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Trichomonas vaginalis: Electrophoretic Analysis and Heterogeneity among Isolates Due to High-Molecular-Weight Trichomonad Proteins

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Department of Microbiology, The University of Texas Health Science Center; San Antonio, Texas 78284, U.S.A.; and †Department of Obstetrics and Gynecology, Hahneman Medical School, Philadelphia, Pennsylvania 19102, U.S.A.

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ALDERETE, J. F., GARZA, G., SMITH, J., AND SPENCE, M. 1986. *Trichomonas vaginalis:* Electrophoretic analysis and heterogeneity among isolates due to high-molecular-weight trichomonad proteins. *Experimental Parasitology* 61, 244–251. The protein composition of *Trichomonas vaginalis* isolates was evaluated using one-dimensional and two-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoresis and fluorography. At least two hundred intrinsically labeled and about 30 major extrinsically labeled proteins of molecular weights less than 120,000 were resolved by isoelectric focusing and electrophoresis. In general, long-term grown and fresh isolates of *T. vaginalis* gave similar fluorograms of the readily detectable proteins. Only a minor variance in a distinct protein was noted among three of the four fresh isolates tested. Labeling with [^{35}S]methionine or ^{3}H-amino acids gave almost identical profiles, ensuring the efficient radiolabeling of trichomonad proteins that dominate quantitatively. Comparative analysis of radioactivity profiles of one-dimensional gels emphasizing the region of high-molecular-weight proteins known to reside in low copy number revealed the presence or absence of the internally synthesized proteins from surfaces of *T. vaginalis* isolates. Finally, immunoblotting of two-dimensional gels demonstrated the highly immunogenic nature of proteins corresponding to quantitatively dominant molecular weight regions of intrinsically labeled proteins.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Trichomonas vaginalis;* Protozoa, parasitic; Protein heterogeneity; Electrophoresis, sodium dodecyl sulfate–polyacrylamide gel (SDS–PAGE); One-dimensional (1-D); Two-dimensional (2-D); Molecular weight (MW); Isoelectric focusing (IEF); Phosphate-buffered saline (PBS); Bovine serum albumin (BSA); NaCl (150 m*M*)-EDTA (5m*M*)-Tris hydrochloride (50 m*M*) (NET); Radioimmunoprecipitation (RIP); Kilodalton (kDa).

INTRODUCTION

Trichomonas vaginalis is an aerotolerant, protozoa which is responsible for one of the most common of sexually transmitted diseases (Ackers 1982; Honigberg 1978). Little is known, however, of specific properties of the organism responsible for disease pathogenesis (Honigberg 1979). Studies which define specific factors of *T. vaginalis* and which may be useful markers common or unique among trichomonal strains are essential. For example, diagnosis of trichomoniasis is presently suboptimal requiring microscopic detection and culturing of the organism (Muller 1983). Thus, generation of antibody reagents which recognize common and stable *T. vaginalis* proteins likely to be found in patients with trichomoniasis (Alderete and Garza 1984) might lead to development of a sensitive diagnostic test.

Analysis of the protein composition and the immunogenic nature of key membrane antigens is an important area of investigation for this microorganism. Recently, comparisons of the various pathogenic human *T. vaginalis* strains using 1-D gel electrophoresis (Alderete 1983a) revealed no differences in their respective protein compositions. Yet, an extensive literature is available which describes a pronounced

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heterogeneity among *T. vaginalis* isolates (Honigberg 1978; Laan 1966; Su-Lin and Honigberg 1983; Warton and Honigberg 1983). Recently, we demonstrated that antigenic heterogeneity among *T. vaginalis* isolates was a property of the ability of some but not all isolates to place a repertoire of high molecular-weight, immunogenic proteins on their surface (Alderete 1985). Furthermore, it appeared that all isolates were capable of biosynthesis of the entire repertoire. In this report, we further test the commonality in the overall protein makeup among the pathogenic human trichomonads. The 2-D electrophoretic patterns of intrinsically labeled proteins show only quantitative differences in a few proteins among several *T. vaginalis* isolates. Only few distinct proteins varied among fresh isolates examined. Dominant protein bands, however, were present in all *T. vaginalis* isolates regardless of length of *in vitro* cultivation. Importantly, data reinforce the previous observation that major differences among isolates may be due to the surface disposition of high-molecular-weight proteins present in low copy number which are synthesized by all isolates.

