Reports

Mechanisms Controlling Phosphorus Retention Capacity in Freshwater Wetlands

Abstract. *Freshwater wetland ecosystems do not effectively conserve phosphorus in the way that terrestrial ecosystems do. The phosphorus retention capacity varies greatly among bogs, fens, and swamps and is concomitant with the amorphous acid oxalate–extractable aluminum and iron content in the soil. However, the phosphorus adsorption potential in wetland ecosystems may be predicted solely from the extractable aluminum content of the soil. Wetlands tested as wastewater filtration systems became phosphorus-saturated in a few years, with the export of excessive quantities of phosphate.*

It has often been hypothesized that freshwater wetlands are nutrient sinks which efficiently process nitrogen and phosphorus, reducing eutrophication of downstream aquatic ecosystems (*1*). High removal rates of many elements have been reported for wetlands, which has led to serious consideration of the use of these ecosystems for wastewater filtration (*2*). However, the amount of total phosphorus lost from many unfertilized wetland ecosystems often exceeds 0.4 kg ha^{-1} $year^{-1}$, nearly twice the average annual output reported for forested terrestrial watersheds (*3*). Moreover, major differences in phosphorus retention capacity among bogs, fens, marshes, and swamps have been suggested (*4*), but the processes controlling these patterns remain largely unexamined.

Storage of phosphorus in wetlands depends on the removal of dissolved inorganic phosphorus (DIP) from the water by microbial and plant uptake, soil adsorption, and incorporation of organic phosphorus into soil peat. The initial removal of DIP under natural loading levels is due largely to microbial uptake, and there is some geochemical adsorption by aluminum and iron minerals in the soil (*5*). This microbial pool is small and quickly becomes saturated (*4, 5*). Rooted emergent wetland vegetation takes up substantial quantities of phosphate, but after tissue death 35 to 75 percent of the plant phosphorus is rapidly released (*6*). Thus, vegetation serves only as a short-term sink for phosphorus unless the biomass is harvested. In temperate wetlands, peat accumulates at rates of 100 to 2000 kg of dry matter per hectare per year (*7*) with a phosphorus concentration of 0.05 to 0.12 percent (*8*). Annual peat accumulations of phosphorus are very limited and range from 0.05 to 2.4 kg ha^{-1}. Studies of a fertilized Michigan fen showed high initial retention capacity for DIP due to microbial and plant uptake, but these pools became saturated within a few years so that long-term storage of phosphorus was dependent on soil adsorption (*9*). Questions remain, however, concerning the reported differences among wetland types to retain phosphorus and the mechanisms controlling soil adsorption of phosphorus.

