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Some properties of the main protein of honeybee (*Apis mellifera*) royal jelly

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Abstract – Royal jelly (RJ) was separated by ultracentrifugation (245 000 × *g* for 5 h at 6 °C) into three physically distinct fractions with different distribution of its components (proteins, sugars and fatty acids): yellowish fluid supernatant (61% w/w of RJ), yellowish-brown gelatinous sediment (32% w/w) and white nearly solid sediment (7%, w/w). Ultracentrifugation of the solvated gelatinous fraction was a suitable method for preparation of MRJP1, the most abundant protein of RJ in the form of gel. MRJP1 was present in RJ in different forms: a monomer (55 kDa), oligomeric subunit (ca. 420 kDa), and water-insoluble aggregates in sediment after its interaction with fatty acids. The oligomeric MRJP1 was well soluble in water and at concentrations of 30 to 50% (w/w) formed a stiff gel. It is suggested that MRJP1 is albumin-like protein. An interesting feature of the oligomeric form of MRJP1 is its ability for self-assembly in water solutions.

honeybee royal jelly / ultracentrifugation / oligomeric albumin-like protein / gel formation / self-assembly

1. INTRODUCTION

Royal jelly (RJ) is a part of the diet of honeybee larvae and it plays a major role in caste differentiation (Moritz and Southwick, 1992). RJ is derived from secretions of both the hypopharyngeal and mandibular glands of nurse honeybees (*Apis mellifera* L.) (Knecht and Kaatz, 1990; Lensky and Rakover, 1983).

Research on RJ has recently focused on the physiological functions of its individual proteins, particularly those of the most abundant protein, named MRJP1 (Major Royal Jelly Protein). It represents 48% of the water-soluble proteins of RJ, while appearing as a single protein on Sodium Dodecyl Sulphate Polyacryl Amide Gel Electrophoresis (SDS-PAGE) with a molecular weight of 55 kDa. With isoelectrofocusing, it shows

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at least eight isoelectrophoretic variants (Hanes and Šimúth, 1992). MRJP1 is synthesized in hypopharyngeal glands of nurse honey bees (Hanes and Šimúth, 1992; Kubo et al., 1996) and its synthesis continues in the heads of foragers (Kaludiny et al., 1994a; Oshati et al., 1997). MRJP1 was characterized as a member of the major protein family of RJ (MRJPs) by using an expression cDNA library obtained from heads of nurse honeybees (Klaudiny et al., 1994b). To date nine members of the MRJPs (49–87 kDa) family have been identified. Five of them (MRJP1 to, MRJP5) represent about 82% of the total protein content of RJ (Schmitzová et al., 1998). The MRJPs gene family encodes a group of closely related proteins that share a common evolutionary origin with the yellow protein of *D. melanogaster* (Albert et al., 1999). It seems that the MRJP1 has more functions than its nutritional role in the larval development of honeybees and it was recently found that MRJP1 is expressed in the mushroom bodies of the adult honeybee brain (Kucharski et al., 1998). A 350 kDa protein with N-terminal sequence identical to MRJP1 (Ohaski et al., 1997; Schmitzova et al., 1998) isolated from RJ has a proliferation stimulating activity for human monocytic cell lines (Kimura et al., 1995).

However, the MRJP1 has never been subjected to detailed molecular study. Such a study is now overdue. The lack of molecular properties for MRJP1 and other proteins of RJ was partly due to the inadequacy of previous preparative procedures. The ordinary methods of isolation of MRJPs led to protein precipitates and aggregates designated as water-insoluble proteins of RJ (Chen and Chen, 1995).

The purpose of the present study was to use ultracentrifugation for the rapid fractionation of RJ with the aim of obtaining MRJP1 in natural form for characterization of its molecular and structural properties.

2. MATERIALS AND METHODS

2.1. Ultracentrifugation of royal jelly

Honeybee RJ was collected 48 h after accepting 1-day larvae and was supplied from an apiary in the Slovak Republic during June 1996 and July 1999. After 3 h of transport at 36 °C, RJ was immediately fractionated by ultracentrifugation at $245\,000 \times g$ in a Sorwal ultracentrifuge (Du Pont; Wilmington, Delaware, USA) using a TH 641 rotor at 6 °C for 5 h. A sample of RJ and the ultracentrifugation fractions of RJ were freeze-dried.

2.2. Microscopic examination

Microscopic examination of RJ was performed by a scanning electron microscope (SEM) (model JSM-580; Jeol, Tokyo, Japan). An individual queen cell of 2-day-old larva was cut out from the frame, the larva was removed and the cell containing RJ was frozen at –20 °C. The RJ samples were applied from the thawed sample and allowed to develop at room temperature as a thin layer on aluminium disc for 72 hours. The samples were then negatively stained with Cu in a vacuum chamber at 10^{-3} Pa and examined. The light microscope (Axiophot; Carl Zeiss, Jena, Germany) or stereomicroscope (Wild; Heerbuug, Schwitzerland) were used for examination of free assembling structures of RJ-gel or MRJP1 formed in diluted solutions after drying in air. Examination of natural RJ-gel-layer was performed with a fluorescent microscope (Fluoval; Carl Zeiss, Jena, Germany).

