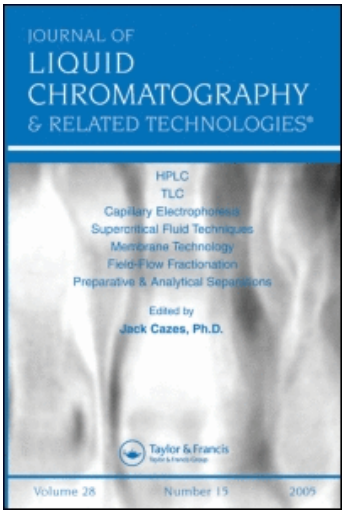

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PEPTIDE MAPPING OF VARIANT GLYCOPROTEINS FROM TRYPANOSOMA RHODESIENSE BY REVERSE PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Peptides of trypsin-digested surface coat glycoproteins isolated and purified from 4 cloned variants of Trypanosoma rhodesiense (Wellcome strain) were mapped by reverse phase high performance liquid chromatography. The peptide maps provide definitive chemical data demonstrating a lack of structural homology among the variant glycoproteins.

INTRODUCTION

The African trypanosomes are parasitic protozoa that cause sleeping sickness in humans and pose a serious threat to the health of 35 million people (1). The disease in animals is known as nagana, the prevalence of which prevents the use of 4 million square miles of Africa for raising livestock (2-4). Trypanosome infections may persist for several months and are often characterized by recurrent waves of parasitemia, in which the parasite population of each successive wave differs antigenically from that of preceding waves. Antigenic variation appears to be the primary mechanism enabling the African trypanosome

to evade the host's immune response (5-7). This phenomenon is generally regarded as a serious obstacle to the development of successful immunoprophylactic measures.

Antigenic variation is mediated through sequential elaboration of variant-specific cell surface glycoproteins (6,7). As each variant-specific glycoprotein (VSG) is expressed, induced host antibodies apparently destroy most of the parasite population. Subsequently, another population arises that carries a new VSG immunologically distinct from the previous VSG. VSG's have been isolated and purified from several species and variants of African trypanosomes and consist of a single polypeptide chain having an apparent molecular weight of 55-65,000 (8-13).

Structural studies suggest that the immunological uniqueness of VSG's results from considerable variation in the sequence of amino acids comprising the polypeptide chain. Partial tryptic hydrolysis of native VSG's has not revealed any common features associated with the cleavage sites (14). Isoelectric points and amino acid compositions have been shown to differ greatly (8,13,15). In addition, analyses of N-terminal amino acid sequences have demonstrated a complete lack of homology (14,16). These findings indicate extensive structural diversity and imply an absence of any significant sequence and conformational homology.

Attempts to determine total sequence variation of VSG's by means of conventional peptide mapping techniques are regarded as inconclusive (7,14). High performance liquid chromatography (HPLC) is a versatile technique for the rapid and effective separation of various biological compounds (17). The application of reverse phase HPLC to the separation of small underivatized peptides was recently described (18,19). Although this procedure was shown to provide a high degree of resolution and extreme sensitivity, only a few investigators have applied the technique to peptide mapping. Hancock and co-workers (20), for example, employed reverse phase HPLC to demonstrate similarities and differences in the sequences of several related proteins. The technique was also used by Fullmer and Wasserman (21) to map and subsequently purify tryptic fragments for amino acid sequence determinations. In the present study, a reverse phase HPLC separation system was developed and employed to map tryptic digests of 4 cloned variant antigenic types (VAT's) of the Wellcome strain (22) of Trypanosoma rhodesiense.

MATERIALS AND METHODS

Surface coat glycoproteins were purified from VAT's CP3B4, 6, 10 and 13 as previously described (13). One mg (dry wt) of each VAT preparation was

denatured by successive treatments including reduction and alkylation (23) and finally suspended in 1 ml of 0.05 M ammonium bicarbonate. TPCK (L-1-tosylamido-2-phenylethyl chloromethyl ketone)-treated trypsin (Millipore) was further purified by HPLC (24) just prior to use. Ten µg quantities of the purified trypsin were added to the 1 ml suspension of denatured glycoprotein at zero time and at each succeeding 24-hr interval of incubation at 25°C. Immediately after the addition of trypsin, 25 µl aliquots were removed from the digestion mixture, mixed with 5 µl of glacial acetic acid to stop trypsinization, and injected directly into the liquid chromatograph.