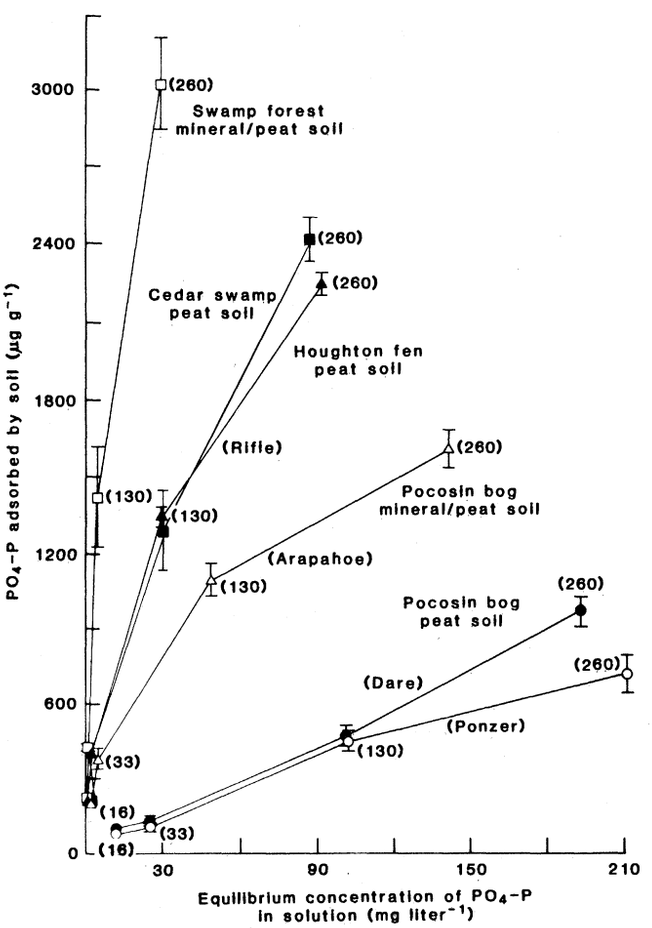

Fig. 1. Isotherms for the adsorption of phosphorus in bog, fen, and swamp wetland soils related to the equilibrium concentration of PO_4-P remaining in solution. The amount of PO_4-P added in solution ranged from 16 to 260 mg $liter^{-1}$ and is shown in brackets at each point on the curves. Error bars represent 1 standard deviation (n = 4).

In the present study undrained soils were collected and analyzed in 1983 from sites spanning a wide range of bogs, fens, and swamps (*10*). Soils were taken from the top 20 cm of each wetland, and four replicates were analyzed for pH, organic matter content, and extractable aluminum, iron, and calcium (*11*). Adsorption isotherms (*12*) relating the phosphate adsorption to the equilibrium concentration of phosphate phosphorus (PO_4-P) remaining in solution among the wetland types revealed that phosphorus sorption capacity was greatest in the fluvial silt loam soils of the swamp forests in Maryland (coarse-loamy, siliceous, acid Thermic Typic Fluvaquents) (Fig. 1). The phosphate retention capacity of the Rifle peat (Euic Typic Borohemists) at the Houghton fen and Tawas peat (Euic Typic Borosaprist) at Bellaire swamp in Michigan was more than double those of the North Carolina Dare pocosin peat (Dysic, Thermic Typic Medisaprists) and Ponzer pocosin peat (loamy, mixed, Dysic, Thermic Terric Medisaprists). The Arapahoe mineral-peat soil (coarse-loamy, mixed nonacid, Thermic Typic Humaquepts) found in some shallow peat pocosin areas had a sorption poten-

tial of nearly double the organic deep peat pocosin sites. Its phosphate adsorption curve was close to that of the fen soil but only up to 130 mg per liter of PO_4-P additions. Both the bog and fen soils showed a decreased phosphate uptake rate at the highest loading level, but the swamp soils with high mineral content displayed no evidence of phosphorus saturation (Fig. 1).

The actual differences in phosphorus adsorption potential between wetland mineral and peat soils are in fact greater than indicated in Fig. 1. When bulk densities of the mineral soils ($\sim 1 \times 10^6$ g m^{-3}) and the peats ($\sim 0.2 \times 10^6$g m^{-3}) are factored in, the peat soils project phosphorus adsorption capacities several orders of magnitude below mineral soils. The massive outputs of phosphorus reported from fertilized pocosin ecosystems with peat soils versus mineral soils (*13*) further substantiate the low phosphorus sorption capacity shown for peat bogs in Fig. 1. The high phosphate adsorption curve displayed by the Houghton fen peat also supports the field results of high initial removal rates of phosphorus from wastewater (*14*).

A phosphate adsorption index (PAI) was developed as an indicator of the capacity of wetland soils to adsorb applied phosphorus (*15*). The PAI values ranged from 163 in the swamp forest soils to 8 in the pocosin bog peats. The fen soils showed intermediate values near 50. Statistical examination of the correlation between PAI, percent soil organic matter, *p*H, and extractable aluminum, iron, and calcium, factors most often related chemically to phosphorus soil fixation (*5*), indicated that PAI was predicted better by concentrations of extractable amorphous (noncrystalline) aluminum ($r = 0.929$) and iron ($r = 0.621$) than by *p*H, extractable calcium, or organic matter. A linear regression model ($r^2 = 0.87$; $P < 0.01$; standard error of estimate = 21.3) of extractable aluminum versus PAI proved the best predictor of the adsorption index. The inclusion of iron and other soil variables in a multiple regression model did not significantly increase the variability explained (*16*). A graph (Fig. 2) of the relation between aluminum and PAI for 20 wetland sites shows a vast range in phosphorus retention capacity among bog, fen, and swamp ecosystems and demonstrates that the phosphorus retention capacity is directly related to the amount of extractable aluminum. High variation in phosphorus sorption was found among different sites in the Maryland swamps, where the standard deviation equaled one-half the mean ($X = 115$; standard deviation = ± 58). High adsorption variability among the swamp sites was not related to differences in earlier loadings of phosphate or site disturbances, but the soils varied in mineral content.