2.3. Biochemical characterization of RJ and MRJP1

Moisture was determined by direct drying at 105 °C and ash content at 600 °C. Crude protein was determined by the micro-Kjeldahl method with a conversion factor

of 6.25. Sugars and fatty acids were determined according to Lercker et al. (1992) at the Instituto Nazionale di Apicoltura, Bologna, Italy.

SDS-PAGE of RJ-proteins was carried out on 12% gels by Coomassie Brilliant Blue staining (Laemmli, 1970) and the concentration of proteins was 5 μg per lane. The gel was calibrated by using protein molecular-weight standards (Gibco BRL; Life Technologies, Wien, Austria). For immunoblotting the samples were diluted 1:10 (v/v) in SDS loading buffer, subjected to electrophoresis, electrophoretically transferred to nitrocellulose membrane (BA85; Schleicher and Schuell, Göttingen, Germany), probed with polyclonal antibodies against recombined MRJP1 (Judova et al., 1998), visualized by using peroxidase-conjugated swine antirabbit IgK (Institute of Sera and Vaccines, Prague), and incubated with solution containing 0.33% 3.3-diaminobenzidine tetrahydrochloride (Fluka, Buchs, Switzerland) and 30 mg/L of hydrogen peroxide in 50 mM Tris-HCl, pH 7.4, for 5 min. The N-terminal amino acid analysis of the protein bands of SDS-PAGE was obtained after their electroblotting into ProBlott PVDF membrane by a gas phase automatic sequencer (done by Argo Bioanalytica; Morris Plains, NJ, USA).

The RJ-gel was fractionated by size-exclusion column chromatography on a Biogel A –1.5 m gel (75–150 μm), (BioRad; Hercules, CA, USA). A sample containing 5 mg proteins of RJ-gel (or protein standard) in 1.0 ml of the elution buffer was applied onto the column (1.6 × 50 cm). Flow rate was 10 ml/h. The elution with 50 mM Tris-HCl, pH 7.0, containing 50 mM NaCl was monitored at 280 nm using a UV recorder at 4 °C. The obtained fractions were dialyzed for 48 h against water and lyophilized. The approximate molecular weights of high molecular (HM) as well as low molecular (LM) protein fractions were determined by comparing their exclusion volumes with Apoferritin from horse spleen (Mr 440 kDa, Sigma) and with bovine serum albumin (Mr 66 kDa, Sigma, Saint Louis, MO, USA).

3. RESULTS

3.1. Fractionation of RJ by ultracentrifugation

RJ was separated by ultracentrifugation (245 000 × *g*; 5 h; 6 °C) into three physically distinct fractions. The upper fraction representing 61% (w/w) of RJ was a green-yellowish fluid, which was named as RJ-supernatant. The mid-layer, a yellowish-brown gelatinous sediment named as RJ-soft-sediment constituted 32% (w/w) of RJ. The white sediment in the bottom (7% of RJ, w/w) appeared to be a nearly solid substance resembling curd and was named as RJ-sediment. In the area between the RJ-sediment and RJ-soft-sediment a golden-yellow viscoelastic stiff gel was observed which was integrated with the top layer of the RJ-sediment. For preparation of this gel the RJ-soft-sediment was resuspended in two volumes of water and stirred for 1 h at 20 °C. The obtained milky-white suspension (pH 3.7) was separated at low speed centrifugation (30 000 × *g*; 30 min; 6 °C) to a sediment of protein aggregates and clear opalescent microemulsion. By high-speed centrifugation of the microemulsion (245 000 × *g*; 5 h; 6 °C) a colorless supernatant separated from a golden-yellow colored stiff gel sediment at the bottom of the tube. This sediment was named RJ-gel and it resembled amber. When the pH of the microemulsion (3.7) was shifted to pH 7.0 or 9.0, i.e. above the isoelectric point of MRJP1 (pH 4.5–5.0) (Hanes and Šimúth, 1992), by dropwise addition of concentrated ammonium hydroxide, RJ-gel in the form of a sediment was not observed after its ultracentrifugation (245 000 × *g*; 5 h; 6 °C). The process of aggregation of protein(s) in the microemulsion was pH dependent.