A Waters Associates (Milfore, MA) liquid chromatograph equipped with two M6000A solvent delivery systems, an M660 solvent flow programmer, a U6K universal injector, an M440 absorbance detector set at 280 nm, an M450 variable wavelength detector set at 215 nm, and an M730 data module were utilized. All separations were performed in a µ Bondapak C_{18} reverse phase column (3.9 mm x 30 mm), also from Waters Associates.

RESULTS AND DISCUSSION

Figure 1 shows the progressive tryptic digestion of VAT 13 glycoprotein. Glacial acetic acid appeared as a large peak at 3-4 min. In the zero time chromatogram, undigested glycoprotein emerged as a peak at 34 min. Following incubation for 24 hr, numerous peaks representing different peptide fragment peaks were clearly evident, although some intact glycoprotein still remained. At 72 hr, digestion was complete as indicated by the absence of the glycoprotein peak. The appearance of several additional peptide fragment peaks was also observed. The height and shape of peptide peaks were generally altered with increasing incubation time. Since further changes were not apparent at 96 hr, subsequent digestions were stopped at 72 hr with glacial acetic acid and the digest lyophilized.

The lyophilized digest of each VAT glycoprotein was dissolved in 400 µl of aqueous 0.1% trifluoroacetic acid, and 100 µl samples used for chromatography. As shown in Figure 2, the number of peaks resolved for the different VAT digests ranged from 30 to 40. Based on the hydrolytic peptide specificity ascribed to trypsin, this number of fragment peaks or cleavage sites is consistent with the quantities of lysine and arginine previously found in each of these VAT glycoproteins (13). To demonstrate reproducibility, three different preparations of each VAT glycoprotein were digested and each digest was chromatographed in triplicate. The within-day peptide fragment retention times of these runs varied by no more than $\pm$ 0.04 min, while day-to-day variation was within a range of $\pm$ 0.14 min. All digests were run under

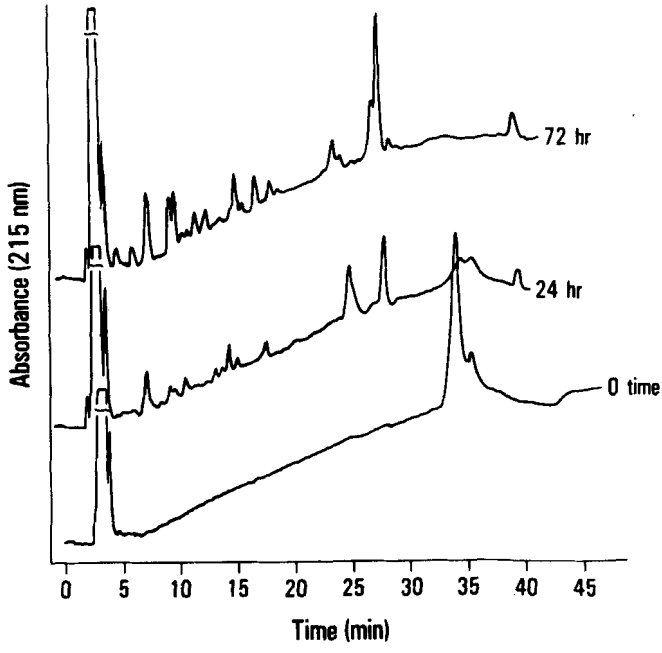

FIGURE 1. High performance liquid chromatographic analysis of the progress in trypsin digestion of VAT 13 glycoprotein. Elution of peptides was achieved by use of a 30 min linear gradient of 12% to 45% acetonitrile-water mixtures containing 0.1% trifluoroacetic acid at a flow rate of 2 ml/min. All runs were conducted at room temperature. Note position of undigested glycoprotein peak at 34 min.

comparable conditions to permit comparative observations. To facilitate comparison of peptide retention times among the four VAT's, a schematic representation consisting of vertical bars is presented in Figure 3. None of the vertical bars were shared by all four VAT's. Moreover, the patterns of retention times were totally different. We conclude from these findings that the purified VAT glycoproteins under present investigation do not contain significant stretches of amino acid homologies and that variation in the sequence of amino acids occurs throughout the polypeptide chains.

