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Molecular Composition of the Louse Sheath

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ABSTRACT: Flash pyrolysis-gas chromatography/mass spectrometry was used to assess the chemical composition of the head louse's nit sheath. The pyrolyzate of the female insect's secretions, which form a cement-like cylinder holding the egg onto the hair, is dominated by amino acid derivatives and fatty acids. No chitin-specific compounds were detected in the sheath. These results, contrary to previous reports, show that the polymeric complex of the sheath is composed of proteinaceous moieties, possibly cross-linked to aliphatic components. This study constitutes the first chemical characterization of the pyrolysis products of insect (louse) glue and unequivocally confirms that louse sheaths are not chitinous, as suggested by earlier histochemical studies. Development of agents that might loosen nits from the hair shaft is dependent on research that addresses the chemical composition of the nit sheath.

Key words: head louse, nit sheath, chemical composition, pyrolysis-gas chromatography-mass spectrometry

Head lice are caused by the host-specific, ectoparasitic insect *Pediculus humanus capitis* and remain a common infestation, as an estimated 6 million American elementary school students, equivalent to 1 in every 4, were infested in 1998 (Anonymous, 1998). The female louse attaches her eggs, i.e., developing embryonated egg, to the hair of the host with a glue-like, water- proof substance produced by the louse's accessory glands (Burgess, 1995). The eggs require 6-10 days to hatch, producing nymphs that develop through 3 instar stages prior to becoming adult lice. Whereas each instar phase of growth is completed in 3-5 days, the adult form lives for 30 days, during which the female lays approximately 10 eggs per day. After the nymph emerges from the egg, the nit, i.e., hatched and empty eggshell, remains adfixed to the hair unless physically removed by fine combing or manual pulling. These empty egg sheaths are highly reflective and visually apparent as white oval specks along the hair shaft.

As a response to the failure of topical insecticides to be 100% ovicidal, nit removal has become an important part of head lice therapy. Furthermore, there are strong movements toward advocating mandatory policies to prevent children from re-entering school after infestation unless all nits have been removed from the entire scalp (Burgess, 1995). Such a "no-nit" policy imposes potentially prolonged absenteeism, as no present commercial product significantly facilitates the task of nit removal (Burkhart and Burkhart, 1998; Burkhart et al., 1998). Indeed, it can take up to 9 h on average for a parent to search thoroughly and remove all nits from a child's scalp (Burgess, 1995). Various commercial chemical formulations based on the belief that the sheath was made of chitin (Barat and Scaria, 1962) have been

marketed (DeFelice et al., 1989; Parish et al., 1989); however, there is no report presenting unequivocal evidence for the chitinous character of the louse nit. Apart from traditional analytical methods, e.g., histochemical tests, arnino acid analysis, used to establish the biochemical composition of insect exoskeletons, recent studies documented application of pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) to the investigation of chitin in fresh and fossil invertebrate cuticles (Stankiewicz et al., 1996; Stankiewicz, Briggs et al., 1997). This method permits rapid determination (using a minute quantity of studied material) of the chemical constituents of insoluble biopolymers. Moreover, Py-GC/MS has been successfully used in combination with biochemical methods such as the colorimetric test in the study of chitin (Bierstedt et al., 1998). Thus, it proved to be a reliable qualitative tool in recognition of proteinaceous moieties in biological materials (Stankiewicz et al., 1996; Stankiewicz, Hutchins et al., 1997). The application of Py-GC/MS allows the determination of the chemical composition of the sheath. The results provided by the present study will hopefully lead to a solution to ameliorate nit removal. Nits were obtained from 5 patients infested with head lice. No attempt was made to designate specific hair from any particular person, as all nits were considered homogeneous for our investigation. The egg was surgically separated from the nit sheath; thereafter, the hair shaft was manually separated from the remaining sheath, so that only the clean sheath material was obtained preventing cross-contamination of sheath and hair. The sheaths and hair (devoid of sheaths) were stored in glass vials and were triple extracted using dichloromethane (CH_2Cl_2) to remove free lipids and possible contamination due to sample handling. Analyses of the extracted nit sheaths (-25 sheaths = 0.12 mg) and hair from the infested individual (-0.1 mg) were performed (in duplicate) using a CDS 120 Pyroprobe (CDS Analytical, lnc., Oxford, Pennsylvania) coupled to an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, Califomia), with an HP 5970 mass selective detector and a 50-m HP-1 GC column (0.2 mm i.d., film thickness 0.33 μm). The samples were pyrolyzed in a flow of He for 20 sec in a platinum coil at 610°C. The GC oven was operated as follows: isothermal hold for 5 min at 40°C; temperature programmed at 5°C/min to 300°C and final hold for 20 min. The MS was operated in full scan mode (50-550 Da, 0.86 scan/sec, 70 eV electron energy). Peaks were identified based on comparison with pyrolyzates of purified proteins and amino acids and published mass spectra (Munson and Fetterolf, 1987; Stankiewicz et al., 1996; Stankiewicz, Hutchins et al., 1997).