Adsorption isotherms or index methods are indicative of phosphorus retention potential but overestimate the actual field adsorption maximum because water movement follows large pores and channels, thus reducing contact with a large

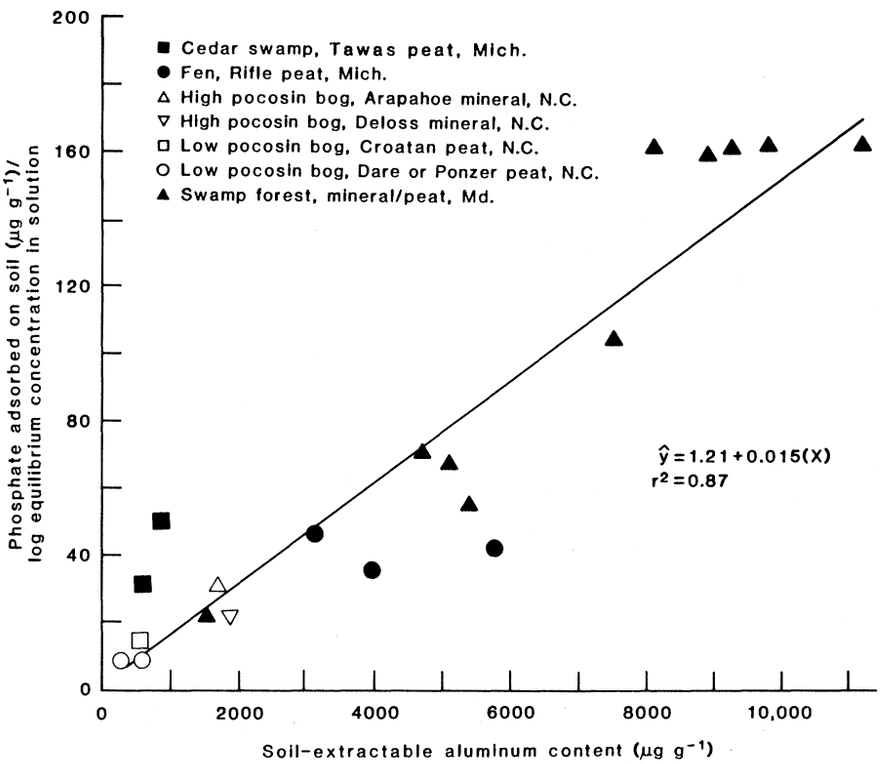

Fig. 2. The relation between extractable amorphous aluminum and a phosphate adsorption index (*15*) for bog, fen, and swamp soils with varying mineral content.