Richards et al. (25) found homology to the extent of 27% in the amino terminal region of two sequentially expressed VSG's in T. congolense, but not in non-sequential T. brucei variants nor between T. brucei and T. congolense VSG's. C-terminal amino acid sequences, as deduced from nucleotide sequencing of T. brucei VSG cDNA's, reveal regions of homology in the final 120-130

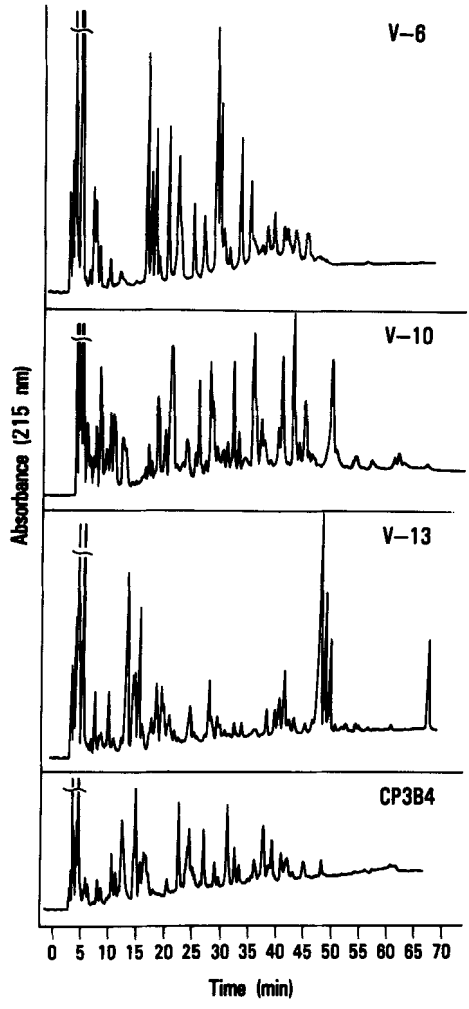

FIGURE 2. Peptide map chromatograms of variant glycoproteins 6, 10, 13 and CP3B4. Separations of peptide fragments of 72-hr trypsin digestion mixtures were obtained by a 60 min gradient as described in Figure 1 at a flow rate of 1.5 ml/min.

amino acids (26,27). A hydrophobic terminus of 17-23 amino acids and an uncharged polar region of 17-18 residues are the longest conserved sequences. However, the hydrophobic terminal sequence is not found on purified mature VSG's (28,29). Since the variants employed in this study, with the exception of CP3B4, were obtained at designated intervals (13) from a single rabbit in

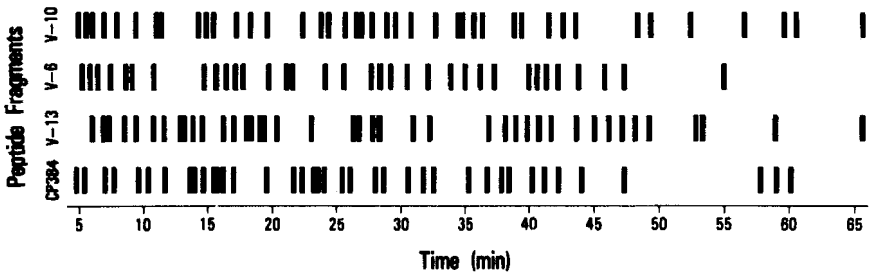

FIGURE 3. Schematic representation of the peptide map chromatograms of variant glycoproteins 6, 10, 13 and CP3B4. The vertical bars represent the average retention times of peptide peaks of triplicate runs (± 0.15 min) for each trypsin-digested glycoprotein.

which the course of infection was characteristically mild and generally uniform throughout the period of our observations, it is not clear whether such variants represent sequential (or non-sequential) populations of trypanosomes. Nonetheless, it is reasonably certain that significant stretches of conserved amino acid homologies would be revealed by the present investigative approach employing reverse phase HPLC peptide mapping.

The present findings suggest that this technique may have significant application in the immunochemical characterization of VSG's. Recently, hybridoma-derived monoclonal antibodies have been prepared against VSG's of T. rhodesiense (30) and T. brucei (31). Coupled with the use of monoclonal antibodies directed against VSG's or any glycoprotein antigens, the technique should permit the recovery of peptides that can be employed as possible inhibitory haptens, thus facilitating the location or definition of immunogenic determinant sites. In addition, immunochemical mapping studies may be designed to probe the relationship of these sites to protective immunity.

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