Materials and Methods

Trichomonas vaginalis isolates NYH 286, JH31A, IR-78, and 30001 have been grown for longer than 2 years by daily passage and have been used previously (Alderete 1983a, 1985; Peterson and Alderete 1984b). These strains, along with the fresh isolate, RU 375, were kindly provided by Miklós Müller (Rockefeller University, New York, NY, USA). Strains labeled JHHZ, JHHW, and JHHR were isolated by inoculating TYI-S-33 medium, pH 6.0, (Diamond *et al.* 1978) with a swab containing vaginal material from an infected individual. Tubes with fresh isolates were placed in a 37 C incubator after addition of gentamycin (10 μg/ml; Sigma Chemical Co., St. Louis, MO, USA) and penicillin (100 units/ml)–streptomycin (100 μg/ml) (M.A. Bioproducts, Walkersville, MD, USA). After growth and multiplication to densities similar to those of long-term grown cultures (Peterson and Alderete 1982), the organisms were passaged daily in trypticase-yeast extract-maltose medium supplemented with serum (Peterson and Alderete 1982). No bacterial contamination was evident after the third serial passage based on standard aerobic and anaerobic culture techniques. Trichomonads grown for no more than three serial passages were cryopreserved by resuspending approximately 5×10^6 organisms in 0.5 ml medium with 10% dimethyl sulfoxide (American Type Culture Collection, Rockville, MD, USA) and storing vials in liquid nitrogen. Parasites from fresh isolate cultures were grown for longer than 1 week after isolation. Only logarithmic phase organisms were employed for these studies (Peterson and Alderete 1982).

Intrinsic radiolabeling of live, motile organisms with [^{35}S]methionine or ^{3}H-amino acids was accomplished as detailed previously (Alderete 1983a). Surface radioiodination of proteins on intact *T. vaginalis* was performed as described recently (Alderete 1983b). For protease treatment of surface-labeled trichomonads, 1 ml of 8×10^6 washed organisms suspended in PBS were treated with 10 mg Pronase (Sigma) at 37 C for 30 min with continuous gentle shaking. Trichomonads were monitored by dark-field microscopy and enumerated with a Neubauer counting chamber to ensure negligible loss of parasites during treatments. Organisms treated with PBS served as controls. After enzyme treatment, the trichomonads were washed twice with PBS prior to trichoroacetic acid precipitation of total proteins for SDS–PAGE (Alderete 1983a).

1-D SDS–PAGE and fluorography were as outlined elsewhere (Alderete 1983a; Peterson and Alderete 1982). Stacking and separating gels consisting of 3 and 7.5% acrylamide, respectively, were employed throughout. Gels were stained with Coomassie brilliant blue (Sigma), destained, and processed for fluorography (Alderete 1983a).

For 2-D SDS–PAGE, IEF of proteins in 12-cm long cylindrical gels was performed using ampholines (LKB Instruments, NC, USA) with the pH range 3.5 to 10 (O'Farrell 1975; O'Farrell *et al.* 1977). A constant voltage of 350 V was applied to the gels for 18 hr followed by a 1-hr exposure of gels to 700 V to achieve complete focusing. Slab gel electrophoresis was performed using a 14-cm 7.5% acrylamide gel with a 2-cm 4% stacking gel. IEF gels were removed from tubes placed on the stacking gel and secured with an overlay of agarose. Electrophoresis was performed as before using 15 mA as initial current until the bromophenol blue penetrated the separating gel, at which time the current was increased to 30 mA per gel. MW standards used for both 1-D and 2-D gels were from Bio-Rad Laboratories (Richmond, CA, USA).

