

Oligopeptide-Based Enzyme Immunoassay for Ovine Lentivirus Antibody Detection

JIMMY KWANG^{1*} AND JOSE V. TORRES²

U.S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, Clay Center, Nebraska 68933,¹ and Department of Medical Microbiology and Immunology, University of California, Davis, California 95616²

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Ovine progressive pneumonia virus (OPPV) is a lentivirus which causes a progressive disease in sheep. Immunodominant epitopes have been identified in the envelope gp40 glycoprotein. Synthetic peptides representing these regions are able to detect the presence of OPPV antibodies in 96% of infected sheep.

Ovine progressive pneumonia (OPP), also known as maedi virus or ovine lentivirus (OLV) pneumonia, is caused by a nononcogenic, exogenous retrovirus of the *Lentiviridae* subfamily (3). The disease is characterized by increasing respiratory distress, chronic weight loss and wasting, and the inevitable death of infected sheep (3). The disease has been recognized in most parts of the sheep-producing world and is endemic in the United States. OPP virus (OPPV) carriers are usually identified by detecting anti-OPPV antibodies in serum by the agar gel immunodiffusion (AGID) test (2). However, this test employs a viral antigen that is produced in an inefficient and time-consuming tissue culture system. Therefore, the AGID test is expensive and insensitive for routine clinical laboratory use (5, 11). Previously, a recombinant polypeptide which represented the N-terminal region of the transmembrane envelope glycoprotein (gp40) and which appeared to be a major serological marker for OPP was found (6, 7). These results have permitted the use of a single recombinant gp40 antigen to develop a specific enzyme-linked immunosorbent assay (ELISA) for the detection of OPPV infection in sheep (8). Synthetic peptides corresponding to the antigenic domains of human immunodeficiency virus gp41 have been incorporated in a clinical diagnostic reagent (1, 13). Therefore, three oligopeptides representing regions encoded by the portion of the gp40 gene corresponding to the N terminus were synthesized and tested in an assay designed to screen sheep sera for the presence of the OPPV antibody.

OLV peptide sequences were obtained from computer translations by the IBI Pustell sequence analysis program of OLV DNA sequences available through GenBank. The focus of the peptide analysis was on the N-terminal segment of the OPPV gp40, which covered amino acids 637 to 731 of the gp135 precursor envelope glycoprotein, situated immediately before the gp40 transmembrane segment. This region was analyzed by calculating the antigenic index, hydrophilicity, flexibility, surface probability, and secondary structure (4, 9). From these analyses, three antigenic sites likely to possess immunodominant epitopes were identified (Fig. 1). These sites corresponded to amino acid residues 648 to 664, 667 to 683, and 703 to 719, designated TGp-X, TGp-Y, and TGp-Z, respectively (Table 1) (12). The three peptides, each 16 amino

acids long, were prepared by F-moc solid-phase synthesis (10, 14). Synthetic peptides were dissolved individually in 0.01 M NaHCO₃ buffer (pH 9.6) and were used to coat 96-well microtiter plates (9 µg per well). The peptides were dried by evaporation overnight at 37°C. Each peptide was tested for reactivity against a panel of sheep sera.

A total of 170 sheep serum specimens were included in this study. Of these specimens, 100 were from known OPP-positive individuals, confirmed by AGID test and recombinant gp40 Western blot (immunoblot) assay. Twenty-two specimens were from the experimental OPP-negative flock of the National Animal Disease Center (Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa), and these sheep serum specimens were employed as negative controls. Forty-eight samples were collected from a flock that had previously been diagnosed with OPP. These 48 serum specimens were used to compare the sensitivities and specificities of the oligopeptide ELISA and the AGID test.

The specificity of each oligopeptide ELISA was evaluated by using the 100 known seropositive and 22 known seronegative sheep serum specimens. None of the 22 OPP confirmed negative samples showed a false-positive reaction with any of the three oligopeptides. However, the TGp-X, TGp-Y, and TGp-Z peptides were recognized by 84, 92, and 89% of 100 seropositive serum specimens, respectively. The data demonstrated that among the three peptides, TGp-Y represents a major antigenic epitope in gp40 of the OPPV. When the TGp-Y peptide ELISA results were compared with the combination of TGp-Y and TGp-Z ELISA results, the complementary effect of the two peptides was demonstrated. Specifically, the TGp-Z peptide detected an additional four serum samples, and antibodies against TGp-Y and TGp-Z epitopes together could be detected in 96% of the OPP-seropositive samples. Therefore, the overall test agreement between the recombinant gp40 Western blot assay and the combination of TGp-Y and TGp-Z peptide ELISAs was 96%. Addition of the TGp-X peptide did not show any complementary effect. The recombinant gp40 Western blot assay detected four serum samples that were negative in the TGp-Y and TGp-Z combination peptide ELISA results. These results indicate that the recombinant gp40 protein has a higher detection rate for OPP than do the synthetic peptides derived from the gp40 region. The reason for this could be that some infected animals had antibodies against epitopes other than TGp-X, TGp-Y, and TGp-Z, which are present in recombinant gp40 protein. Alternatively, these sheep could be infected with OPPV strains showing variation at these epitopes.

