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Getting the feel of food structure with atomic force microscopy

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ABSTRACT

This article describes the progress in the development of the atomic force microscope as an imaging tool and a force transducer, with particular reference to applications in food science. Use as an imaging tool has matured and emphasis is placed on the novel insights gained from the use of the technique to study food macromolecules and food colloids, and the subsequent applications of this new knowledge in food science. Use as a force transducer is still emerging and greater emphasis is given on the methodology and analysis. Where available, applications of force measurements between molecules or between larger colloidal particles are discussed, where they have led to new insights or solved problems related to food science. The future prospects of the technique in imaging or through force measurements are discussed.

1. Introduction

This review is dedicated to Prof. G. O Phillips on the occasion of his 90th birthday. During his extensive research career there has been substantial progress in the understanding of food structure, partly due to the development and application of new experimental methods. Prof Phillips has contributed greatly to the dissemination, development, and application of this knowledge through his own research group, the origination and editorship of the Food Hydrocolloids journal, and the initiation and running of the highly successful Gums and Stabilisers & International Food Colloids meetings. In celebration of this contribution this article reviews developments in the use of one of these new techniques, namely atomic force microscopy (AFM), both as a microscope and as a force transducer. This is a technique we have been intimately involved with over this period. Since its inception in the 1980s AFM has led to considerable insights into a range of food structures. The review will focus on uses in food science and on new developments and, in particular, highlight the increasing use of AFM as a force transducer, which is likely to open up new areas of understanding in the near future.

2. Atomic force microscopy – a microscopic tool

An atomic force microscope (AFM) scans a tiny and extremely sharp tip that is mounted on the end of a flexible cantilever over the surface of samples – it is similar to the action of a stylus on a record player, but in terms of microscopy effectively a nano-profilometer. Unlike all other forms of microscopy it has no lenses and does not image the sample by 'viewing', rather it does so by 'feeling' the surface of the sample (Morris, Kirby, & Gunning, 2009).

2.1. Imaging food molecules and structures: early days

The development of atomic force microscopy (AFM) as a tool for imaging biological systems offered considerable promise for imaging at molecular, or sub-molecular level, in a liquid environment (Morris et al., 2009). Realisation of these challenges principally involved the development of an understanding of image contrast in AFM, and development of imaging procedures, which eliminated, or at least minimised, the damage to the sample by the imaging probe. Considerable advances in instrumentation have aided the identification and elimination of artifacts, and there have been major advances in the software produced to run the microscopes and process the images. The AFM offers comparable resolution to that of the transmission electron microscope with the advantage of imaging under natural conditions. This has been achieved for molecules and macromolecular complexes through devising methods for immobilising samples on suitable substrates without

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destroying or deforming their native structures (Morris et al., 2009). For larger more complex biological systems the challenge is to image the surface, or surfaces of sections of these more complex structures. Through these developments AFM is now becoming used widely to solve problems for biological samples rather than just obtaining good images. Despite widespread use the technique is still not entirely routine and, like other microscopic methods, will always require skill and expertise in modifying sample preparation and imaging methods for particular samples (Morris et al., 2009).

2.1.1. Imaging molecules and complexes: new understanding

AFM has provided new information on molecular size and shape under more natural conditions. For helix-forming polysaccharides, which function as gelling and thickening agents, it has not only been possible to characterise the molecules but to investigate their functional role. Gellan gum is a model system for investigating polysaccharide gelation, and through the imaging of the molecules, gel pre-cursers and the surface of intact hydrated gels it has been possible to visualise the molecular networks formed in the gels and infer the mode of association (Gunning, Kirby, Ridout, Brownsey, & Morris, 1996). Microgel formation observed in preparations of the thickening agent xanthan gum, provide a basis for understanding the weak gel properties of this material (Morris et al., 2009). Continual progress in instrumentation has, and is still allowing enhanced resolution of molecular structure: in the case of xanthan this has allowed the identification of the arrangement of individual chains in annealed xanthan helices (Fig. 1), providing direct visual evidence for a double, rather than single helical structure (Moffat, Morris, Al-Assaf, & Gunning, 2016).

Microscopic techniques such as AFM allow the characterisation of heterogeneity at the molecular level. An example is the observation of branching in semi-flexible molecules such as arabinogalactans (Adams, Kroon, Williamson, & Morris, 2003). An analysis of AFM images of the binding of inactivated enzymes to arabinogalactans has been used to confirm the random distribution of branches, plus enzymatic creation of non-random distribution of blocks of unsubstituted backbone following enzymatic removal of arabinose branches (Adams, Kroon, Williamson, Gilbert, & Morris, 2004). In the case of pectin extracts AFM revealed (Fig. 2a) irregular branching of the polygalacturonic backbone (Round, Rigby, MacDougall, Ring, & Morris, 2001), which led to a new proposed model for the internal structure of plant cell walls (Vinchen et al., 2003). The enhanced characterisation of pectin extracts has been employed in studies of transgenic strawberry mutants to study the role of ripening enzymes, and has revealed novel structures, previously not seen in cell wall extracts (Pose, Kirby, Mercado, Morris, & Quesada, 2012). AFM showed (Fig. 2b) the nature of pectin – protein complexes (Kirby, MacDougall, & Morris, 2006; Morris, MacDougall, & Kirby, 2008), suggested to be responsible for the emulsifying action of sugar beet pectin.