3.2. Biochemical characterization of the fractions of RJ obtained by ultracentrifugation

The content of proteins, sugars and fatty acids of RJ used for this study (Tab. I) corresponded to average values of the typical composition of RJ (Lercker et al., 1992; Schmidt and Buchmann, 1992; Takenaka, 1982). The RJ-supernatant contained a high amount of sugars (50.6% w/w) but only 1.2% of fatty acids. A significant part of the fatty acids of RJ was concentrated in fractions with lower water content, i.e. in the RJ-soft-sediment (11.5%) and in the water-insoluble RJ-sediment (48.1%). There was a high amount of proteins as in the RJ-soft-sediment (57.1%). The RJ-gel represented a relatively pure protein fraction containing 88.4% protein, 4.8% sugars, trace amounts of fatty acids (0.4%) and 2.1% water (Tab. I). Comparison of the protein patterns of MRJPs on SDS-PAGE (Fig. 1A, lane 1) with the protein patterns of RJ-soft-sediment (Fig. 1A, lane 3), RJ-sediment (Fig. 1A, lane 4) and RJ-gel (Fig. 1A, lane 5) respectively, showed in all fractions the presence of an abundant protein with a molecular weight of 55 kDa. On the other hand, in RJ-supernatant (Fig. 1A, lane 2) there were mainly proteins with MW of 49 kDa (MRJP2) and 60–70 kDa (MRJP3s). The RJ-supernatant had some unique hydrophilic and adhesive properties. In the air the lyophilized powder RJ-supernatant changed to a sticky mass during 24 hours at 22 °C and 45% relative humidity. The powder character of the lyophilized RJ and its other

Table I. Content of sugars, fatty acids and proteins of RJ and its ultracentrifugation fractions after lyophilization (% of dry matter).

	Royal Jelly (RJ)	RJ-supernatant	RJ-soft-sediment	RJ-sediment	RJ-gel
SUGARS					
Fructose	14.0	21.8	10.6	3.8	1.7
Glucose	18.8	27.2	12.1	4.1	1.1
Saccharose	1.0	0.9	0.4	0.1	1.7
Trehalose	0.2	0.2	0.4	0.1	0.06
Other	0.7	1.1	0.39	0.03	0.24
Total	34.3	50.6	23.4	8.13	4.8
FATTY ACIDS					
10-H-2-DA	10.5	0.7	6.4	36.6	0.13
10-H-DA	2.7	0.4	2.1	8.6	0.04
Other	2.0	0.1	3.0	2.9	0.23
Total	15.2	1.2	11.5	48.1	0.4
CRUDE PROTEIN	39.3	35.3	57.1	40.3	88.4
ASH	2.8	0.85	4.5	0.56	0.21
DRY MATTER (%)					
Lyophilized sample	93.5	90.3	96.8	97.6	97.9
Original sample	33.8	21.5	49.1	65.7	34.2

10-H-2-DA: 10-hydroxy-2-decanoic acid.
10-H-DA: 10-hydroxy-decanoic acid.

ultracentrifugation fractions, including RJ-gel, did not change under the same conditions.

Further fractionation of RJ-gel by size exclusion column chromatography showed its separation into two fractions (Fig. 2). The high molecular fraction (HM), representing the substantial part of RJ-gel (95% w/w of total proteins of RJ-gel), was eluted in the region corresponding to a MW ca. 420 kDa. The low molecular weight fraction (LM) was eluted in the region of