Total trichloroacetic acid-precipitated proteins of *T. vaginalis* were obtained as detailed previously (Peterson and Alderete 1982). Eighty microliters of a total protein preparation boiled for 4 min in dissolving buffer were added to 32 mg urea (O'Farrell 1975) in 13 × 100-mm borosilicate glass tubes. After the urea was completely dissolved, 200 μl lysis buffer (9.5 *M* urea, 2% (w/v) NP-40, 2% ampholines composed of 1.6%

pH 5–7 and 0.4% pH 3.5–10, and 5% β-mercaptoethanol) was added to the sample, and the 40–70 μl volumes loaded on a typical IEF. Unused portions were frozen at −20 C in lysis buffer for several weeks with no noticeable changes in gel patterns.

Electrophoretic transfer of most or all proteins from the acrylamide gels was performed as described recently (Peterson and Alderete 1983) by a modification of the method of Towbin *et al.* (1979) with a Trans Blot Cell (Bio-Rad). Blotting was carried out at 300 mA for 12 hr in a solution consisting of 25 m*M* Tris base, 192 m*M* glycine, and 20% methanol (pH 8.3). After 2-D electrophoresis, the slab gel is sandwiched between four layers of Whatman No. 40 filter paper and two porous polystyrene sheets, and enclosed in a hinged plaster cover. One layer of nitrocellulose cut to the appropriate slab gel size is placed on the cathode face of the gel. Special precaution was taken to eliminate any trapped air between the gel and nitrocellulose-filter papers. Control blots were stained in a 0.1% amido black stain in a methanol–acetic acid (45:10, vol/vol) solution for 5–10 min and destained in a methanol–acetic acid (90:2, vol/vol) solution.

The nitrocellulose blots were then removed and incubated with 5% BSA (Sigma) prepared in NET buffer, pH 7.2, (Alderete 1983a) for 90 min at 37 C to block any nonspecific binding sites. Finally, appropriate control serum or antiserum to *T. vaginalis* diluted 1/10 in NET buffer to a final volume of 100 ml were added to the blots. After a 2-hr incubation at room temperature with constant gentle swirling, the blots were washed well for 15 min with NET buffer followed by twice further for 30 min each wash with NET-0.05% NP-40 detergent (Gallard-Schlesinger, Carle Place, NY, USA). Another two individual washes with NET buffer alone were followed by addition to the blots of NET-5% BSA containing ^{125}I-protein A (Sigma) (sp act, 1.2×10^7 cpm/μg protein) radioiodinated by a modified chloramine T procedure (Dorval *et al.* 1975). The blots were then washed with NET buffer overnight with at least three buffer changes. The nitrocellulose blots were finally dried in

