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minireview

A Proteomic Approach for Investigation of Bee Products: Royal Jelly, Propolis and Honey

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Summary

Bee products such as royal jelly, honey and propolis have been reported to possess several biological activities. In order to better understand their mechanism of action and, consequently, their efficiency and safety, 'omic' approaches are used. Here cases with proteomic approach are indicated. In addition to studying biological activity at a proteome level, a proteomic approach for investigation of bee products has also been applied in analyzing proteins as their (bioactive) components.

Key words: proteomics, bioactive components, propolis, royal jelly, honey, yeast

Introduction

Consumers are showing increased interest in bioactive components with health-promoting effects derived from various sources. Among them, bee products such as propolis, honey and royal jelly have generated considerable interest. They contain many bioactive components that have been shown to possess several biological properties. Honey has been used since ancient times as part of traditional medicine. Several functions such as antibacterial, antioxidant, antitumour, anti-inflamatory, antibrowning, and antiviral have been reported. Propolis is a resinous substance that bees collect from the exudates of plants and use to seal holes in the beehive. This substance has been used in folk medicine since ancient times, due to its many biological properties such as antitumour, antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory effects, among others. Royal jelly as exclusive food of the queen honeybee has been demonstrated to possess numerous functional properties such as antibacterial, anti-inflammatory, vasodilative and hypotensive, disinfectant, antioxidant, antihypercholesterolemic, and antitumour activities (*1–3*). Biological activities of propolis are mainly attributed to the phenolic compounds, while in honey in addition to phenolic compounds, proteins (peptides) have also been reported to show similar properties (*3–5*). Biological activities of royal jelly are mainly attributed to bioactive fatty acids, proteins and phenolic compounds (*1*).

A proteomic approach in investigating bee products is focused either on analyzing proteins as (bioactive) components of bee products or studying their biological activity at proteome level.

Proteins as (Bioactive) Components of Bee Products

A proteomic approach in analyzing proteins as (bioactive) components of royal jelly has been widely reported. Since up to 50 % (dry mass) of royal jelly is protein, proteome analysis is a promising starting point for attempts to identify the proteins that provide health-promoting effects (*6*). Many studies have used either a gel-based proteomic approach, including sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

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and two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), mostly in combination with mass spectrometry (MS) (*7–16*), or gel-free proteomics (*13*) merely to analyze and/or to identify royal jelly proteins. Furthermore, Schönleben *et al.* (*6*) applied various methods for the pre-fractionation and separation of royal jelly proteins in order to circumvent the shortcomings of individual techniques such as 2-D PAGE or multidimensional chromatography, which can only yield certain subpopulations of a proteome due to the specific bias of each method. In this way they achieved a high coverage of the royal jelly proteome and were able to identify a total of 20 different proteins, as well as to demonstrate a very high degree of cleavage of different proteins of the major royal jelly protein (MRJP) family. Furthermore, they investigated the protein phosphorylation of royal jelly proteins, and identified and located two phosphorylation sites within venom protein 2. Similarly, Furusawa *et al.* (*11*) reported potentially post-translationally modified (phosphorylated and glycosylated) royal jelly proteins based on Pro-Q diamond phosphoprotein and emerald glycoprotein gel stains using 2-D PAGE. Zhang *et al.* (*17*) analyzed post-translational modifications (PTM) of royal jelly proteins by using complementary proteome strategies of two-dimensional gel electrophoresis (2-DE), shotgun analysis in combination with high performance liquid chromatography-chip/electrospray ionization quadrupole time-of-flight/tandem mass spectrometry and bioinformatics. Phosphorylation was characterized in MRJP 1, MRJP 2 and apolipophorin-III-like protein, and a new site was localized in venom protein 2 precursor. Methylation and deamidation were also identified in most of the MRJPs. The results indicate that methylation is the most important PTM of MRJPs that triggers the polymorphism of MRJP 1–5 in the royal jelly proteome. The study by Bíliková *et al.* (*18*) illustrated multifunctionality of proteins of honeybee royal jelly and how their neofunctionalization results from various PTMs of maternal proteins.

Honey is another bee product containing proteins, so SDS-PAGE, 2-D PAGE and MS have been used to analyze and identify major honey proteins (*19,20*). Di Girolamo *et al.* (*5*) analyzed honey from chestnut, acacia, sunflower, eucalyptus and orange for its proteome, using SDS PAGE and 2-D PAGE in order to investigate the origin of its proteins. It was shown that all the proteins identified (except one, the enzyme glyceraldehyde-3-phosphate dehydrogenase from *Mesembryanthemum crystallinum*) were not of plant origin but belonged to the *Apis mellifera* proteome. Among the total proteins identified five belonged to the family of major royal jelly proteins 1–5 and were also the most abundant ones in any type of honey, together with α-glucosidase and defensin-1. It thus appears that honey has a proteome resembling the royal jelly proteome (but with considerably fewer species), except that its protein concentration is lower by three to four orders of magnitude as compared to royal jelly. Similarly, Kwakman *et al.* (*4*) demonstrated by using SDS-PAGE and Western blotting that honey contains an antimicrobial peptide, bee defensin-1, and that this peptide substantially contributes to the bacterial activity. Baroni *et al.* (*21*) reported on the development of a novel alternative method for assessing the floral origin of honey samples based on the study of honey proteins, using SDS-PAGE immunoblot techniques.

