field cases of lactation failure. These results suggest that endotoxins may play a pivotal role in the pathogenesis of some cases of lactation failure in the sow. Sources of endotoxin may well be foci of bacteria, for example, *E. coli*, in one or more mammary glands of affected sows. This hypothesis is supported by Morkoc [(*12*); see also (*13*)] who reported a significantly higher incidence of detectable endotoxin concentrations in hypogalactic sows than in paired control animals. Although the endotoxins exert several effects that are deleterious to normal lactation, for example, disruption of the microvasculature, immunologic stimulation, and alterations in the endocrine profile of the animals, the relative importance of each to the disruption of lactation is unknown. Since prolactin is an apparent prerequisite for normal lactation in the pig (*14*), the magnitude of the observed prolactin decline is sufficient to produce lactation failure in the absence of the other changes induced by endotoxins. We therefore propose that small amounts of *E. coli* endotoxins are capable of suppressing prolactin concentrations in the sow and are a significant factor in the pathogenesis of lactation failure in the periparturient animal.

BRADFORD B. SMITH
W. C. WAGNER

Department of Veterinary Biosciences, College of Veterinary Medicine, University of Illinois at Urbana Champaign, Urbana 61801

References and Notes

1. A. D. Leman *et al.*, *J. Am. Vet. Med. Assoc.* **161**, 1248 (1972); W. R. Threlfall and C. E. Martin, *Vet. Med. Small Anim. Clin.* **68**, 423 (1973); L. Backstrom *et al.*, *Proceedings of the International Pig Veterinary Society Congress* (1982), Abstract 175; P. Dziuk, *Anim. Reprod. Sci.* **2**, 335 (1979).
2. H. W. Dunne and A. H. Leman, *Diseases of Swine* (Iowa State Press, Ames, 1975).
3. R. H. Penny, *Austr. Vet. J.* **46**, 153 (1970); N. Ringarp, *Acta Agric. Scand. Suppl.* **7**, 1 (1960).
4. T. J. McDonald and J. S. McDonald, *Cornell Vet.* **65**, 73 (1975); R. F. Ross *et al.*, *Am. J. Vet. Res.* **42**, 949 (1981).
5. R. G. Elmore, C. E. Martin, J. N. Berg, *Theriogenology* **10**, 439 (1978); O. Ladefoged, *J. Vet. Pharmacol. Therap.* **2**, 209 (1979).
6. W. R. Threlfall *et al.*, *Am. J. Vet Res.* **35**, 313 (1974).
7. R. F. Nachreiner *et al.*, *ibid.* **33**, 2489 (1972); I. Hermansson *et al.*, *ibid* **32**, 1065 (1971).
8. B. B. Smith and W. C. Wagner, unpublished observations; E. Benjaminsen, *Acta Vet. Scand.* **22**, 67 (1981).
9. P. S. Li and W. C. Wagner, *Biol. Reprod.* **29**, 11 (1983).
10. A. L. Mulloy and P. V. Malven, *J. Anim. Sci.* **48**, 876 (1979).
11. P. Bratusch-Marrain *et al.*, *Acta Endocrinol.* **99**, 352 (1982); J. R. Sowers *et al.*, *J. Clin. Endocrinol. Metab.* **44**, 237 (1977); G. Schwinn *et al.*, *Acta Endocrinol.* **82**, 486 (1976).
12. A. C. Morkoc, thesis, University of Illinois (1982).
13. A. C. Morkoc, L. Backstrom, L. A. Lund, *Proceedings of the International Pig Veterinary Society Congress* (1982), Abstract 174.
14. M. D. Whitacre and W. R. Threlfall, *Am. J. Vet. Res.* **42**, 1538 (1981).