* Corresponding author. Mailing address: USDA, ARS, U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933. Phone: (402) 762-4372. Fax: (402) 762-4390.

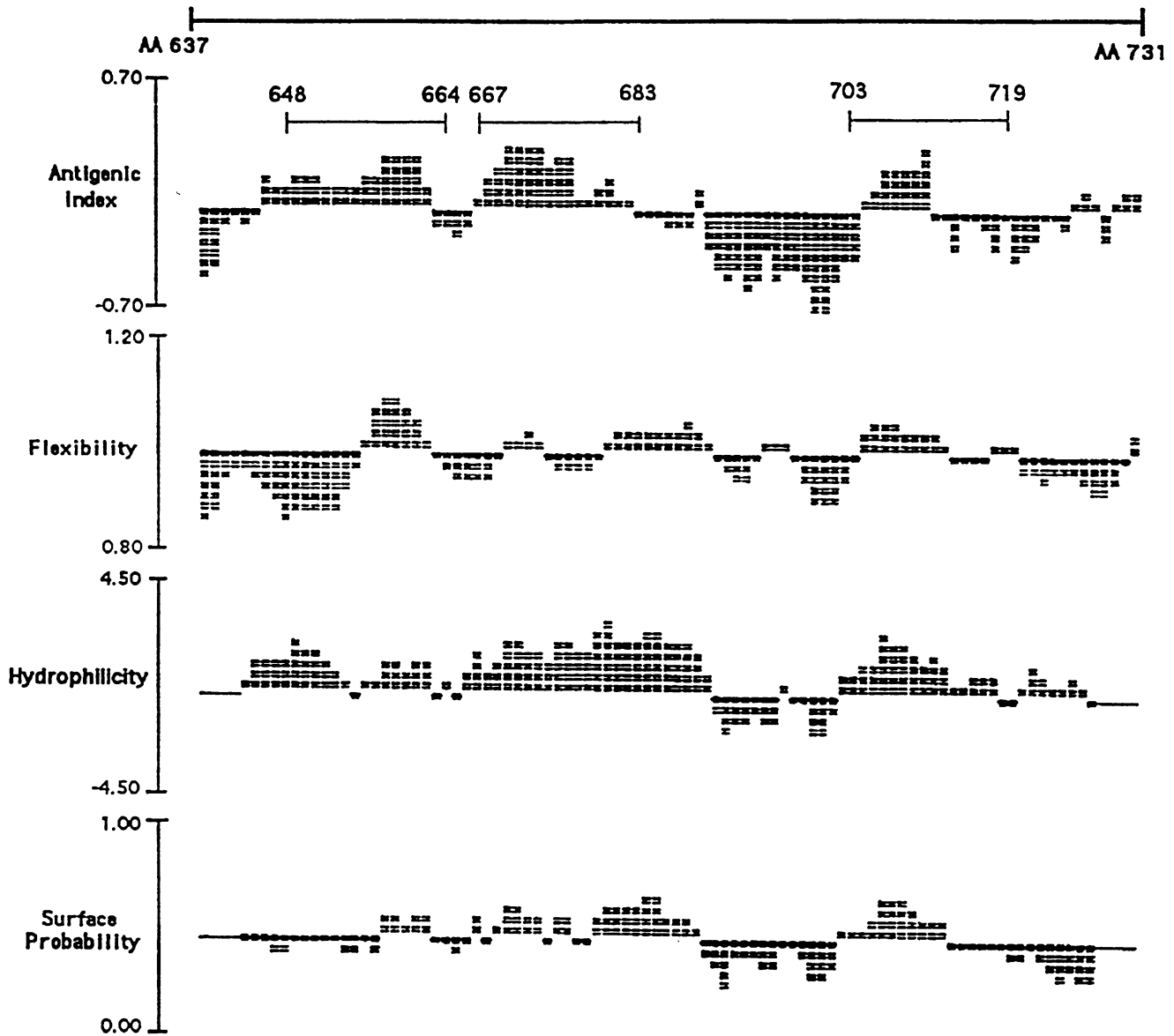


FIG. 1. Computer algorithm analysis of antigenic index, flexibility, hydrophilicity, and surface probability profile for the N terminus of OPPV gp40. AA, amino acid.