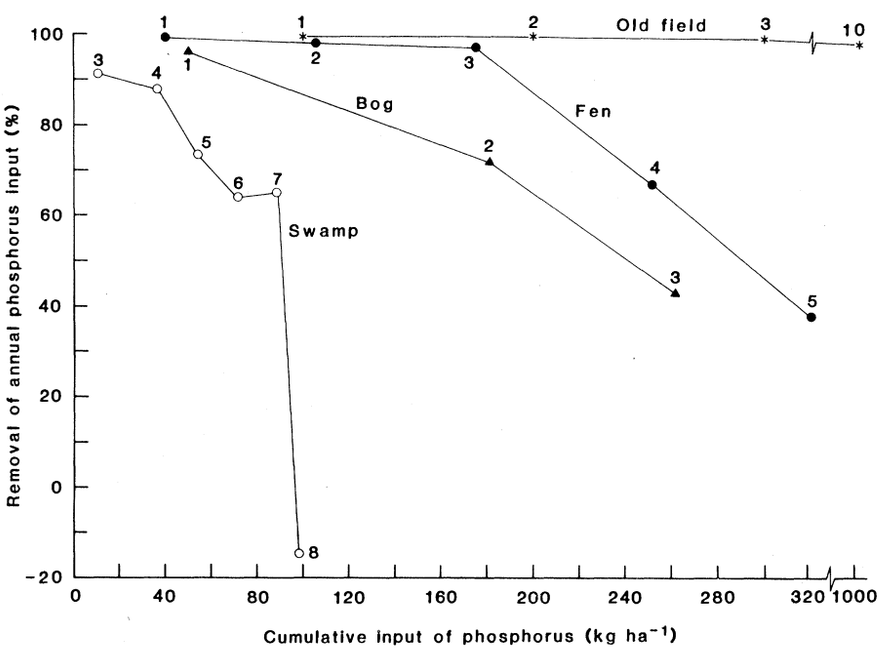

Fig. 3. The change in phosphorus removal efficiency for the Houghton Lake fen, a white cedar swamp forest, an Irish blanket bog, and an abandoned old field in Pennsylvania as a function of cumulative phosphate inputs. The numbers along each line indicate the number of years of phosphorus addition. The extractable aluminum contents for the fen and cedar swamp are shown in Fig. 2.

portion of the soil matrix (*4*). In an effort to determine the actual phosphorus retention capacity of several wetland types and compare this to terrestrial exports, I analyzed input-output data from the Houghton fen (*1, 4, 14, 17*), a Michigan white cedar *(Thuja)* swamp (*18*), a blanket bog in Ireland (*19*), and an old field ecosystem in Pennsylvania (*20*). All these ecosystems had received high loadings of phosphorus from wastewater over several years. The differences in phosphorus loading rates among these ecosystem types precludes an exact comparison of removal efficiencies, but the data are inconsistent with the hypothesis that wetlands are more efficient at removing and storing phosphorus than terrestrial ecosystems.

Indeed, the abandoned old field on a Hublersburg silt loam to clay loam soil (Typic Hapludalf) continued to remove 96 percent of the added phosphate in year 10 even though the total phosphorus loading of 1044 kg ha^{-1} was more than three times the phosphate received by any of the wetlands (Fig. 3). The high phosphate retention by this site was related to the large sesquioxide content of the soil (*21*), which was also found to be the factor controlling phosphorus geochemical fixation in a northern hardwood forest (*22*). In contrast, the percentage of phosphorus retained by the bog and fen dropped significantly after the first and third year of wastewater additions, respectively. The white cedar swamp received the lowest loading rates of phosphorus and continued to remove more than 60 percent of the phosphorus through year 7 (Fig. 3); in year 8 the cumulative loadings to the swamp resulted in a phosphorus loss of 12.7 kg ha^{-1} $year^{-1}$, which exceeded that year's inputs of phosphate by 25 percent (*18*). The peatland system with the highest phosphate removal was the fen, which had significantly higher concentrations of soil-extractable aluminum than the swamp or bog.

Collectively, these data indicate that high initial removal rates of phosphorus by freshwater wetlands will be followed by large exports of phosphorus within a few years. Wetland types with predominately mineral soils and high amorphous aluminum content (for example, swamps) are better phosphorus sinks than peatlands but apparently retain much less phosphate than terrestrial ecosystems. Higher soil phosphorus sorption in terrestrial ecosystems is related to the larger pools of aluminum and iron sesquioxides that are found in conjunction with mineral soils and aerobic soil conditions (*16, 22*). The fact that many wetlands have accumulated massive quantities of phosphorus in peat over time has also been misconstrued as implying high phosphate retention. The annual storage rate of phosphorus in peat in temperate freshwater wetlands is very low. The selection of natural freshwater wetlands with peat soils for efficient processing and storage of phosphorus, especially at high concentrations, is thus unwarranted.