14 March 1983; accepted 3 November 1983

Antigens on HTLV-Infected Cells Recognized by Leukemia and AIDS Sera Are Related to HTLV Viral Glycoprotein

Abstract. *Cross-reactive antigens of molecular size of 61,000 to 68,000 daltons are found on the surface of human cells infected by human T-cell leukemia-lymphoma virus (HTLV). They are recognized by antibodies from patients with adult T-cell leukemias and lymphomas, from healthy carriers of HTLV, and from patients with the acquired immunodeficiency syndrome (AIDS). The latter finding has been one of the major reasons for suggesting an association of HTLV with AIDS. However, whether these antigens are of cellular or viral origin has not been clear. These antigens have now been shown to be associated with the presence of viral proteins in the cells, and a cross-reactive glycoprotein of molecular size of 46,000 daltons has been found to be a consistent structural part of viruses purified from several HTLV-producer cell lines. The findings thus suggest a viral (HTLV) origin of these antigens.*

Human T-cell leukemia-lymphoma viruses (HTLV) are a family of exogenous T-lymphotropic type C retroviruses strongly associated with adult T-cell leukemia (ATL) (*1*). Recently, additional attention has been given to these viruses as a result of the demonstration that a high proportion of patients with acquired immunodeficiency syndrome (AIDS) have antibodies that react with antigens on the surface of HTLV-producing transformed human T cells, such as the Hut 102 (*2*) and the MT 2 (*3*) cell lines (*4*). In some cases, HTLV was isolated from these patients or was shown to be integrated in the cellular genome (*5, 6*). In particular, one of the surface antigens, which is also widely recognized by antibodies from ATL patients (*7*), appears to be gp 61, a glycoprotein of molecular size 61,000 daltons (*4*). Whether this antigen is of viral or cellular origin is, however, still unknown. If it is cellular in origin, its expression might be induced by HTLV infection. However, another etiological agent yet to be identified might induce the same cellular protein. Therefore, it is unclear whether the antibodies indicate a direct involvement of HTLV in AIDS, and it has become imperative to define the origin of these antigens.

Using an HTLV-transformed virus-producing cell line we call G-25/MI (*6*), we found an antigen of 63,000 to 67,000 daltons (p65) which, like gp 61 in Hut 102 cells (*4, 7*), reacted strongly with sera from both ATL patients and healthy carriers of HTLV but did not react with sera from seronegative normals, as shown by a strip radioimmunoassay (RIA) based on the "Western blot" technique (*8*) (Fig. 1A). The G-25/MI cell line was established from the leukemic T cells of a black ATL patient from the Caribbean (*6*) and produces HTLV of type I. Although all of the sera from ATL patients or seropositive normals also detected the viral *gag*-related antigens p24 or p19 (or both) (*9, 10*), the recognition of p65 was much stronger in many sera. An antigen of 54,000 daltons was previously identi-

Table 1. Competition of p65 and *gag*-related antigens by various cellular and viral extracts. Competition RIA's were done on G-25/MI cell strips as described in Fig. 1. Symbols indicate that competition was +++, complete; ++, strong; +, marginal; or −, absent.

Competing agent	Competition with	
	p65	*gag* antigens
Cells (50 μg of protein)		
G-25/MI	+++	+++
JM (Jurkat)	−	−
Normal human T cells	−	−
MT 2	+	+++
Hut 102	+	+++
G-11/MJ	+	+++
Purified viruses (10 μg of protein)		
G-25/MI	−	+++
MT 2	++	+++
Hut 102	++	+++
G-11/MJ	++	+++
Virus-producer and nonproducer cells (500 μg)		
G-11/MJ	+++	+++
NIH82/C2 (HTLV-producer)	+++	+++
NIH82/15B (HTLV-infected but nonproducer)	−	−

fied as the precursor of the viral *gag* proteins $Pr54^{gag}$ (*11*). Moreover, antigens of about 41,000 and 80,000 daltons are detected by sera from some ATL patients or carriers but not by sera from normals.

A comparison of the intensities of the bands in Fig. 1A suggested that p65 is not related to *gag*. This was confirmed by the failure of large amounts of purified p24 (*9*) and p19 (*10*) to compete with p65 and by the failure of antibodies to p24 and p19 to bind to p65 (Fig. 1B). A competition RIA on G-25/MI cell strips, with glutaraldehyde-fixed G-25/MI cells as the competing agent, showed that p65, or a cross-reactive antigen, is located on the cell surface (Fig. 1C). The surface of the fixed cells, which cannot be penetrated by antibodies, selectively absorbed antibodies to p65 and to some *gag*-related antigens, while leaving others uninfluenced, among them antibodies to a nonmembrane antigen of 28,000 daltons (*12, 13*), thus ascertaining that the competition is not based on nonspecific absorption.