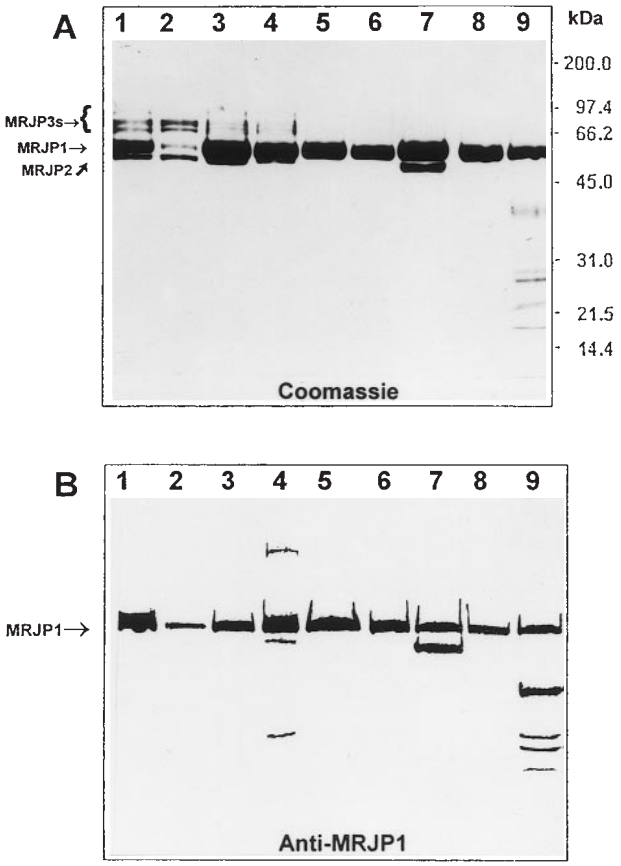

Figure 1. Characterization of MRJP1 obtained by ultracentrifugation of RJ. (**A**) Electrophoretic analysis of RJ-proteins by SDS-PAGE electrophoresis was carried out on 12% gels according to Laemmli (1972) by Coomassie Brilliant Blue staining. The concentrations of proteins were 5 μg per lane. The lanes represented: Native RJ (lane 1). Ultracentrifugation fractions of RJ: RJ-supernatant 2 μg of proteins (lane 2), RJ-soft-sediment (lane 3), RJ-sediment (lane 4) and RJ-gel (lane 5). Fractionations of RJ-gel by size exclusion chromatography: high molecular fraction (HM) (lane 6) and low molecular fraction (LM) (lane 7). Stability of MRJP1: oligomeric MRJP1 of HM-fraction (lane 8) and monomeric MRJP1 of LM-fraction (lane 9). The stability of MRJP1 was investigated at a concentration of 0.5 mg protein per 1 ml of buffer (50 mM Tris-HCl; pH 7.0 and 50 mM NaCl) at 37 °C for 12 h. The gel was calibrated by using protein molecular weight standards (Gibco BRL). (**B**) Immunoblot of samples corresponding to the SDS-PAGE in panel A probed with polyclonal antibodies against recombined MRJP1.

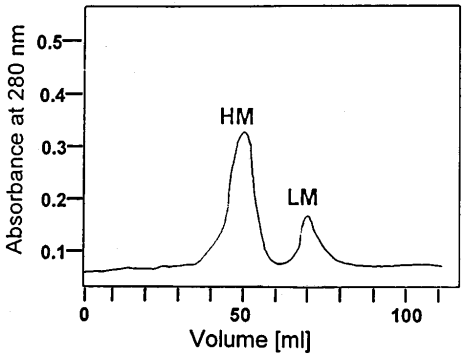

Figure 2. Size exclusion column chromatography of RJ-gel. The approximate molecular weight of high molecular (HM) as well as low molecular (LM) protein fractions under investigation was determined by comparing their exclusion volume with that of the molecular weight protein standards. Exclusion volume for Apoferritin from horse spleen (Mr 440 kDa) was 42 ml and for bovine serum albumin (Mr 66 kDa) was 61 ml.

66 kDa indicating that the proteins of this fraction were in the form of monomers. The SDS-PAGE of HM (Fig. 1A, lane 6) showed the presence of a 55 kDa protein as a single protein band. Besides a 55 kDa protein, the LM fraction also contained a protein with 47 kDa MW (Fig. 1A, lane 7). The N-terminal sequence of the 55 kDa protein of RJ-gel was N-I-L-R-G-E, which is identical to the N-terminal sequence of the most abundant protein of RJ, that is, MRJP1 (Oshahi et al., 1997; Schmitzová et al., 1998). Both the 55 kDa (Fig. 1A,B lanes 1–9) and the a 47 kDa proteins (Fig. 1A,B lanes 8) were immunoactive against polyclonal antibodies of recombined MRJP1. The presence of a 47 kDa protein (Fig. 1A,B lane 7) immunoactive against MRJP1 in the LM fraction suggested the possibility that MRJP1 was partially degraded.

The stability of the oligomeric MRJP1-HM fraction (Fig. 2) was investigated at a concentration of 0.5 mg protein per 1 ml buffer (50 mM Tris-HCl; pH 7.0 and 50 mM NaCl). Degradation was not observed even after 12 h at 37 °C (Fig. 1A,B, lanes 8). On the other hand, the MRJP1 and the 47 kDa degradation product of monomeric-LM fraction (Fig. 2) were degraded under the same conditions as low-molecular fragments, immunoactive against MRJP1 (Fig. 1A,B, lanes 9). The components of the LM fraction were weakly bound to the oligomeric subunit of MRJP1 and were separable from MRJP1 by size exclusion chromatography. The oligomeric MRJP1 obtained by column chromatography (Fig. 2) was very soluble in water and at concentrations of 30 to 50% (w/w) formed a stiff gel. The solubility of MRJP1 in water and formation of gel at pH 3.9 were not influenced by freezing to –20 °C and thawing. From the amino acid sequence of MRJP1 it was possible by computer analysis to predict 20% α-helix segments and 17.6% β-conformation of the total amino acids residues.

3.3. Microscopic investigation of RJ and self-assembly of MRJP1 in RJ-gel

Examination by SEM showed relatively large globular particles in some areas of the freely developed RJ layer. Their sizes ranged from 20 to 80 μm and were connected with each other by a system of filamentous networks (Fig. 3A). Higher power magnification of an individual globule (Fig. 3B) showed filaments radiating from the shell-like surface of the globule. The diameter of the filaments was about 2 μm and their length varied.

