Impact Objectives

- Create an evidence base for the use of biotechnology in asbestos testing
- Develop a protein within a bacterial protein library that can be used to bind asbestos

Breaking new ground in asbestos identification

__Professor Akio Kuroda__, from __Hiroshima University__, talks about taking a unique approach to asbestos testing - using protein engineering to develop targeted probes for use in assays to identify the presence of asbestos

Can you share what inspired you to become involved in protein engineering?

I initially completed my undergraduate and graduate degrees in Fermentation Technology at the Faculty of Engineering, Osaka University. This laid the groundwork for my research background in protein engineering. At graduate school, my research title was "Protein engineering of penicillinase as affinity ligands for bioprocessing", and this was published in the *Journal of Fermentation Bioengineering*, 67, 315-320 (1989). Penicillinase is an enzyme that specifically acts to break down penicillin. It acts by first capturing penicillin in an enzyme pocket, then hydrolysing it. There are several amino acids in the active centre of penicillinase that are involved only in the hydrolysis step of this process. I experimentally modified these amino acids, which resulted in the creation of a modified penicillinase that still bound penicillin but no longer hydrolysed it. I created a penicillin binding protein from the penicillin hydrolysing enzyme that could be used as an affinity ligand in a practical penicillin purification step. I was so excited about my success in changing a protein's function through protein engineering. This was a real turning point for me!

From your perspective, what are the big gaps in global knowledge generally about biotechnology that need addressing?

No one has been using biotechnology for asbestos assays and, as far as I know, no one knows anything about asbestos-binding proteins. There has been a lot of resistance from researchers in this field towards accepting this new technology. However, I hope to break down this resistance and prove that biotechnology has a very real place in asbestos testing. So far, biosensing has mainly been used to detect biomolecules such as antigens, nucleic acids, including DNA, and toxic organic molecules. I wanted to expand the field of biosensing in inorganic materials using proteins. In short, my collaborators and I have found a protein within a bacterial protein library that can be used to bind asbestos! However, this protein also binds to other fibres. There are several domains within these proteins that bind to asbestos while others are more specific to different fibres. Using this knowledge, we have identified a domain involved in asbestos-binding and have created a truncated protein from this that specifically binds to asbestos. This is also a form of protein engineering and is extremely useful in creating tailored proteins that can be used in assays and other diagnostic applications.

What type of collaborations do you have? Can you talk about the importance of these to your research?

Collaborations have been vital in our work so far and the most important collaboration was in developing a portable and robust fluorescent microscope. We first used a fluorescent microscope in the laboratory. However, it was not suitable on-site at a demolition, where it is both shiny and dusty outside. We worked with a manufacturer to develop a robust, portable fluorescent microscope.

You are involved in several societies and councils, including the Japan Society for Biotechnology and the American Society for Microbiology. How valuable is this involvement to your research and in supporting wider research outcomes?

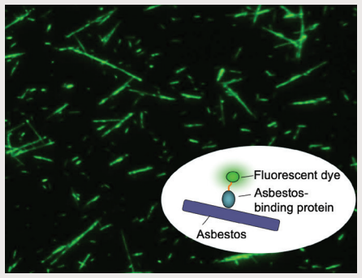
The Japan Society for Biotechnology is kind of my home ground. They have been really helpful and supportive in my work on developing targeted proteins. However, when I expanded my work from biotechnology into new areas (in this case, asbestos detection), I needed to also reach out to more specific asbestos-related groups. These groups are a fantastic way for researchers to tap into a network of experts in different areas.

Shining the light on asbestos

Asbestos is a toxic substance that is found in older buildings, as well as in cosmetics and products for children. As testing for its presence can be problematic __Professor Akio Kuroda__ has been working on a novel solution

Asbestos is a naturally occurring silicate (silicon- and oxygen-containing) mineral that has a fibrous structure. These fibres are composed of microscopic fibres that can become airborne when the asbestos is disturbed, making asbestos easily inhaled. Formerly enjoying widespread use, especially in construction, for its insulation and fireproofing qualities, asbestos has since been banned in many countries. This is due to the discovery, in the 1970s, that asbestos fibre inhalation can cause lung cancer, asbestosis and other lethal lung conditions.

Professor Akio Kuroda, from the Graduate School of Integrated Sciences for Life at Hiroshima University, is an expert in the field. 'While asbestos has been banned in most developed countries, large quantities of asbestos-containing materials remain in old buildings, and people are at risk of exposure to asbestos during demolition,' he explains. Kuroda confirms that it is not just building materials that pose a risk. 'In addition, natural minerals, such as talc, a raw material used in the manufacture of cosmetics, pharmaceuticals and baby powder, may also contain asbestos. It has recently been highlighted that asbestos-contaminated talc may cause cancer,' he says.



The fluorescently labeled asbestos-binding proteins visualise asbestos fibres, but not man-made asbestos substitutes (rockwool, glasswool, etc.).