Competition strip RIA's with cellular and viral extracts (Table 1) revealed that antigens that cross-react with p65 are found in all tested human cell lines producing HTLV-I, such as Hut 102 (*2*), MT 2 (*3*), and G-11/MJ (*6*) (Table 1). The cell line G-11/MJ, a T-cell line producing HTLV-I, was initiated from a patient with cutaneous T-cell lymphoma (*6*). In comparison to G-25/MI, the competition with p65 by these cell lines was weak. No competition was found with phytohemagglutinin-activated normal human T cells or with JM, a human T-cell line not infected by HTLV (*14*). In comparison with the HTLV-producing cells, the viruses purified from them competed much better, with the exception of the G-25/MI virus that predominantly consists of particles lacking the envelope, as judged from the banding pattern in sucrose gradient centrifugation (data not shown). Antigens that cross-react with p65 are thus enriched in the virus preparations. An HTLV-transformed nonproducer cell line, NIH 82-15B, which had been established from human bone marrow by cocultivation with G-11/MJ cells, did not compete with p65, whereas a virus-producer cell line, NIH 82-C2, established from cord blood by cocultivation with the same G-11/MJ cells, competed well. The presence of p65 is thus associated with the expression of viral structural proteins.

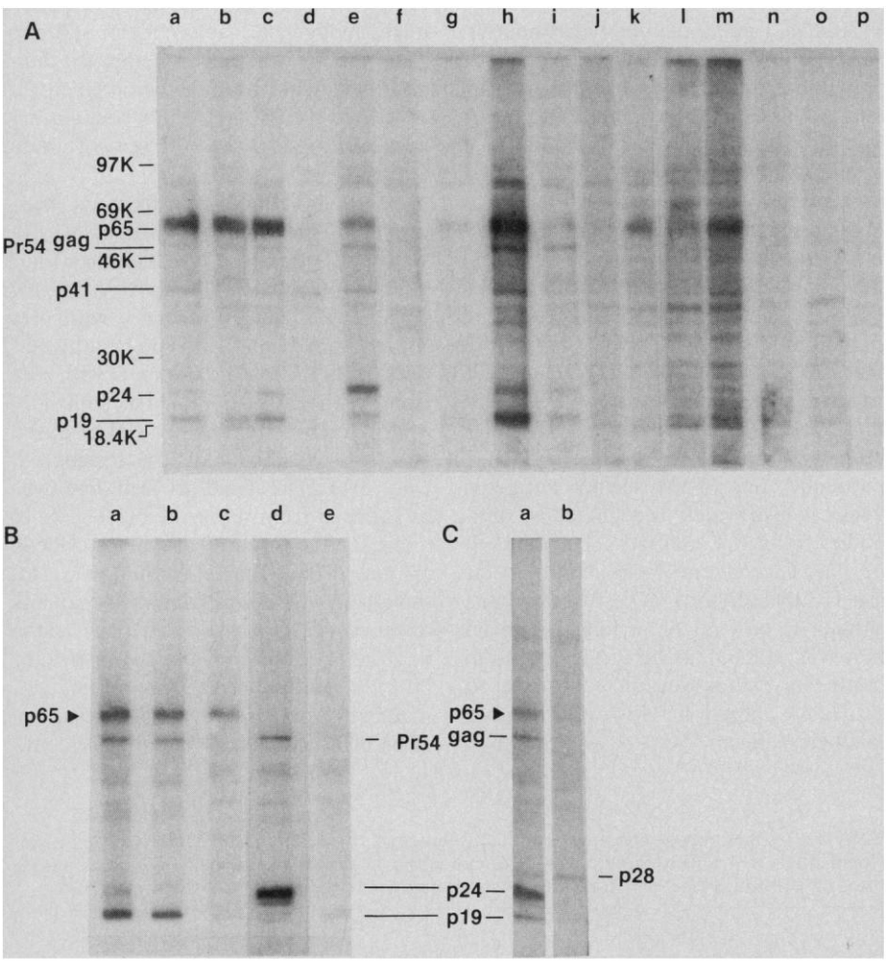


Fig. 1. (A) Recognition of p65 in HTLV-producer cells. Sera from patients with HTLV-positive ATL (lanes a to g), HTLV carriers (lanes h to m), and uninfected controls (lanes n to p) were analyzed in a strip RIA based on the Western blot technique (*8*), as described elsewhere (*11*). Briefly, lysates of G-25/MI cells were subjected to electrophoresis under reducing conditions on a preparative sodium dodecyl sulfate (SDS)–polyacrylamide slab gel (*17*) and transferred electrophoretically to a nitrocellulose sheet (*8*). Strips cut from the sheet were reacted with human test serum at a dilution of 1:500. Bound antibodies were made visible with radiolabeled goat immunoglobulin G directed against human