The RJ-gel obtained by ultracentrifugation was further microscopically investigated. Complex filamentous networks with globular particles that were formed on cover glass immediately after solvating the RJ-gel in water were observed by light microscopy (Fig. 3C), resembling structures detected in RJ (Fig. 3A,B). The mode of sample application and concentration of the protein determined the architecture of MRJP1. An interesting self-assembly of network filamentous structures of MRJP1

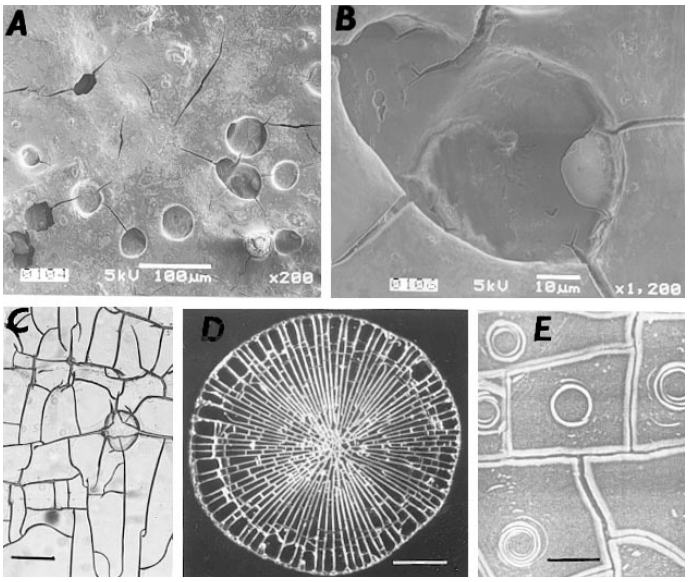

Figure 3. Microscopic examination of RJ and morphological appearance of assembled MRJP1. (**A**) The scanning electron microscopy (SEM) view of globular particles in RJ obtained from an individual queen cell of 2-day-old larva. (**B**) Higher SEM magnification of a globular particle in RJ presented in Figure 3A. (**C**) The visualization of spherical-membranous-filamentous structures of RJ-gel at a protein concentration of a 190 mg per 1 ml water using a light microscope. Bar 100 µm. (**D**) The free assembling of regular filamentous structures of MRJP1. The MRJP1 was obtained by dialysis of HM (Fig. 2) against water and lyophilization. The light microscopy view was performed 20 min after applying a drop (3 µl) of MRJP1 (80 mg per 1 ml water) on a cover slip. Bar 500 µm. (**E**) The structure of RJ-gel layer and demonstration of its autofluorescent properties. A thin layer was formed after a gentle touch of RJ-gel from a centrifugation tube by a glass stick on cover a slip. Examination of this layer was performed after 24 hours drying at room temperature. Bar 50 µm.

prepared from RJ-gel (HM fraction, Fig. 2) was observed (Fig. 3D). The process of self-assembly of MRJP1 was entirely spontaneous and the arrangement of fibrils presented in Figure 3D had symmetrical features. The fluorescent microscopic investigation of the original RJ-gel sample obtained by ultracentrifugation showed the yellow autofluorescent lanes surrounding irregular quadrilateral planes figure with an autofluorescent circle in the centre (Fig. 3E).

4. DISCUSSION

To eliminate the changes of RJ caused by storage (freezing, lyophilization) a fresh RJ was used in these experiments. Ultracentrifugation was a suitable method for preparation of the MRJP1, the most abundant protein of RJ, in the form of gel. Significant amounts of fatty acids were concentrated in fractions with lower water content: 11.5% in RJ-soft-sediment and 48.1% in RJ-sediment. Hence, the content of fatty acids was assumed to be a factor influencing the physical properties of RJ sediment fractions. It has been suggested (Sasaki et al., 1987) that an interaction between RJ water-soluble protein and 10-hydroxy-2-decanoic acid (10-H-2-DA) was the main factor in the loss of its fluidity and it was associated with the whitening of RJ. The white, semisolid, water-insoluble RJ-sediment

contained 40.3% protein (Tab. I) corresponding to MRJP1 (Fig. 1A, lane 4), 36.6% of 10-H-2-DA and 8.6% of 10-hydroxydecanoic acid (10-H-DA). It is reasonable to suggest that an interaction between MRJP1 and fatty acids resulted in the formation of the water-insoluble protein fraction of RJ.

MRJP1 was present in RJ in different forms: a monomer, oligomeric subunits (Fig. 2), and water-insoluble aggregates in sediment after its interaction with fatty acids. On the other hand, the localization of monomeric MRJP3s and MRJP2 in the supernatant fluid fraction (Fig. 1A, lane 2) could be determined by their hydrophilic properties influenced mainly by C-terminal of the repetitive region (Schmitzová et al., 1998). The repetitive conserved polymorphic pentapeptide (14–28 repeated) pattern XQNXX of MRJP3s was composed mainly of polar amino acids, cationic (arginine/lysine) and anionic (aspartic acid) (Albert et al., 1999), and therefore MRJP3s was present as a main soluble protein component of RJ-supernatant (Fig. 1A, lane 2). The MRJP1 localized mainly in sediment fractions (Fig. 1, lanes 3–4) was only one member of the MRJPs protein family without a hydrophilic C-terminal repetitive region (Schmitzová et al., 1998).