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2. R. K. Bastian and S. C. Reed, Eds., *Aquaculture Systems for Wastewater Treatment: Seminar Proceedings and Engineering Assessment* (Publication MCD-67, Environmental Protection Agency, Washington, D.C., 1979).
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10. Four replicate soil samples were taken from ten swamp sites in Maryland, three locations in the Houghton fen in Michigan, three soil types in the pocosin bogs of North Carolina, and two locations in the Lake Bellaire white cedar swamp in Michigan. Soils were maintained at field moisture by storage in airtight plastic containers that were kept cool prior to analyses.
11. I determined the extractable noncrystalline (amorphous) aluminum and iron, using 100 ml of acid oxalate solution and 5.0 g dry weight equivalent of soil [W. M. Saunders, *N.Z. J. Agric. Res.* **8**, 30 (1965); M. I. Karim and W. A. Adams, *Soil Sci. Soc. Am. J.* **48**, 406 (1984)]. I determined *p*H electrometrically on a thin paste of the soil in water. Calcium was extracted with 1*M* ammonium acetate. An extraction time of 1 hour on a rotary shaker preceded filtration. Solubilized elements were determined by atomic absorption spectrophotometry [*Analytical Methods for Atomic Absorption Spectrophotometry* (Perkin-Elmer, Norwalk, Conn., 1982)]. After drying, organic matter was determined as loss on ignition by ashing for 4 hours at 450°C.
12. I developed phosphate adsorption isotherms for each soil by equilibrating 2.0 g dry weight equivalent of fresh soil with 25 ml of 0.01*M* $CaCl_2$ solutions containing 16, 33, 130, and 260 mg per liter of phosphorus added as KH_2PO_4 [L. E. Sommers, D. W. Nelson, L. B. Owens, M. Floyd, *Technical Report 99* [Purdue University, West Lafayette, Ind., 1977)].
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15. An indicator of each soil's phosphate sorption capacity was indexed as: $X/\log C$, where X is the quantity of phosphorus adsorbed by the sample (milligrams of phosphorus per 100 g dry weight of soil) and C is the concentration of phosphorus in an equilibrium solution (130 mg $liter^{-1}$) [B. W. Bache and E. G. Williams, *J. Soil Sci.* **22**, 289 (1971)].
16. I. C. R. Holford and W. H. Patrick, Jr. [*Soil Sci. Soc. Am. Proc.* **43**, 292 (1979)], have pointed out that the actual role of amorphous iron oxides or hydroxides in phosphorus sorption may be diminished since reduced soil conditions (for example, redox of −150 mV), often found in undrained wetlands, can lead to a decrease in phosphate adsorption because of the reduction and dissolution of ferric hydrous oxides.
17. R. H. Kadlec [*1982 Operations Summary, Houghton Lake Wetlands Treatment Project* (Univ. of Michigan, Ann Arbor, 1983)] found that the peat soil and biota near the wastewater discharge pipe had reached saturation and nutrient uptake capacity, respectively. This localized overloading of the ecosystem has resulted in a phosphorus gradient which now extends some

300 m from the wastewater discharge pipe. The average annual concentration of total dissolved phosphorus in the wastewater effluent at the outflow pipe in the wetland is 3.3 ± 0.6 mg liter^{-1} (85 percent of the total dissolved phosphorus is available to organisms as DIP). Approximately 6300 kg of phosphorus had been placed in the fen during the treatment period (1978 to 1982) (Kadlec, 1983). This represents phosphorus additions of nearly 9 kg ha^{-1}, but the wastewater was not distributed evenly over the entire 716-ha wetland. The large size of the fen in comparison to the input volumes of water and phosphate has resulted in the confinement of all phosphate additions within the ecosystem. Outputs, however, calculated for the entire peatland, do not give a realistic picture of the removal efficiency per hectare. An estimate of the phosphorus removal capacity of a portion of the fen was determined by a mass-balance approach for an area of 19.5 ha adjacent to the discharge pipe during 1978 to 1982. This size area was chosen since the average area of wetlands used for wastewater disposal in the midwest is 16 ha (*4*). The hydrologic budget and phosphorus concentrations for inputs and outputs were estimated for this area from data reported by Kadlec (*14*, *17*) and D. E. Hammer and R. H. Kadlec [in *Sixth International Peat Congress* (International Peat Society, Helsinki, Finland, 1981), pp. 563–569.

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Studies of the Putative Transforming Protein of the Type I Human T-Cell Leukemia Virus

Abstract. *The putative transforming protein of the type I human T-cell leukemia virus (HTLV-I) is a 40-kilodalton protein encoded by the X region and is termed p40^{xI}. On the basis of both subcellular fractionation techniques and immunocytochemical analysis, it is now shown that p40^{xI} is a nuclear protein with a relatively short half-life (120 minutes). It is synthesized de novo in considerable quantities in a human T-cell line infected with and transformed by the virus in vitro, and it is not packaged in detectable amounts in the extracellular virus.*

