Identity of *Streptococcus mutans* Surface Protein Antigen III and Wall-Associated Protein Antigen A

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Preparations of *Streptococcus mutans* surface proteins AgIII and antigen A from different laboratories were compared with regard to amino acid composition, N-terminal amino acid sequence, electrophoretic mobility, and antigenic similarity. Despite previous observations of differences in physical properties, data indicate that these two preparations represent the same protein.

The antigenic structure of the surface of the oral bacterium *Streptococcus mutans* has attracted considerable interest because of the possibility of developing a vaccine against dental caries, and consequently a number of antigenic proteins have been identified (12, 17, 19). However, some questions concerning the relationship between antigens discovered by different investigators remain. Our laboratories independently described antigens, named AgIII (11) and antigen A (14), which could be extracted from the surface of *S. mutans* and also found free in culture supernatants. Both antigens showed the same distribution among the serotypes of *S. mutans*, but there were differences in the reported physical properties (Table 1).

The *wapA* gene that encodes antigen A has been cloned and sequenced (3). The *wapA* gene encodes a mature protein (WapA) with a predicted M_r of 45,000, and it was proposed that the antigen A found in culture supernatants of *S. mutans* is derived from proteolytic cleavage of WapA in the region of residues 330 to 340, where a cluster of four Arg residues and one Lys residue occurs (3). The deduced amino acid composition of the cleaved N-terminal fragment closely resembled that previously reported for AgIII (11). Thus, we undertook a direct comparison of the preparations of these two antigens from our independent laboratories.

AgIII was prepared by column chromatography as previously described (11) and subjected to automated N-terminal sequence analysis over 12 cycles. The resulting sequence was identical to the first 12 residues of mature WapA as determined by nucleotide sequencing (Fig. 1). AgIII has been shown to have an internal pepsin cleavage site which generates an antigenic fragment of approximately 22 kDa, designated pep AgIII (11). Analysis of the N terminus of this fragment revealed that it corresponded to residues 170 to 179 of WapA (Fig. 1).

The preparations of AgIII and antigen A (produced as described previously [16]) showed identical mobility by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), though the calculated molecular mass varied with the concentration of acrylamide used in the separating gel (Fig. 2), ranging from 29 kDa on 8% acrylamide to 34 kDa on 15% acrylamide (Fig. 2). Both antigen preparations displayed the same phenomenon of resolving into a doublet or triplet when

separated in lower concentrations of acrylamide. Both antigens reacted identically in Western blot (immunoblot) experiments with polyclonal rabbit antiserum raised against either antigen or monoclonal mouse antibody raised against antigen A (kindly provided by M. Hughes) (Fig. 3a), and in Ouchterlony immunodiffusion experiments, AgIII and antigen A showed a reaction of complete identity (Fig. 3b).

The data discussed above leave no doubt that AgIII and antigen A are identical but raise the question as to why physical methods of size determination have yielded such discrepant results. Even within our individual laboratories, we have observed size variations as determined by SDS-PAGE when minor changes have been made to electrophoretic conditions, and the migration of WapA is markedly at variance with its predicted size (3). The antigen's atypical behavior may, we believe, be explained by a number of characteristics. First, the protein is subjected to posttranslational modification by the removal of the signal peptide and also by cleavage in the region of residues 330 to 340; in addition, it seems probable that it is cleaved near the LPSTGE motif near the C terminus since this region is highly conserved in gram-positive bacteria and has been proposed as a target for specific proteolysis (9). Such multiple sites of cleavage would generate a range of molecular sizes, with the preponderance of each form determined by mechanisms not yet understood. Secondly, the primary amino acid sequence contains an extremely hydrophilic region rich in threonine and serine, amino acids which bind SDS poorly and hence give rise to anomalous migration during SDS-PAGE (7); thus, intact, extracellular, and pepsin-derived fragments will differ in the ratio of hydrophobic residues to hydrophilic residues. Thirdly, it has been reported that a high-molecular-weight form of WapA has binding properties which may contribute to interaction with separation matrices (10).

Although it is now clear that AgIII and antigen A are the same protein, the results concerning their potential as protective vaccines in animal models of caries immunity vary. M. W. Russell and his colleagues found no correlation between re-

TABLE 1. Comparative properties of AgIII and antigen A

Antigen	M_r	pI	Distribution (serotypes)	Reference(s)
AgIII	39,000	3.95	b, c, e, f	11
Antigen A	29,000	4.3	b, c, e, f	14, 15

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AgIII	(D)Q V T N Y T N(T)A S I
WapA	D Q V T N Y T N T A S I
	30
Pep AgIII	(V)K D K D I T P I S
WapA	V K D K D I T P I S
	170

FIG. 1. N-terminal sequences of AgIII and pep AgIII compared with corresponding sequences of WapA. Residues shown in parentheses are the most probable of two or three amino acids identified.

sponses to AgIII and protection of rhesus monkeys immunized with *S. mutans* and no protection after immunization of rhesus monkeys with purified AgIII (6, 13), whereas R. R. B. Russell et al. reported protection of cynomolgus monkeys after immunization with antigen A (18). Antigen A also protected mice against caries and has been proposed as a candidate human vaccine (5). The function of this antigen also remains unknown. It is located over the entire cell surface (8) but, in contrast to a number of other wall proteins, does not appear to contain any regions of α -helix which would give it a fibrillar structure. Antibodies to this antigen do not interfere with aggregation induced by sucrose or dextran (1) or with adherence to saliva-coated hydroxyapatite (2). Qian and Dao (10) reported that inactivation of *wapA* in *S. mutans* resulted in a significant decrease in sucrose-dependent adherence to surfaces, though Harrington and Russell (4) found no such effect. However, inactivation of *wapA* has an indirect "knock-on" effect on other surface components (4). It is possible that the consequences of this may vary in different strains of *S. mutans* and may account for discrepancies in the results obtained by different laboratories with regard to the adherence of different *S. mutans* strains, especially since adherence assays are generally regarded as difficult to standardize.

It is hoped that this paper clarifies confusions in the literature about the nomenclature of this antigen and stimulates investigation of the structure, function, and immunological significance of this major surface protein of *S. mutans*.

FIG. 2. SDS-PAGE of AgIII (lanes 1) and antigen A (lanes 2) on gels which contained 8 or 15% acrylamide. Molecular weight standards were run in lanes 3.

FIG. 3. (a) Western blot of AgIII (lane 1) and antigen A (lane 2) after SDS-PAGE (8% acrylamide), developed with monoclonal antibody to antigen A. (b) Ouchterlony immunodiffusion of AgIII (well 1) and antigen A (well 2) against antiserum to antigen A (well 3).

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