Despite this the oligomeric MRJP1 purified from RJ-gel by column chromatography (Fig. 2) was soluble in water in the gelatinous form even at a concentration of 50% (w/w). This stiff and colorless gel of MRJP1 was not influenced by freezing to –20 °C and subsequent thawing. Ovalbumin for example loses part of its solubility during the freezing-thawing treatment (Koseki et al., 1990). It is assumed that MRJP1 belongs to the albumin-like proteins and, therefore it is named as apalbumine. From the amino acid sequence of MRJP1 (Ohashi et al., 1997; Schmitzova et al., 1998) it was possible to predict (Rost and Sander, 1993) its preliminary secondary structure. According to this prediction MRJP1 contained 20% α-helix segments and 17.6% β-conformation of the total amino acids residues, that is, a relatively less ordered secondary structure than ovalbumin containing 30.6% α-helix and 31.4% β-sheets segments (Stein et al., 1991; Tatsumi and Hirose, 1997).

MRJP1 has an ability to form in the air regular filaments and four-branched shell structures (Fig. 3C,D,E). These structures resemble in macrodimension the self-assembly of some protein subunits in forming closed structures such as rings, tubes, or spheres (Alberts et al., 1994). Quaternary structures are formed by spontaneous, but ordered aggregation of individual peptide chains (identical or different) to form multisubunit structures that are stabilized by noncovalent forces (Franks, 1993).

It is still not clear how some of the self-assembly processes are regulated. Evaluation of the architecture of the multi-subunit of MRJP1 in RJ-gel seems to be an overwhelming task when one compares the results from relatively indirect approaches to its function during the period of larval feeding. The formation of oligomers from monomers would, of course, lead to large proteins thereby diminishing their permeability through membranes and also decreasing osmotic pressure in various organelles containing a large concentration of proteins. For many proteins, oligomer formation undoubtedly would confer enhanced stability relative to their monomeric constituents (Eisenstein and Schachman, 1989). On theoretical grounds, one would not expect the major part of the MRJPs to break into amino acids in vivo because it could produce an intolerably high osmotic pressure (Tsao and Shuel, 1968). This general feature of protein oligomers was confirmed by stability of the oligomeric form MRJP1 (Fig. 1A, B, lanes 8) and sensitivity of the monomeric form of MRJP1 to degradation (Fig. 1A, B, lanes 9) during long incubation at 37 °C. It was suggested that some protease(s) of RJ (Chen and Chen, 1995) could also be present in RJ-gel.

It was demonstrated (Crailsheim, 1991) that nurse honeybees distributed the RJ proteins produced by hypopharyngeal glands to all hive mates. We have detected by imunoblotting using polyclonal antibodies against recombined MRJP1 (Júdová et al., 1998) that MRJP1 was the most abundant protein in the water soluble fractions of the pollen pellet and honeybee's pollen bread (Šimúth et al., unpublished data). In addition to nutrition function, MRJP1 could also play a particular role in a honeybee colony in processing honeybee products because mRNA for MRJP1 was detected in hypopharyngeal glands of foragers (Kaludiny et al., 1994a; Ohashi et al., 1997). The structural properties, mainly flexible self-assembly of MRJP1 presented in Figure 2C,D,E, as well as biochemical stability of RJ-gel in water (Fig. 1A,B, lanes 8) may participate in immobilization of the pollen dust dispersed over the entire honeybee and formation of pollen pellet during processing of the pollen grains.

Few generalizations can be made about physiological properties of MRJPs. The behavioral evidence, though suggestive, still does not tell us directly whether MRJPs do serve the similar function in early as well as in later larval development. The study on breakdown of RJ protein in the midgut of the larvae (Tsao and Shuel, 1968) showed that some RJ proteins may have passed through the gut epithelium unchanged. Recently it was found that MRPJ1 was expressed in the brain of adult honeybees from a few hours after emergence to the 21st day at the sites where the learning and memory centres are located (Kucharski et al., 1998). It means that MRJP1 is in the hypopharyngeal glands of the nurse honeybee and is secreted into RJ as the main source of protein nutrition and, at the same time, the same honeybee synthesizes MRJP1 in the brain. The polyfunctional features of MRJP1 and other MRJPs could also be extrapolated from the analysis of the *Drosophila yellow* locus. Recently, it has been found that the *Drosophila* genome encodes at least seven members of the Yellow family (Maleszka and Kucharski, 2000). The yellow locus in *Drosophila* is involved in both the cuticle development and behavior. Yellow protein functions in the polymerization of melanin precursors in adult cuticular structures of *D. melanogaster* (Kornezos and Chia, 1992). We have found that MRJPs are related to yellow proteins of *D. mellanogaster* and have no relatives in other non-insect metazoan species (Albert et al., 1999). A characteristic feature of the MRJPs family, apparently absent in the yellow proteins, is the presence of the repetitive region at their C-terminal (Albert et al., 1999).