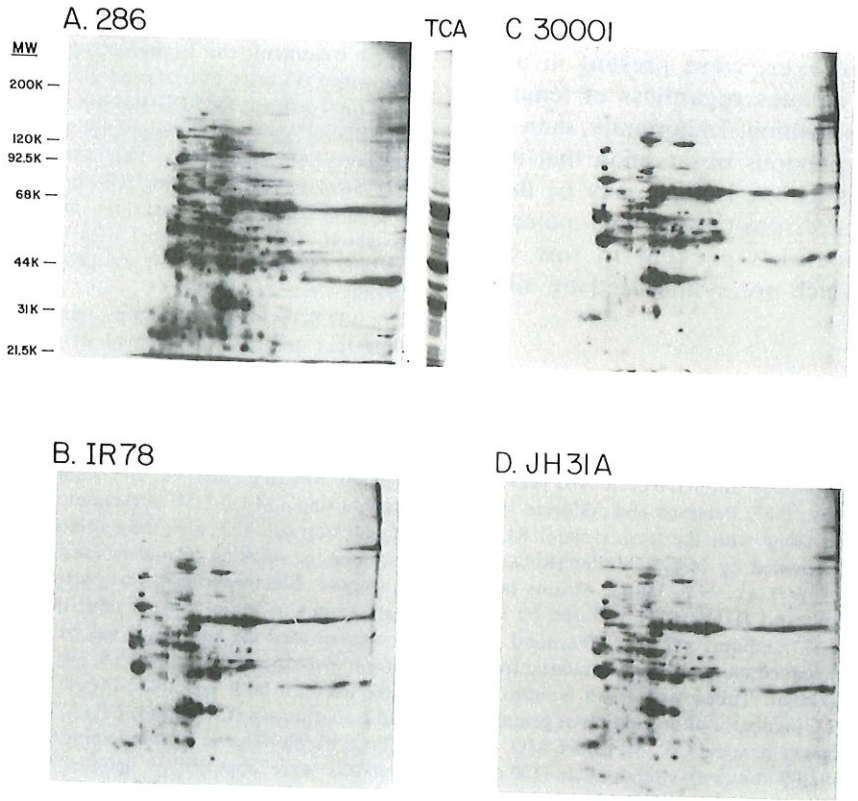

FIG. 1. Two-dimensional gel electrophoretic fluorograms of total proteins of [^{35}S]methionine-labeled, long-term grown *Trichomonas vaginalis* isolates 286, IR78, 30001, and JH31A. TCA refers to trichloroacetic acid-precipitated proteins shown in a representative one-dimensional (1-D) gel pattern. Numbers at right of 1-D gel indicate bands which correspond to highly immunogenic membrane proteins (ALDERETE 1983b). MW refers to molecular weights of protein standards (K = 1000).

warm air and then exposed to X-ray film for autoradiography. 2-D gels of intrinsically radiolabeled parasites were always run simultaneously and stained and exposed to X-ray film after blotting to ensure efficient transfer of proteins.

Prebled control serum and antiserum against *T. vaginalis* were generated in New Zealand white rabbits. Immunization protocols and testing of sera for antibody to *T. vaginalis* have also been described (Alderete 1983a). Additionally, these sera did not react with medium or serum components based on double diffusion and immunoelectrophoresis experiments.

RESULTS

A 2-D fluorographic profile of [^{35}S]methionine-labeled NYH 286 (Fig. 1A) is compared with 2-D patterns of other longterm grown *Trichomonas vaginalis* isolates (Fig. 1). The fluorogram was exposed to X-ray film longer for NYH 286 to show the complexity in protein composition of trichomonads and revealed up to 200 individual trichomonad protein spots as compared to only 30–40 protein bands resolved by 1-D SDS–PAGE of the same preparation. Only minor differences are obvious among the long-term grown isolates of *T. vaginalis* evaluated by 2-D electrophoresis. Further exposure of X-ray film to gels, however, yielded patterns almost equal to NYH 286 (Fig. 1A). 1-D gel patterns as presented for NYH 286 (Fig. 1A) were typical for all long-term grown and fresh isolates of *T. vaginalis*. The pH gradient employed for 2-D gels was from pH 4 (left of gel) to pH 10 (right side). IEF gel electrophoresis of trichomonads labeled with ^{3}H-amino acids yielded a profile identical to ^{35}S-labeled proteins (data not shown).

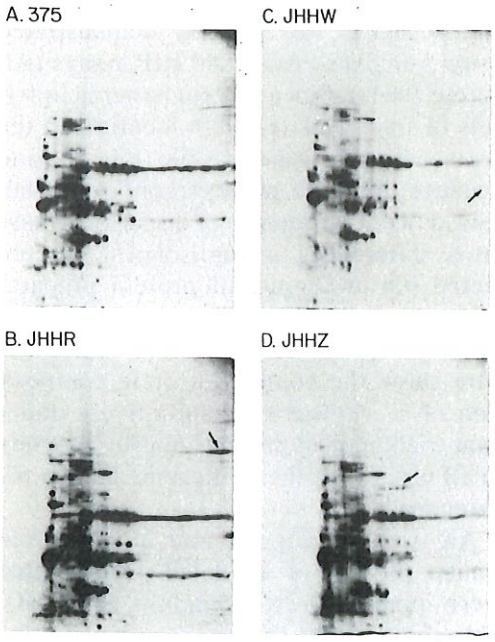

FIG. 2. Two-dimensional protein patterns of [^{35}S]methionine-labeled *Trichomonas vaginalis* isolates 375, JHHZ, JHHR, and JHHW grown for less than 1 week after isolation.