immunoglobulin. (B) Unrelatedness of p65 to *gag*. Serum h [lane h in (A)] was diluted 1:1000 and used in strip RIA either without competing material (lane a) or after being incubated for 3 hours at 37°C with 30 μg of purified HTLV p24 (lane b) or p19 (lane c). No competition with p65 was found. Furthermore, p65 was not detected by an affinity-purified goat antibody to p24 (lane d) or by a monoclonal antibody to p19 (lane e). (C) Localization of p65 cross-reactive antigen on the cell surface. A human serum diluted 1:500 was used either without competing material (lane a) or after being incubated for 3 hours at 37°C with 5×10^7 glutaraldehyde-fixed (*18*) G-25/MI cells (lane b). Antibodies to p65 and *gag*-related proteins were specifically absorbed by the cell surfaces, whereas antibodies to an antigen of 28,000 daltons not present on the cell surface and not related to *gag* (*12*) remained unaffected.

To determine the nature of the p65–cross-reactive antigen (or antigens) in viruses, we made use of the observation that the G-25/MI virus absorbed all *gag*-related antibodies from serum h (lane h in Fig. 1A) while leaving antibodies to p65 uninfluenced (see Table 1). This absorbed serum was used to detect the antigens related to p65 on strips made from G-11/MJ virus, which was a good competitor for p65 (Table 1). The unabsorbed serum (lane b in Fig. 2A) detected p19, p24, and antigens of 32,000, 36,000, and 54,000 daltons, all of which are *gag*-related (*11*). In addition, two fainter bands of 46,000 and 51,000 daltons were detected, but no band of 65,000 daltons. The preabsorbed serum (lane c in Fig. 2A) detected two bands only, p46 and p51. Neither of these antigens was reactive with normal human serum (lane a in Fig. 2A). Thus, at least one of them had to account for the competition with p65 G-25/MI cell strips.

Table 1 also shows the presence of material that cross-reacts with p65 in MT 2 cells and MT 2 virus. A glycoprotein extract made from MT 2 cell culture fluids by adsorption to and elution from lentil lectin–agarose completely blocked the detection of p65 on G-25/MI cell