The physiological function of RJ-proteins during larval development is not fully known because of absent fundamental sequential data on their genes. Therefore, we started with sequential characterization of the MRJP1 gene and its regulation region with the methods used in mapping the human genome.

The presented method opens the possibility of obtaining large quantities of homogeneous MRJP1 in the form of gel. The polyfunctional properties and molecular mechanisms of self-assembly of MRJP1 makes this protein an important object of investigation, not only in the context of bee studies, but also from a basic molecular standpoint.

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Résumé – Quelques propriétés de la protéine la plus abondante de la gelée royale d'abeille (*Apis mellifera*). La recherche sur la gelée royale (GR) s'est concentrée sur les fonctions physiologiques de ses protéines principales (GRPPs), particulièrement la

protéine la plus abondante de la GR désignée par GRPP1. Le but de cette étude était d'utiliser l'ultracentrifugation pour fractionner la GR afin d'isoler sa protéine la plus abondante (GRPP1) dans la forme naturelle permettant une caractérisation de ses propriétés moléculaires et structurales. La GR a été séparée par ultracentrifugation ($245\,000 \times g$, 5 h, 6 °C) en 3 fractions physiquement distinctes : un surnageant liquide jaunâtre (61 % p/p de la GR), un sédiment mou gélatineux jaunâtre-brun (32 % p/p de la GR) et un sédiment blanc presque solide (7 % p/p de la GR). L'ultracentrifugation de la fraction gélatineuse a fourni la GRPP1 sous forme de gel (gel de GR). La répartition des principaux constituants de la GR (protéines, glucides et acides gras) dans les fractions d'ultracentrifugation était différente de celle de la GR originale (Tab. I). Le surnageant de la GR contenait une quantité plus élevée de glucides (50,6 %) mais seulement 1,2 % d'acides gras. La majorité des acides gras de la GR étaient concentrés dans les fractions ayant une faible teneur en eau, i.e. dans le sédiment mou de la GR (11,5 %) et dans le sédiment solide (48,1 %). Ces fractions contenaient principalement de la GRPP1. On suppose que l'interaction entre la GRPP1 et les acides gras aboutit à la formation du sédiment de la GR insoluble dans l'eau.

La comparaison des spectres protéiniques des GRPPs de la GR obtenus par SDS-PAGE avec les spectres protéiniques du sédiment mou, du sédiment solide (Fig. 1A, voie 3) et du gel de GR (Fig. 1A, voie 5) a montré que la GRPP1 était abondante dans toutes les fractions avec un poids moléculaire de 55 kDa. Par contre, dans le surnageant (Fig. 1A, voie 2) on n'a trouvé que des protéines avec poids moléculaire de 49 kDa (GRPP2) et 60–70 kDa (GRPP3). L'ultracentrifugation de la fraction gélatineuse de la gelée apparaît comme une méthode convenable pour isoler la GRPP1, protéine la plus abondante de la GR sous forme de gel (gel de GR). La GRPP1 était présente dans la GR sous des formes différentes : monomère (55 kDa), sous-unité oligomérique (environ 420 kDa) (Fig. 2) et agrégats insolubles dans les sédiments après interaction avec les acides gras. La GRPP1 oligomérique est fortement hydrosoluble et formait un gel solide dans les concentrations de 30 % à 70 % (p/p). On suppose que la GRPP1 fait partie des protéines à l'albumine.

Une propriété intéressante de la forme oligomérique de la GRPP1 est sa capacité d'assemblage dans les solutions aqueuses. Des filaments réticulaires avec des particules globulaires formés dans le gel de GR ont été observés par microscopie optique (Fig. 3C,D,E).

On a montré que la protéine GRPP1 existe dans le miel, les pelotes de pollen et le pain de pollen (Šimúth et al., résultats non publiés). En dehors de la fonction nutritive, la GRPP1 dans la colonie des abeilles peut jouer un rôle particulier dans la traitement du pollen floral en pelotes de pollen. Les propriétés structurales, spécialement l'autoassemblage flexible de la GRPP1 ainsi que la stabilité biochimique du gel de GR dans l'eau peuvent participer à la fixation du pollen sur le corps de l'abeille et à la formation des pelotes de pollen lors de l'humidification des grains de pollen.