Kuroda is keen to focus on the importance of fast and accurate asbestos testing. In January 2022, the US Food and Drug Administration (FDA) released a report written by the Interagency Working Group on Asbestos in Consumer Products outlining an improved scientific assessment for asbestos in order to better protect the public. Kuroda and his team has been developing testing techniques to accurately pinpoint the presence of asbestos. Their approach to establishing better testing for asbestos has been twofold, firstly with the development of a fluorescent microscopy (FM) method that offers increased sensitivity as well as convenience, as well as the creation of an asbestos-specific protein probe combined with a fluorescent marker that allows users to easily visualise asbestos fibres, but not non-asbestos ones, under a fluorescent microscope.

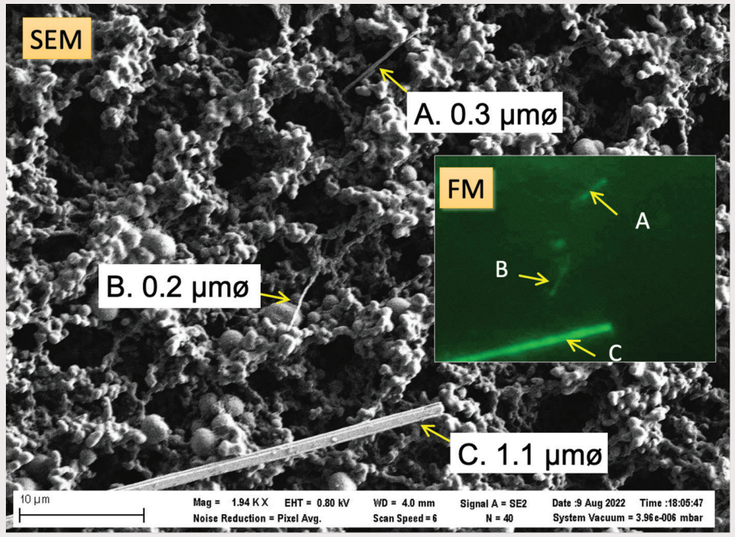
A NEED FOR IMPROVED TESTING

Testing for asbestos is currently often accomplished using polarised light microscopy (PLM). While this method is convenient and cheap to run, the sensitivity is insufficient for the detection of fine fibres. The FDA recommends transmission electron microscopy (TEM) due to its higher sensitivity. However, it is labour-intensive and therefore testing becomes more costly. Other popular testing methods include phase contrast microscopy (PCM) and scanning electron microscopy (SEM). All of these have significant drawbacks that keep the search for a better option going.

Kuroda's method using FM offers much higher sensitivity that comes close to rivalling that of electron microscopy, and it is also far more convenient for users than TEM. A US National Institutes of Health (NIH) study published in 2012 found that FM was able to detect fibres that were almost ten times smaller than those found by PCM testing, making it a much more sensitive testing method, approaching the accuracy of even electron microscopy. The study concluded that FM demonstrated exciting potential to offer a more accurate option for identifying

asbestos fibres without the need for time-consuming, labour-intensive laboratory testing.

Fine asbestos fibres (A, B and C) are visible under FM and SEM.



Developing a faster, more accurate and convenient way to test for the presence of asbestos fibres is vital as testing is often required quickly due to the toxic nature of the substance. Many incidents arise when potential asbestos is released into the air during construction or repair works on older buildings, requiring a speedy result to allow steps to be taken quickly to neutralise the contamination. Thus, SEM and TEM have their drawbacks in these situations, although they do provide reliable, accurate readings. Despite their provision of clear, accurate results, both generally require samples to be sent to a laboratory to be processed. PCM, on the other hand, can be carried out *in situ*, enabling faster results. However, this method, in turn, has drawbacks. PCM identifies the presence of airborne fibres but is not able to distinguish between asbestos and other types of fibres.

OVERCOMING OBSTACLES

Asbestos detection is an area that certainly has room for improvement in finding a method that meets the need for both speed and accuracy. Kuroda believed that existing methods of testing failed to cover these requirements. 'I needed to show evidence that the new technology can be used for asbestos detection,' he says. His approach has been to target FM as a highly specific way to identify asbestos.

The most difficult challenge he faced, having developed a specific probe for asbestos, has been to quantify what percentage of fluorescent fibres made visible in the test samples are asbestos. 'To verify the identity of the fibres that were fluorescently tagged, we analysed them under an electron microscope to identify their elemental composition,' Kuroda outlines. 'However, it was very difficult to find the individual fibres under both microscopes in order to verify them.' In a sample that may contain thousands, or even hundreds of thousands of fibres, this task was like finding the proverbial needle in a haystack. Fortunately, Kuroda was able to turn to recent advances in imaging technology to solve this issue. 'We were able to use a newly developed correlative microscopy system where the sample stage is shared among the two microscopes,' he explained. This means that the location information of fibres therefore remained. Kuroda says the results were astounding. 'We demonstrated that over 95 per cent of fluorescent fibres observed in the practical samples are really asbestos,' he confirms.

> *"We demonstrated that over 95 per cent of fluorescent fibres observed in the practical samples are really asbestos"*

CHANGING THE LANDSCAPE

Kuroda's work on developing and modifying the asbestos-binding protein with fluorescent dye has been truly ground-breaking. By creating this probe that is highly specific for asbestos, it is now possible to visualise this lethal substance in samples of construction materials, cosmetics and even baby products. 'Before this finding, no one knew that asbestos could be fluorescently visualised,' points out Kuroda.