To compare the sensitivities of the peptide ELISA and the AGID test, we analyzed another 48 sheep serum samples by both methods. Among these 48, the AGID test detected 9 and the TGp-X, TGp-Y, and TGp-Z peptide ELISAs detected 13, 19, and 14 seropositive samples, respectively. Peptides TGp-Y and TGp-Z also showed complementary effects and increased

the detection rate to 21 of 48. These positive and negative ELISA results agree with the results of the Western blot assay. This comparison confirmed that the oligopeptide ELISA was more sensitive than the AGID test.

Economic OLV testing is important in the sheep industry, which has experienced only marginal profitability in recent years. There are three different test generations for diagnosis of OLV infection, and each test relies on either virus lysates, recombinant proteins, or synthetic peptides as the antibody-detecting antigen. Compared with antigens prepared from whole-virus lysates, both recombinant proteins and synthetic peptides have potential purity, sensitivity, availability, and cost advantages.

A major problem for the first-generation AGID test and whole-virus ELISA is that viral antigen preparation in cell culture systems is time-consuming, expensive, and tedious. The

TABLE 1. N terminus of gp40-derived synthetic peptides

| Peptide | Amino acid positions ^a | Sequence |
|---------|-----------------------------------|------------------|
| TGp-X | 648-664 | QHYCVTSTRSEVANYV |
| TGp-Y | 667-683 | RFKDNCTWQQWEEIE |
| TGp-Z | 703-719 | QRDARRIPDAWKAIQE |

^a The amino acid position numbers are based on the published sequence (12).

relative and absolute viral protein compositions are difficult to reproduce from one virus culture preparation to the other. Diagnostically relevant epitopes, especially *env* gene products, are often not available in sufficient quantities. Host cell proteins are incorporated into the virus membrane and either cannot or can only incompletely be removed. Thus, false-positive tests may result from immune reactions with cellular proteins which copurify with viral antigens, while false-negative tests may result from the absence or insufficient quantity of specific immunogenic viral proteins in the whole-virus antigen preparation.

Second-generation recombinant protein assays and third-generation synthetic oligopeptide assays have been developed to circumvent these problems. Properly selected and prepared recombinant and synthetic peptides used in solid-phase ELISA formats will increase the density of diagnostically relevant epitopes compared with viral lysates, resulting in increased test sensitivity. High compositional purity decreases the likelihood of nonspecific (false-positive) reactions. Furthermore, well-defined, uniform, and reproducible recombinant or synthetic peptides have distinct quality control advantages over virus culture-derived proteins when used as antigens in serodiagnostic assays. The requirement to maintain the virus itself is also eliminated by recombinant or synthetic antigen production.

While the initial development of recombinant OLV proteins was a biotechnologically intensive and time-consuming process (7, 8), it represented a one-time investment of resources and effort. After that, it was possible to produce and purify sufficient recombinant antigen in 2 days to test several thousand sheep serum specimens. Moreover, the growth and technical requirements for bacterial culture of *Escherichia coli* to produce recombinant proteins are simpler and less expensive than those for tissue culture of lentiviruses to produce conventional antigens. Thus, given the inherently faster and simpler in vitro culture of bacteria than that of viruses, recombinant proteins offer the potential for reduced cost per animal tested. Similarly, identification of diagnostically relevant synthetic peptide epitopes was a one-time resource investment. However, peptide synthesis is much more expensive than recombinant protein production. Furthermore, recombinant proteins appear to be more sensitive for OLV serodiagnosis than either viral lysates or synthetic peptides. Also, the signal-to-noise ratio tended to be higher for the recombinant transmembrane ELISA than for the synthetic peptide assay among samples that were positive by both assays, facilitating discrimination between negative and positive animals by the second-generation antigen.

Thus, our overall conclusion is that OLV second-generation recombinant proteins offer the best combination of economy and test performance compared with their first- or third-generation counterparts.

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