La méthode présentée permet d'obtenir de grandes quantités de GRPP1 native, homogène et sous forme de gel. La GRPP1 devient un modèle attractif pour les études d'autoassemblage des protéines ainsi que pour les recherches physiologiques de la GRPP1 au cours du développement larvaire.

gelée royale / ultracentrifugation / protéine semblable à l'albumine oligomérique / formation de la gelée / autoassemblage

Zusammenfassung – Einige Eigenschaften der häufigsten Proteine im Weiselfuttersaft (royal jelly) der Honigbiene (*Apis mellifera*). In der Forschung über Weiselfuttersaft (royal jelly-RJ) standen bislang die physiologischen Funktionen der

Hauptproteine (MRJPs) im Zentrum, hauptsächlich das am häufigsten vorkommende als MRJP1 (Major Royal Jelly Protein) bezeichnete Protein des RJ.

Das Ziel der vorliegenden Studie war, durch Ultrazentrifugation RJ sparsam zu fraktionieren und damit das reichlich vorhandene Protein MRJP1 in seiner natürlichen Form zu Charakterisierung seiner molekularen und strukturalen Eigenschaften zu isolieren. Das RJ wurde durch Ultrazentrifugation (245 000 × *g*; 5 Stunden; 6 °C) in drei physikalisch gesonderte Fraktionen getrennt, eine gelbliche Flüssigkeit als Überstand (61 % W/W von RJ), eine gelblich-braune gelatineartige mittlere Schicht (32 % W/W von RJ) und ein weiβer, fast fester Rückstand (7 % W/W von RJ). Die ultrazentrifugierten Schichten von RJ enthielten im Vergleich zum ursprünglichen RJ (Tab. I) unterschiedliche Anteile an Protein, Zucker and Fettsäuren. Der RJ-Überstand enhielt eine gröβere Menge von Zucker (50,6 %), aber nur 1,2 % Fettsäuren. Der Hauptteil der Fettsäuren von RJ war in den Fraktionen mit dem geringeren Anteil an Wasser enhalten, dem RJ-gelatineartigen Rückstand (11,5 %) und dem festen Rückstand (48,1 %). Diese Fraktionen enhielten vorwiegend MRJP1. Der Vergleich der Proteinmuster von MRJP1 in RJ auf SDS-PAGE (SDS-Polyacrylamid Gel-Electrophoresis) mit Proteinmustern des RJ-gelatineartigen Rückstands (Abb. 1A, Linie 3) und RJ-gel (Abb. 1A, Linie 5) zeigte MRJP1 als ein häufiges Protein mit einem Molekulargewicht von 55 kDa. Anderseits waren in dem Rückstand von RJ (Abb. 1A, Linie 2) hauptsächlich Proteine mit einem Molekulargewicht von 49 kDa (MRJP2) beziehungsweise 60–70 kDa (MRJP3s) enthalten. Eine erneute Ultrazentrifugation des gelösten Gelfraktion Rückstands war eine geeignete Methode um MRJP1, dem in RJ am häufigsten enthaltenen Protein in Form von Gel (bezeichnet als RJ-Gel) zu isolieren. MRJP1 war in RJ in verschiedenen Formen enthalten: als ein Monomer (55 kDa), als eine oligomere Untereinheit (etwa. 420 kDa) (Abb. 2) und als wasserunlösliches Aggregat in Rückstand wegen seiner Wechselwirkung mit den Fettsäuren. Das oligomere MRJP1 war sehr gut wasserlöslich und bildete in der Konzentration von 30 bis 50 % (W/W) ein festes Gel. Wir schlagen vor, MRJP1 zu den Albumin-ähnlichen Proteinen zu zählen. Eine interessante Eigenschaft der oligomerischen Form von MRJP1 war seine Fähigkeit zur Spontanaggregation. Mit dem Lichtmikroskop wurden faserartige Netzwerke mit globulären Teilchen beobachtet, die sich in der Lösung von RJ-Gel oder MRJP1 auf mikroskopischem Deckglas geformt hatten (Abb. 3C,D,E).

Es wurde festgestellt, dass MRJP1 in Honig, in Pollenkügelchen und in Pollenbrot vorkommt (Šimúth et al., unveröffentlichte Ergebnisse). Auβer der Nahrungsfunktion des MRJP1 im Bienevolk könnte es ebenfalls eine besondere Rolle bei der Verarbeitung von Blütenpollen zu Pollenkügelchen spielen. Die strukturellen Eigenschaften, hauptsächlich die bewegliche Spontanaggregation von MRJP1, so wie die biochemische Stabilität von RJ-Gel in Wasser könnte an diesem Prozess der Verfestigung des Pollenstaubes beteiligt sein.

Die vorgestellte Methode eröffnet die Möglichkeit, groβe Mengen von homogenem natürlichen MRJP1 in Form von Gel zu erhalten. Es macht MRJP1 zu einem attraktiven Modell um strukturelle Studien von Protein-Spontanaggregaten, sowie physiologische Untersuchungen zur Rolle von MRJP1 während der Larvenentwicklung durchzuführen.

Weiselfuttersaft / Ultrazentrifugierung / oligomerische Albumin-ähnliche Proteine / Gel-Formierung / Spontanaggregation

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