The human T-cell leukemia viruses, HTLV-I and HTLV-II, are closely associated with specific malignancies of T cells in humans (*1*) and are capable of transforming normal peripheral blood T lymphocytes in vitro (*2*). The putative transforming gene of these viruses, termed *x*, is located between the *env* gene and the 3′ long terminal repeat (LTR) (*3*). The proteins encoded by this gene in both HTLV-I and HTLV-II have been identified (*4*, *5*). A 40-kilodalton (kD) protein called p40^{xI} and a 37-kD protein called p37^{xII} were found in cells infected with HTLV-I and HTLV-II, respectively (*4*). The same proteins have been called p42^{lor} and p38^{lor} (*5*). Attention has been focused on the biology of these proteins because of their possible role in induction of T-cell malignancies. In this report we describe studies on the amount of p40^{xI} in infected cells, the kinetics of intracellular turnover of the protein, and its subcellular localization.

Previously, we used synthetic peptides representing determinants of the predicted translation products of the *x* genes of HTLV-I and HTLV-II to generate antisera to these proteins (*4*). Earlier studies had shown that antibodies to proteins produced in bacteria via expression vectors are useful in detecting the products of viral transforming genes (*6*, *7*). Here, we generated antibodies to the COOH-terminus of the p40^{xI} protein. The antiserum was tested in an immunoprecipitation assay with cells that had been infected with HTLV-I (SLB-I) and labeled with [^{35}S]methionine as described (*4*). As with the antisera to the peptides, the antiserum to the COOH-terminus recognized a 40-kD protein in HTLV-I–infected cells (Fig. 1). The antibody titers achieved in rabbits that had been injected with either the synthetic peptides or the bacterially promoted protein (fusion protein) were similar (*8*). However, in experiments with a fixed amount of isotopically labeled cell lysate and an equivalent amount of antiserum, antiserum to the fusion protein was three to five times better at immunoprecipitating the p40^{xI} protein than antiserum to the peptide (Fig. 1, lanes b, c, e, and f). This may have been due to the greater number of potential epitopes in the bacterially promoted 54-amino-acid x^{I} polypeptide compared to the 14- or 17-amino-acid synthetic peptides. The antiserum to the promoted protein, however, did not recognize the p37^{xII} protein in HTLV-II–infected cells (JLB-I). It is known that the proteins encoded by the *x* genes have less sequence homology at the COOH-termini than at the NH_2-termini (*9*).

The level of p40^{xI} protein synthesis compared to that of other cellular and viral proteins in HTLV-I–infected cells was estimated by immunoprecipitation. A known amount of isotopically labeled cell lysate was assayed and the radioactivity in the 40-kD protein band was eluted, counted, and compared to the total trichloroacetic acid–precipitable material in the cell lysate (*10*). Approximately 0.15 percent of the total [^{35}S]methionine incorporation was asso-

Fig. 1. Immunoprecipitation with antibodies against a p40^{xI} fusion protein. The derivative "runaway" plasmid pCFM516 (*25*), containing a bacterial tryptophan synthetase promoter and a synthetic bovine growth-hormone (bGH) gene (*26*), was used to generate a bGH-p40^{xI} fusion protein in *Escherichia coli*. A Sca I–Hinc II DNA fragment coding for the COOH-terminus of the p40^{xI} protein was ligated into M13 mp11 (*27*). The COOH-terminal p40^{xI} DNA fragment was excised from M13 mp11 with Sst I and Bam HI and placed in the bGH expression vector to form a fusion protein containing the NH_2-terminal 76 amino acids of bGH and the COOH-terminal 54 amino acids of the p40^{xI} protein. The fusion protein was purified from bacterial whole cell lysate by SDS-PAGE (*28*). The purified bGH-p40^{xI} fusion product was then used to immunize rabbits by the method previously described (*4*). Antisera were tested in an immunoprecipitation assay with HTLV-I–infected cells (SLB-I) metabolically labeled with [^{35}S]methionine for 4 hours (*4*). (Lane a) SLB-I cell lysate and sera from unimmunized rabbits; (lane b) SLB-I cell lysate and 5 μl of antiserum to p40^{xI} fusion protein; (lane c) SLB-I cell lysate and 15 μl of antiserum to the p40^{xI} fusion protein; (lane d) SLB-I cell lysate and 5 μl of sera from unimmunized rabbits; (lane e) SLB-I cell lysate and 5 μl of antiserum to the pX IV-6 peptide (*4*); and (lane f) SLB-I cell lysate and 15 μl of antiserum to the pX IV-6 peptide.