In the case of propolis, protein profiling using a proteomic approach has not been reported.

Investigating the Biological Activity of Bee Products in the Cell at Proteome Level

To investigate the biological activity of bee products, studies have mainly been conducted at the level of whole experimental animals and cells, while biological activity at a molecular level is less known (*1–3*). Both food as a whole and individual bioactive compounds interact with our body at system, organ, cellular and molecular levels (*22*). At a molecular level, they can essentially affect every step in the flow of genetic information, from gene expression to protein synthesis and protein degradation, and thereby alter metabolic functions in the most complex way (*23,24*).

Omic technologies enable elucidation of the molecular basis for the activity of bioactive components of functional foods or other sources, leading to a better understanding of their mechanism of action. Among them, proteomics has an important advantage; it investigates the proteins that carry out functions. Proteins are the real players of life; they build our cellular and extracellular structures, provide and respond to messages (growth factors, hormones, receptors, cytosol and nuclear proteins), enable the transport of nutrients and metabolites in blood, to and in the cells, enable metabolism (enzymatic reactions), protect the organism (antibodies, blood coagulation factors) and regulate gene expression. Signalling pathways are concerned with changes in protein expression, and with most pathophysiological conditions. Using proteomic technology, it is possible to determine changes in protein expression and to identify the proteins themselves. Moreover, information about the presence of isoforms, post-translational modifications and protein interactions can also be obtained (*23,25*).

The effect of bioactive components on proteins generally occurs as an effect on protein abundance, an effect on post-translational modification or as an interaction (bioactive component-protein). Post-translational modifications of proteins, as well as their direct interaction with bioactive components, can result in changes of the three-dimensional structure of such affected proteins. Their original functions are thus modulated, resulting, for example, in reduced activity in the case of enzymes or changes in recognition ability between molecules, such as in protein-protein interactions or ligand-receptor interactions (*26,27*).

As already mentioned, proteomic tools enable the detection of changes that have occurred in proteins targeted by bioactive compounds. In order to gain a full picture of the proteome of any cell, and consequently possible targets, it is essential to use a subcellular proteomic approach (*28,29*). Subcellular proteomics has the advantage not only of relating proteins to functional compartments within eukaryotic cells, but also of reducing the complexity of a whole cell or a tissue protein extract, which can often prevent satisfactory proteomic analysis. Specifically, it allows the identification of novel and low-ab-

undance proteins that can otherwise remain masked by those expressed at higher levels when the total proteome of a cell or tissue is investigated (*30*). Particularly in the case of certain regulatory proteins, such as kinases, transcription factors and some membrane receptors, analysis of subcellular compartments is the strategy of choice (*31*). This approach is powerful enough to select with high confidence the proteins truly involved in a given biological process (*30*).

In the case of bee products, such as royal jelly, propolis and honey, some studies have been carried out at a molecular level, but mostly by analyzing gene expression for a limited number of candidate genes (*1–3*). This is a limitation, since a reduced number of genes may not provide insight into the causative relationship between a bioactive food component and its biological effect (*24, 32*). Analysis of global gene expression at a proteome level offers better possibilities. However, the application of proteomics in studying the mechanism of activity of bioactive components of bee products is still fairly limited.

Barlak *et al*. (*33*) investigated the effect of dimethyl sulphoxide and water extracts of Turkish propolis on the proteome of a prostate cancer cell line using surface-enhanced laser desorption ionization/time-of-flight mass spectrometry (SELDI-TOF MS). It was concluded that dimethyl sulphoxide and water extracts of Turkish propolis may have an antiproliferative effect through differentiating the protein expression profile in PC-3 prostate cancer cell lines, together with their antioxidant capacity. Two studies (*34,35*) are summarized below that illustrate the effect of propolis and royal jelly on a proteome, using yeast *Saccharomyces cerevisiae* as a model organism. This lower eukaryote is an appropriate model organism for the study of fundamental eukaryotic cellular processes, such as their stress responses and metabolic pathway (*36–39*). Since many genes from the yeast *Saccharomyces cerevisiae* are conserved in humans and a number of powerful genomic tools and methodologies have been developed for this model system, yeast is making a major contribution to the field of chemical genetics (*40*). In drug research, where one of the major challenges is to identify the proteins and cellular pathways affected by a drug, several different approaches are being developed using *Saccharomyces cerevisiae* as a model organism (*41*). Comparative genomic studies have shown that 40 % of yeast proteins share amino acid sequence similarity with at least one human protein (*42*), and 30 % of genes with a recognized involvement in human disease have a yeast orthologue (*43*). Furthermore, yeast has many technical advantages over human cells. It is well suited to high-throughput methods because of its rapid life cycle, it can grow as dispersed cells in liquid or as colonies on solid media, and its culture requires neither an elaborate sterile technique nor expensive media. It is highly amenable to genetic modifications such as gene disruption, deletion and replacement (*44*). Experiments with yeast will always be technically easier, more rapid and much less costly than experiments with human cells, and it is anticipated that yeast will remain a good first line of attack in the hunt for drugs (*41*), as well as bioactive compounds from different sources.