Figure 2 presents 2-D patterns of four representative fresh isolates of *T. vaginalis*. Overall, except for indicated minor variances in single spots, very similar if not identical gel patterns were obtained depending on the length of X-ray film exposure to fluorograms. For example, only JHHZ possessed a readily discernible quartet of proteins, and JHHW did not appear to have a protein prominent in the other isolates. JHHR also contained an intense protein spot absent among the other fresh isolates.

Figure 3 shows comparative 2-D profiles for [^{35}S]methionine-labeled versus radioiodinated trichomonads along with a typical 1-D pattern of ^{125}I-labeled proteins of isolate NYH 286. At least 40 prominent radioiodinated spots or regions (Fig. 3B) were readily resolved and clearly represented a subset of total proteins from intrinsically labeled trichomonads (Fig. 3A). All isolates (Figs. 1 and 2) gave equivalent 2-D patterns of surface proteins for the proteins $\leqq$70 kDa. The surface location of these radioiodinated polypeptides was demonstrated by the removal or decrease in the intensity of protein bands in both 1-D (Fig. 3D) and 2-D gels of protease-treated, iodinated organisms. The iodinated proteins in the 1-D gel pattern were similar to those obtained recently using radioimmunoprecipitation assays (Alderete 1983b).

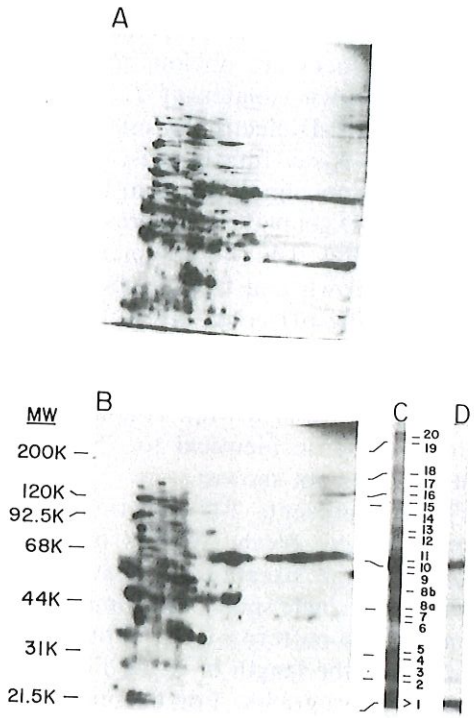

FIG. 3. Comparative gel patterns of two-dimensional analysis of intrinsically (A) versus surface-labeled (B) *Trichomonas vaginalis* NYH 286 proteins. A typical one-dimensional autoradiogram of TCA-precipitated, iodinated proteins is shown in (C) and (D) shows the bands of iodinated proteins of pronase-treated trichomonads. Numbers refer to immunogenic and antibody binding membrane proteins of *Trichomonas vaginalis* NYH 286 (ALDERETE 1983b). MW refers to molecular weights of protein standards (K = 1000).

It is noteworthy that few ^{125}I-labeled proteins were detected at or above a MW of 100 kDa. The low copy number of these molecules (Alderete 1983b) relative to other proteins may result in undetected spots. Radioactivity patterns of 1-D acrylamide gels were, therefore, performed to examine this molecular weight region. To our surprise, major divergence in ^{125}I cpm profiles were observed in the high-molecular-weight (≧70K) protein region of *T. vaginalis* isolates (Fig. 4A, area I). All isolates, however, gave similar if not identical cpm scans of intrinsically labeled proteins (Fig. 4B). Only the IR-78 isolate also gave a cpm profile of iodinated proteins like that seen for RU 375.

We finally wanted to determine the number and complexity of protein antigens by the immunoblot technique of proteins separated by 2-D gels. About 40 proteins were readily detected using antiserum to NYH 286 serum (Alderete 1983a) known to possess antibody to most protein antigens (Fig. 5). A correlation was observed between the intensity of some bands in the 2-D blot (Fig. 5) and the patterns of iodinated proteins (Figs. 3B,C). Importantly, proteins bound by antibody possessed electrophoretic mobilities similar to antigens identified by immunoprecipitation assays (Alderete 1983a,b) and are numbered on the corresponding 1-D gel (Fig. 5). Finally, all isolates yielded similar 2-D immunoblots, and no reactivity was ever observed when prebleed serum was used.

