood and other bodily fluids in medical laboratories, and testing for microbial contamination of liquids in restaurants and industrial food-processing facilities. A sample can be prepared and analyzed by use of this process and apparatus within minutes, whereas an equivalent analysis performed by use of other processes and equipment can often take hours to days.

The process begins with the conjugation of near-infrared-fluorescent dyes to antibodies that are specific to a particular protein. Initially, the research has focused on using near-infrared dyes to detect antigens or associated proteins in solution, which has proven successful vs. microbial cells, and streamlining the technique in use for surface protein detection on microbes

would theoretically render similar results. However, it is noted that additional work is needed to transition protein-based techniques to microbial cell detection. Consequently, multiple such dye/antibody pairs could be prepared to enable detection of multiple selected microbial species, using a different dye for each species. When excited by near-infrared light of a suitable wavelength, each dye fluoresces at a unique longer wavelength that differs from those of the other dyes, enabling discrimination among the various species.

In initial tests, the dye/antibody pairs are mixed into a solution suspected of containing the selected proteins, causing the binding of the dye/antibody pairs to such suspect proteins that may be present. The solution is then run through a microcentrifuge that includes a membrane that acts as a filter in that it retains the dye/antibody/protein complexes while allowing any remaining unbound dye/antibody pairs to flow away.

The retained dye/antibody/protein complexes are transferred to a cuvette, wherein they are irradiated with light

from a miniature near-infrared laser delivered via a fiber-optic cable. The resulting fluorescence from the dye(s) is measured by use of a miniature spectrometer, the output of which is digitized, then analyzed by laptop computer. The software running in the computer identifies the protein species by the wavelengths of their spectral peaks and determines the amounts of the proteins, and thus, one day, microbes of the various species from the intensities of the peaks. The above-mentioned removal of the unbound dye/antibody pairs during centrifugation prevents false positive readings. The process proves successful in detecting proteins in solution and thus can now be employed for use in microbe detection.

This work was done by Maximilian C. Scardelletti and Vanessa Varaljay of Glenn Research Center.

Inquiries concerning rights for the commercial use of this invention should be addressed to NASA Glenn Research Center, Innovative Partnerships Office, Attn: Steve Fedor, Mail Stop 4-8, 21000 Brookpark Road, Cleveland, Ohio 44135. Refer to LEW-18148-1.

Recombinant Collagenlike Proteins

These proteins can be tailored to have specific biological properties.

Lyndon B. Johnson Space Center, Houston, Texas

A group of collagenlike recombinant proteins containing high densities of biologically active sites has been invented. The method used to express these proteins is similar to a method of expressing recombinant procollagens and collagens described in U. S. Patent 5,593,859, "Synthesis of human procollagens and collagens in recombinant DNA systems."

Customized collagenous proteins are needed for biomedical applications. In particular, fibrillar collagens are attractive for production of matrices needed for tissue engineering and drug delivery. Prior to this invention, there was no way of producing customized collagenous proteins for these and other applica-

tions. Heretofore, collagenous proteins have been produced by use of such biological systems as yeasts, bacteria, and transgenic animals and plants. These products are normal collagens that can also be extracted from such sources as tendons, bones, and hides. These products cannot be made to consist only of biologically active, specific amino acid sequences that may be needed for specific applications.

Prior to this invention, it had been established that fibrillar collagens consist of domains that are responsible for such processes as interaction with cells, binding of growth factors, and interaction with a number of structural proteins

present in the extracellular matrix. A normal collagen consists of a sequence of domains that can be represented by a corresponding sequence of labels, e.g., D1D2D3D4. A collagenlike protein of the present invention contains regions of collagen II that contain multiples of a single domain (e.g., D1D1D1D1 or D4D4D4D4) chosen for its specific biological activity. By virtue of the multiplicity of the chosen domain, the density of sites having that specific biological activity is greater than it is in a normal collagen. A collagenlike protein according to this invention can thus be made to have properties that are necessary for tissue engineering.