Our study did not reveal the presence of chitin-derived components in the pyrolyzate of the nit sheath but rather shows that it is proteinaceous with a relatively high contribution of C_{14} (myristic) and C_{16} (palmitic) saturated fatty acids and other aliphatic components, e.g., alkanols (Fig. 1). Close examination of the pyrolysis products shows a high relative abundance of components derived from amino acids, such as phenylalanine (peaks 2, 4, 5, 9, 13), tyrosine (peaks 7, 8, 8', 12), tryptophan (peaks 15, 17), and glutamic acid (peak 20). Moreover, 2,5- diketopiperazines indicative of dipeptides such as Pro-Ala, Pro-Gly, Pro-Val, Pro-Arg, and Pro-Pro were also observed (Fig. 1) and are known to be present in nondegraded proteins (Stankiewicz, Hutchins et al., 1997). Compounds apparently derived from leucine and isoleucine (Boon and de Leeuw, 1987) were also observed (Fig. 1), together with products found in pyrolyzates of human hair (characterized by ions m/z 100) and characteristic of keratin (Munson and Fetterolf, 1987; B. A. Stankiewicz, unpubl. obs.). These results indicate that the overall composition of the protein building the nit sheath, although structurally different, may be chemically similar to that of keratin. In fact comparison of sheath pyrolyzate to that of the hair from infested individuals, apart from striking differences in abundance of many pyrolysis products, e.g., 7, 8', 15, 18, 18', 20, 21-26, shows remarkable similarity in distribution of several protein-derived products (Fig. 1). However, further research is needed to substantiate unequivocally the possible keratin-like structure of the louse sheath. Furthermore, the relatively high abundance of Tyr and Phe may

suggest a high degree of sclerotization (Hopkins and Kramer, 1992) of the sheaths. The latter process provides insect cuticles with high mechanical strength and could explain the resistance of the nit sheath to chemical degradation. The involvement of melanins (as indicated by relatively high proportion of indoles in the pyrolyzates) in the formation of covalent linkages with other cuticular components (Hopkins and Kramer, 1992) is a possibility and would lead to a stiffening of the sheath. The presence of relatively abundant lipids in the lice sheaths is noteworthy. Because the free nature of fatty acids, thus contamination, can be easily ruled out (samples were extracted with polar solvent prior to pyrolysis), their macromolecular nature remains a possibility (lipid-protein bonds are known to exist; Bortz et al., 1990). For example, the fully keratinized epidermal cells were shown to be bounded by a cross-linked protein envelope with a monolayer of lipids covalently linked to the outer surface (Swartzendruber et al., 1987; Wertz and Downing, 1987; Wertz et al., 1989). These bound lipids, consisting of fatty acids, hydroxyacids, and ceramides, are believed to form a covering on the outer surface of the cells contributing to the chemical and enzymatic resistance of these cells (Swartzendruber et al., 1987; Wertz and Downing, 1987; Wertz et al., 1989; Bortz et al., 1990).

In conclusion, our study unequivocally demonstrates that the macromolecular structure of the human louse nit sheath is mainly composed of protein, with specific composition dominated by aromatic amino acid, perhaps associated with lipids, i.e., fatty acids. In addition, it provides convincing results showing that an abundance of the amino acids responsible for the sclerotization process, e.g., Tyr, Phe, aids in the formation of a molecular complex relatively resistant to chemical degradation. This study should aid in development of effective treatment for human lice.

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F1GURE 1. Total ion chromatogram (pyrolysis at 610°C for 10 sec) of (**A**) specimen of human louse (*Pediculus humanus capitis*) sheath and (**B**) human hair from the individual that sheaths were collected. Protein markers: 1 = pyrrole; 2 = toluene; 3 = methylpyrroles; 4 = ethylbenzene; 5 = styrene; 6 = C_2-pyrroles; 7 = phenol; 8, 8' = methylphenols; 9 = methylbenzonitrile; 10 = methyl-2,5-pyrrolidinedione; 11 = dimethylphenol; 12 = vinylphenol; 13 = ethylbenzonitrile; 14 = amine; 15 = indole; 16 = alkylpyrimidine; 17 = methylindole; 18, 18' = derivatives found to be especially abundant in pyrolyzate of keratin (m/z 100); 19 = histidine marker (m/z 133); 20 = glutamic acid and glutamine marker (m/z 84); 21 = 2,5-diketopiperazine of Pro-Ala; 22 = 2,5-diketopiperazine of Pro-Gly; 23 = diketodipyrrole (Hyp marker); 24 = 2,5-diketopiperazine of Pro-Val, Pro-Arg; 25 = 2,5-diketopiperazine of Pro-Pro; 26 = 2,5-diketopiperazine; ▢ = Leu-Ile markers; $FA_{14:0}$ = tetradecanoic acid; $FA_{15:0}$ = pentadecanoic acid; $FA_{16:0}$ = hexadecanoic acid; ◯ = other aliphatic components, * = contaminants (phthalate).