DISCUSSION

An extensive commonality among proteins of long-term grown *Trichomonas vaginalis* isolates was recently demonstrated using 1-D SDS-PAGE and RIP assays (Alderete 1983a). Since protein patterns in 1-D gels of fresh isolates were identical to the previously established profile (Fig. 1A) and because this type of electrophoretic analysis may not be capable of discerning qualitative differences among isolates, we subjected our trichomonad protein preparations to sequential IEF and SDS–PAGE and fluorography (Figs. 1–4). While our data show the complex protein composition of *T. vaginalis,* quantitatively dominant trichomonad proteins appear common to all isolates of the pathogenic human trichomonads.

All isolates (Figs. 1 and 2) possessed similar profiles of iodinated proteins that were quantitatively dominant (Fig. 3B). This showed that the same major proteins of ≦70 kDa comprise the surface of all isolates. An interesting finding, however, was that high-molecular-weight proteins,

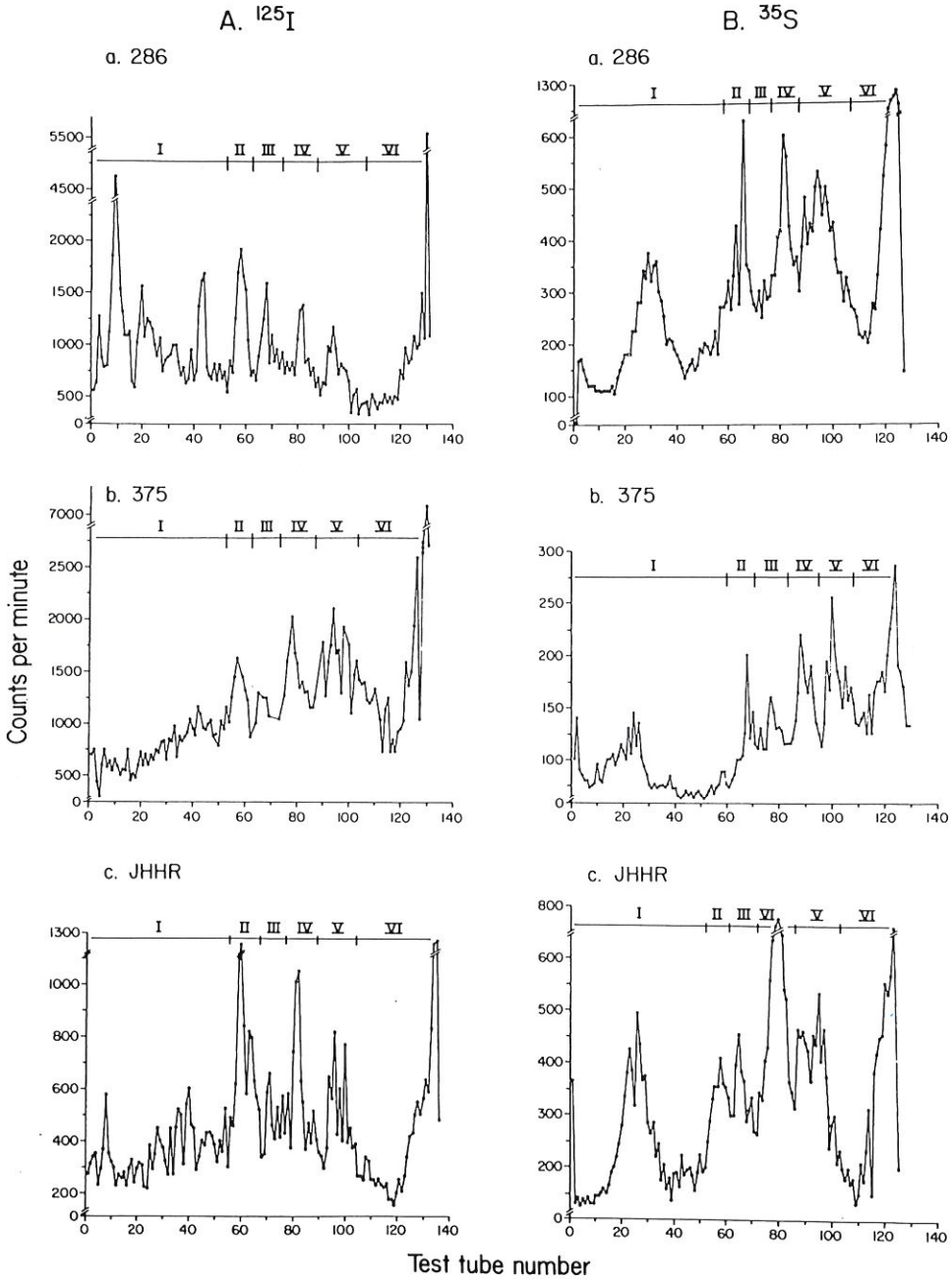

FIG. 4. Representative radioactivity patterns of one-dimensional 7.5% acrylamide gels of surface-iodinated (A) versus intrinsically labeled (B) *Trichomonas vaginalis* isolates.

though synthesized by all isolates (Alderete *et al.* 1985), are not externalized on surfaces of some isolates (Fig. 4, area I). These data reinforce the idea that the surface disposition of key protein immunogens in this same high-molecular-weight region may be the principal mediators of antigenic heterogeneity (Alderete *et al.* 1985) as re-

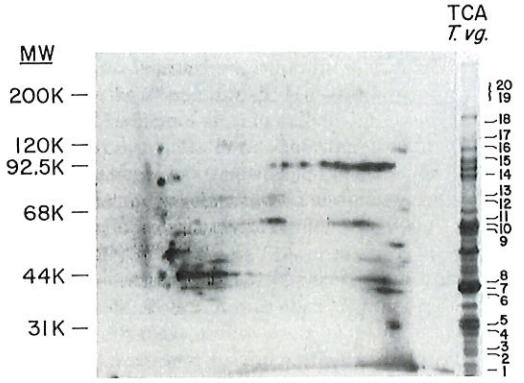

FIG. 5. Immunoblot with rabbit antiserum against *Trichomonas vaginalis* NYH 286 to proteins of NYH 286 separated by two-dimensional SDS-PAGE (Fig. 1A). Prebleed control serum did not bind to any proteins in a duplicate immunoblot. MW shows the molecular weights of protein standards (K = 1000).