In the study of Cigut *et al.* (*34*), the effect of ethanolic extract of propolis (EEP), as well as its particular fraction, was investigated at cellular and proteome levels. While the effects of both of these treatments on the total proteome were not significant, intensive changes were found in the mitochondrial proteome. Thus, using a subcellular proteomic approach, the α subunit of mitochondrial F_0F_1-ATP synthase (Atp1), Mn superoxide dismutase (Mn SOD) and peroxiredoxin (Prx1) were found to be targets; decreased levels of all proteins were observed. F_0F_1-ATP synthase has been suggested as a good molecular target for drugs in the treatment of various diseases and in the regulation of the energy metabolism (*45*). In the case of phenolic compounds, it has been shown that these compounds often target F_0F_1-ATP synthase (*45–47*). Mn SOD and Prx both belong to endogenous antioxidant defence systems and their decreased levels might be due to reduced intracellular oxidation after exposure to exogenous antioxidants, which was also measured in the study. Similarly, Jamnik *et al*. (*35*) showed a reduced level of Cu/Zn SOD in the yeast *S. cerevisiae* when exposed to royal jelly, which decreased intracellular oxidation. The reduced levels of endogenous antioxidant proteins and reduced intracellular oxidation measured in both studies indicate that both propolis and royal jelly are likely to be acting directly as radical scavengers, and not indirectly as pro-antioxidants. The latter would lead to decreased intracellular oxidation due to increased levels of endogenous antioxidant proteins (*48*). In yeast cells treated with royal jelly, a total of 22 spots were found to be differentially expressed compared to untreated cells. The densities of six spots were decreased and five spots were found up-regulated, while eleven protein spots were detected only in cells treated with royal jelly. Among the identified spots, in addition to Cu/Zn SOD, there were a regulating protein (transcription modulator WTM1) and proteins that play an important role in proteolysis and several metabolic pathways (vacuolar protease B, glycine cleavage T protein GCV1, vacuolar H+-ATPase) (*35*). Both these studies (*34,35*) offered insight into the mechanism of action of propolis and royal jelly also at a proteome level, specifically in terms of antioxidant activity.

Besides the antioxidant and antiproliferative activity of bee products, antimicrobial effect studied by using proteomic approach has been reported. The effect of manuka honey on protein expression was investigated in meticillin-resistant *S. aureus* (MRSA). Namely, honey has been used to eradicate MRSA strains from wounds, but its mode of action is not yet understood. Using 2-D PAGE combined with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), a downregulation of universal stress protein in honey-treated cells compared to untreated samples was identified. This protein is involved in the stress stamina response and its downregulation could help to explain the inhibition of MRSA by manuka honey (*49*). Similarly, Packer *et al*. (*50*) studied the non-peroxide antibacterial effects of this honey on the proteome of the common wound pathogen *Staphylococcus aureus*. Treatment with manuka honey resulted in a significant decrease in the bacterial cell growth rate as well as downregulation of ten and upregulation of two proteins. The differentially expressed proteins have roles in ribosomal function, protein synthesis, metabolic processes and transcription. Manuka honey uniquely caused downregulation of two

proteins (dihydrolipoamide dehydrogenase (DLD) and elongation factor Tu (EF-Tu)) associated with two of these pathways as well as upregulation of one stress-related protein (cold shock protein C (CspC)). The proteomic profile following a treatment with manuka honey differed from the profiles of other antibacterial agents, indicating a unique mode of action and its potential value as a novel antimicrobial agent.

Conclusions

Knowledge of the profiles and characteristics of proteins of bee products gives valuable information concerning their quality. Changes related to proteins, such as contamination, adulteration, degradation, oxidation or polymorphism can be readily identified from proteomic analysis. Analysis of major proteins in honey and royal jelly is thus a useful method for discriminating honey and royal jelly produced by different honeybee species (*20,51*). On the other hand, proteomic approach in the field of bee products is oriented towards studying their biological activity at a proteome level, especially in explaining antioxidant, antiproliferative and antimicrobial activity. The characterization of changes at a proteome level with functional data from the established biochemical and physiological methods can result in a better understanding of the mode of action of bioactive compounds. Changes in protein profile may alter fundamental cellular processes and metabolic pathways, leading to changed cell functions. Therefore, knowledge of proteins targeted by bee products could be the basis for the development of biomarkers for validation of their efficacy and/or safety (*26*).

Proteomics integrated with other advanced technologies (genomics, transcriptomics, metabolomics and bioinformatics) will greatly facilitate elucidation of the effects of bioactive components on the global expression of genetic information and cell function, without making assumptions about what to look for in terms of risk (*52*).

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