With results published in the *Annals of Occupational Hygiene*, Kuroda's methods were approved in the Asbestos Monitoring Manual, a guide published by Japan's Ministry of the Environment. The probes he developed in this study have been made commercially available (http://siliconbio.co.jp/siliconbio_en.html) and are currently used by several companies for fast and accurate monitoring of asbestos.

In addition to developing the probe and validating his results, Kuroda has also worked with a commercial manufacturer of microscopes to create a fluorescent microscope that was suitable for use *in situ*. Existing fluorescent microscopes were unwieldy devices that needed to be used in a laboratory setting and were quite unsuitable for transport around dusty demolition sites. Aware of the desire for faster testing, Kuroda and his industry partners were able to design a robust and portable microscope that could be taken on site, thus making testing faster and more accurate.

By tackling multiple problems that had beset the arena of asbestos testing and control, Kuroda has revolutionised the industry, bringing faster, more accurate testing to contaminated sites worldwide. In the UK, where the use of asbestos has been banned since the late 1990s, it is still present in many buildings, with almost 50 per cent of buildings, including over 75 per cent of schools affected. In terms of the benefits, this means greater safety not just for construction workers labouring on potentially hazardous sites, but also for all citizens (including children) living and working in polluted homes and buildings. Kuroda's advances in better testing and better equipment means greater safety for all. ●

A robust and portable fluorescence microscope has been developed for the on-site asbestos detection. Magnification (approximately ×300) can be digitally increased (until ×1,000).

Using protein-engineering to detect asbestos

By modifying bacterial cellular proteins to develop specific probes, ***Professor Akio Kuroda*** *has designed a high-accuracy technique for asbestos testing*

Greater awareness of the dangers of asbestos has driven a more urgent requirement for better methods of testing for the presence of asbestos. The FDA in the US has issued a report supporting better assessments for testing to better protect the public. With a recent landmark case in which cosmetics giant Johnson & Johnson was ordered to pay out a total of $2.1 billion to users of its products over cancer claims, fast, accurate testing is crucial. Found not only in many older buildings, including schools and homes, asbestos has also been detected in talc-containing products, including cosmetics, and children's products. Not only must testing be accurate, but speed is also of the essence in many instances, such as accidental damage to buildings during construction and demolition works.

Professor Akio Kuroda has a background in protein engineering. He has taken a unique approach to addressing the need for a highly specific, accurate and fast means of testing for the presence of asbestos.

MODIFYING PEPTIDES

Peptides, the building blocks of proteins, have been engineered to bind specifically to various targets to highlight their presence. They have been notably used in the medical field, where they can be developed to target specific proteins within samples, to diagnose diseases such as cancer, infectious diseases and even genetic markers. Coupling the use of specific peptide markers with fluorescent tags that allow materials to be visualised using a FM has been a further development that has proved highly effective in many applications, including the identification of inorganic materials.

There have been fewer examples seen using these methods on testing environmental pollutants. However, Kuroda has, using engineered peptides, developed a highly specific probe that binds to asbestos fibres, flagging up the presence of even minute fibres of the material. Kuroda and his colleagues turned to existing cellular protein libraries to select from material-binding proteins as a springboard to manufacturing their own. 'During these experiments, the sample material was mixed with a bacterial cell protein lysate mixture, with the proteins that were adsorbed into the sample then selected for co-precipitation and identification using the peptide mass fingerprinting method,' Kuroda describes. 'A deletion mutant library for the binding protein was then used to identify particular sequences or binding domains,' he says.

FOUNDATIONS FOR SUCCESS

There are several types of asbestos, each of which differs in both crystal structure and toxicity. As a result, the surface of the fibres of the different types are both chemically and behaviourally different, requiring at least two different probes to be used to cover all the types of asbestos. 'The first of the probes (DksA) was developed to target chrysotile-type asbestos, while proteins GatZ and H-NS were used as a basis to target amphibole asbestos types,' Kuroda explains. 'All of these proteins were based on samples from the *Escherichia coli* cellular protein library.' Kuroda and his colleagues verified the efficacy of these probes on asbestos and non-asbestos fibres, including several samples of asbestos substitutes, all of which were provided by the Japan Fibrous Material Research Association.

Kuroda confirms that while DksA was found to be highly selective to serpentine asbestos (chrysotile), GatZ and H-NS bound to not only amphibole asbestos, but also to the asbestos substitutes. 'Working on the basis that presenting a multiple display of a binding site can increase the affinity of the peptide when compared to that of the single peptide, I used a streptavidin tetramer as a scaffold to display four molecules of the biotin-labelled amosite-binding peptide,' outlines Kuroda. He found that this tetramer had an affinity approaching 250 times higher than that of the single peptide. 'By using multiple probes with fluorescent tagging, both chrysotile and amphibole asbestos types can be identified simultaneously in the same sample,' he confirms. Combining these engineering techniques with existing FM technology is another key factor towards developing the new gold standard asbestos detection methodology. ●

Project Insights

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