ported in the literature (Honigberg 1978; Su-Lin and Honigberg 1983). The trichomonad origin of these iodinated proteins was demonstrated by using a whole cell radioimmunoprecipitation assay (Alderete 1983b) which detected protein antigens binding to antibody of intrinsically or extrinsically labeled *T. vaginalis* (Alderete 1983b; Alderete *et al.* 1985). Further chemical characterization of these proteinaceous molecules will be of interest. For example, a glycoprotein nature of components present or absent from surfaces of *T. vaginalis* isolates would also help clarify heterogeneity based on lectin recognition of trichomonads (Warton and Honigberg 1983).

Many proteins detected with rabbit antiserum to *T. vaginalis* in immunoblot experiments (Fig. 5) correspond in molecular weight to immunogenic proteins identified previously by RIP (Alderete 1983a,b; Alderete *et al.* 1985). The diminished intensity in X-ray films of proteins present in low copy number (Figs. 1–3) but recognized as major immunogens (Fig. 5) by immunoblot shows the usefulness of combined techniques in identifying possibly significant protein antigens. This information may be useful for future experimental strategies involving vaccine development. In addition, such data may be prerequisite to successful development of reagents such as monoclonal antibodies for diagnosis of trichomoniasis via antigen detection in patient body fluids (Alderete and Garza 1984; Connelly *et al.* 1985). In summary, we hope this and other studies in progress will ultimately help us to understand the nature and complexity of the *T. vaginalis* antigenic makeup and heterogeneity.

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