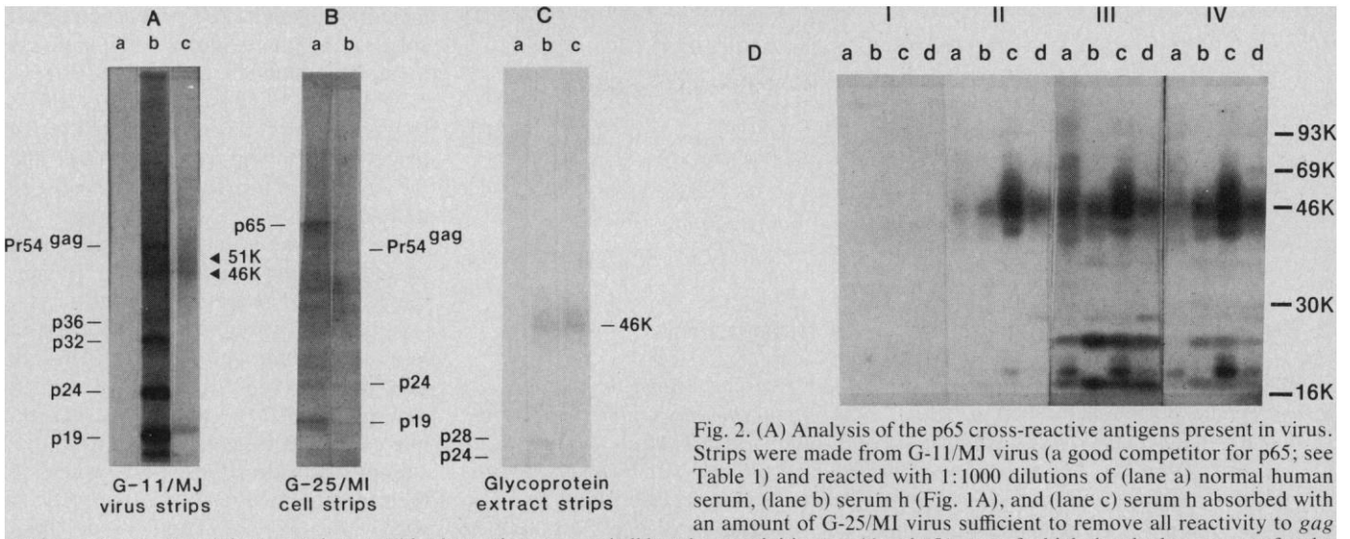


Fig. 2. (A) Analysis of the p65 cross-reactive antigens present in virus. Strips were made from G-11/MJ virus (a good competitor for p65; see Table 1) and reacted with 1:1000 dilutions of (lane a) normal human serum, (lane b) serum h (Fig. 1A), and (lane c) serum h absorbed with an amount of G-25/MI virus sufficient to remove all reactivity to *gag* while leaving the reactivity to p65 intact. This absorption removed all but the reactivities to p46 and p51, one of which thus had to account for the cross-reactivity to p65. (B) Competition with p65 by a glycoprotein extract. Strips made from G-25/MI cells were reacted with a 1:1000 dilution of serum h (Fig. 1A) either without competing material (lane a) or absorbed with 100 μl of a glycoprotein extract made from the cell culture fluids of MT 2 cells (lane b). This extract completely blocked the antibodies to p65. (C) Analysis of the p65 cross-reactive antigens in the MT 2 glycoprotein extract. Strips were made from the glycoprotein extract used in (B) and reacted with 1:1000 dilutions of (lane a) normal human serum, (lane b) serum h of Fig. 1A, and (lane c) serum h incubated with the G-25/MI virus to make it "monospecific" for p65. The only antigen not affected by this absorption is a glycoprotein of 46,000 daltons, thus responsible for the cross-reactivity with p65. (D) gp46 is a consistent component of purified viruses. Virus from the HTLV-I producer cell lines (lanes a) G-11/MJ, (lanes b) NIH 82-C2, (lanes c) Hut 102, and (lanes d) MT 2 was subjected to electrophoresis on an SDS–polyacrylamide gel and analyzed by Western blot with human sera diluted 1:1000. (I) Normal human serum; (II to IV) sera from ATL patients (III is serum e and IV is serum a of Fig. 1A).

strips (Fig. 2B). The glycoprotein responsible for the competition was shown to have a molecular size of 46,000 daltons by the technique used above. Unabsorbed serum incubated with strips made from the glycoprotein extract (Fig. 2C) strongly reacted with an antigen of 46,000 daltons (gp 46) and faintly with antigens of 24,000 and 28,000 daltons (lane b in Fig. 2C), the latter two of them reactive with antibody to p24 (not shown). When incubated with G-25/MI virus, the serum reacted with gp 46 only (lane c in Fig. 2C), which is therefore responsible for the competition with p65. Experiments with the viruses purified from four different cells—G-11/MJ, NIH82-C2, Hut 102, and MT 2—showed that all of them have a strongly reactive gp46 (Fig. 2D).

Experiments analogous to those described in Fig. 2 were also done with strips made from Hut 102 cells. The serum rendered unreactive to *gag* still recognized a band comparable in intensity to p65 but somewhat smaller (not shown). This antigen, called p61 or gp61, is a cell surface glycoprotein unrelated to *gag* and the principal target of antibodies from patients with ATL and AIDS (*4, 7*). Our data show that p61 of Hut 102 and p65 of G-25/MI cells are homologous proteins.