AFM images revealed for the first time irregular low level branching Fig. 2c) of the starch polysaccharide amylose (Gunning et al., 2003). Furthermore, studies of complexes of amylose with mutants of the starch degrading enzyme glucoamylase, revealed how the starch-binding domain (SBD) of the enzyme can bind to amylose helices (Fig. 3). As shown in the AFM image SBDs formed ring-shaped complexes with single amylose chains. Quantification of the chain lengths in the images enabled interpretation of how the rings were formed. Despite the fact that amylose is a polydisperse polymer the distribution of the perimeter length of the rings were half that of the distribution of the contour lengths of the linear chains in all of the images which suggested the amylose chain bound to both binding sites of the SBD. It was known that the binding sites on the SBD are oriented at 90° relative to each other (Sorimachi et al., 1996) and this combination of factors suggested that the SBDs act as substrates for an expanded double helix. This identified the fact that the SBD can recognise, bind and distort the amylose double helix on starch crystal surfaces. This led to a suggested mechanism for the selective degradation of crystalline starch by glucoamylase (Morris et al., 2005).

2.1.2. Imaging molecular assembles: new understanding

The internal structure of more complex biological structures can be investigated by imaging flat cut surfaces or sections of the samples: examples include the study of plant cell walls or starch granule structure (Morris et al., 2009).

In order to investigate the internal structure of starch it was found necessary to embed isolated starch granules in non-penetrating resins, in order to avoid artifacts induced by penetrating resins (Ridout, Gunning, Wilson, Parker, & Morris, 2002), and to cut sections or produce polished flat blocks for imaging (Morris, Ridout, & Parker, 2005; Ridout, Parker, Hedley, Bogracheva, & Morris, 2004). The contrast in the images was shown to be due to different levels of adsorption of water into amorphous and crystalline regions within the granule (Morris, Ridout, et al., 2005;

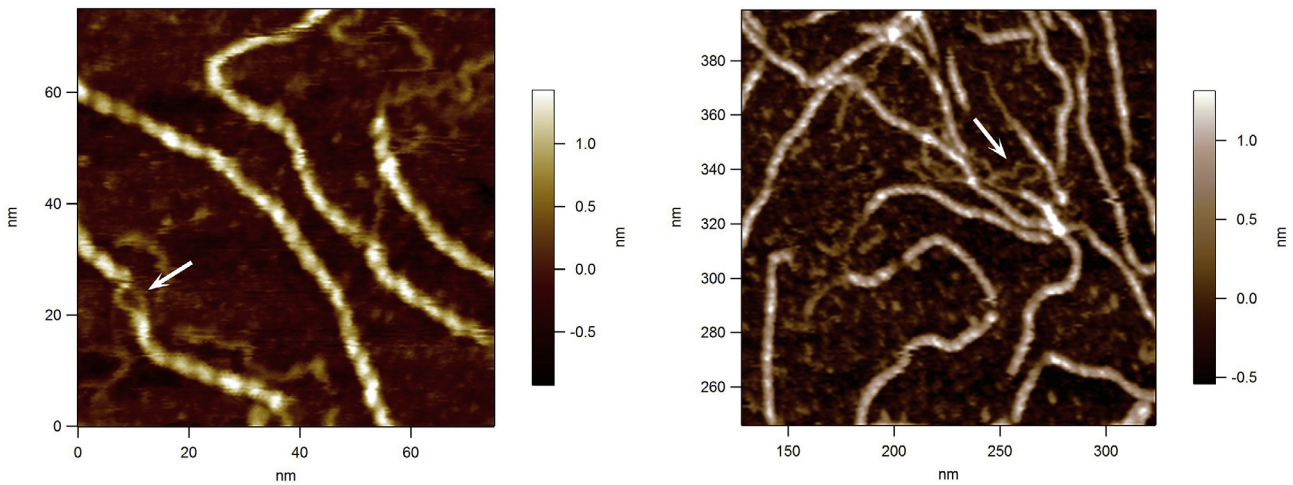

Fig. 1. High resolution AFM images of xanthan double helices. The loops at the ends and along the molecule provide evidence for intra- and intermolecular double helix formation. For details see Moffat et al. (2016).

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