In conclusion, we have established a relationship of homology between surface antigens of 61,000 and 65,000 daltons present in human T cells producing HTLV-I, but absent in HTLV-I–transformed nonproducer cells. Cross-reactive antigens are enriched in viruses purified from these cells. Taken together, the findings strongly suggest a viral origin of these antigens, though not definitely excluding the possibility that the presence of viral protein in the cell might lead to the expression of a cellular p65, which then copurifies with the virus. However, this theoretical possibility is rendered unlikely by the demonstration that the cross-reactive antigen in virus is not p65, but a glycoprotein of 46,000 daltons that contains all of the highly immunogenic determinants of p65 (Fig. 2B). These findings are typical for the products of the envelope gene (*env*) of animal retroviruses (*15*). The *env* gene codes for a glycosylated precursor, which is then cleaved into the larger, highly immunogenic, main envelope glycoprotein and the smaller transmembrane glycoprotein, which is less immunogenic. Therefore, p65 and p61 probably represent the viral envelope precursor, which is readily detected in the cellular extracts because of the immunogenic envelope glycoprotein it contains. In the virus, the envelope glycoprotein may not be detected at the high serum dilution used. Alternatively, p65 or p61 may represent the major *env* glycoprotein, and the gp46 found in the virus may be a breakdown product of p65 and p61, as suggested for glycoproteins of 45,000 daltons often found in animal retrovirus preparations (*16*). In either case, the interpretation indicates a viral origin of p65 and p61.

JÖRG SCHÜPBACH
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205
M. G. SARNGADHARAN
Department of Cell Biology, Litton Bionetics, Inc., Kensington, Maryland 20895
R. C. GALLO
Laboratory of Tumor Cell Biology, National Cancer Institute

References and Notes

1. For recent reviews, see R. C. Gallo and M. Essex, Eds., *Cancer Cells*, vol. 3, *Human T-cell Leukemia Viruses* (Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., in press).
2. B. J. Poiesz, F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980).
3. I. Miyoshi, I. Kubonishi, S. Yoshimoto, Y. Shiraishi, *Gann* **72**, 978 (1981).
4. M. Essex *et al.*, *Science* **220**, 859 (1983).
5. R. C. Gallo *et al.*, *ibid.*, p. 865; E. P. Gelmann *et al.*, *ibid.*, p. 862.
6. M. Popovic *et al.*, *ibid.* **219**, 856 (1983).
7. M. Essex *et al.*, *ibid.* **221**, 1061 (1983).
8. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
9. V. S. Kalyanaraman, M. G. Sarngadharan, P. A. Bunn, J. D. Minna, R. C. Gallo, *Nature (London)* **294**, 271 (1981).
10. V. S. Kalyanaraman, M. Jarvis-Morar, M. G. Sarngadharan, R. C. Gallo, *Virology*, in press.
11. J. Schüpbach *et al.*, in preparation.
12. This 28,000-dalton protein was also found in another HTLV-infected cell line, G-11/MJ, and is, in contrast to a *gag*-related p28 of Hut 102 (*4*) and MT 2 (*13*) cells, unrelated to *gag* (*11*), and not part of the cell membrane, as shown by competition RIA.

13. N. Yamamoto and Y. Hinuma, *Int. J. Cancer* **30**, 289 (1982).
14. U. Schneider, H.-U. Schwenk, G. Bornkamm, *ibid.* **19**, 621 (1977).
15. For a review, see J. R. Stephenson, in *Molecular Biology of RNA Tumor Viruses*, J. R. Stephenson, Ed. (Academic Press, New York, 1980), p. 245.
16. J. H. Elder, J. W. Gautsch, F. C. Jensen, R. A. Lerner, J. W. Hartley, J. W. Rowe, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4676 (1977); H. Marquardt, R. V. Gilden, S. Oroszlan, *Biochemistry* **16**, 710 (1977); M. J. Krantz, M. Strand, J. T. August, *J. Virol.* **22**, 804 (1977).
17. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
18. J. Schüpbach *et al.*, *Blood* **62**, 616 (1983).
19. J.S. is a Fogarty International Fellow of the National Cancer Institute and also supported by the Swiss National Science Foundation.

12 December 1983; accepted 12 March 1984

A Pure Enzyme Catalyzing Penicillin Biosynthesis

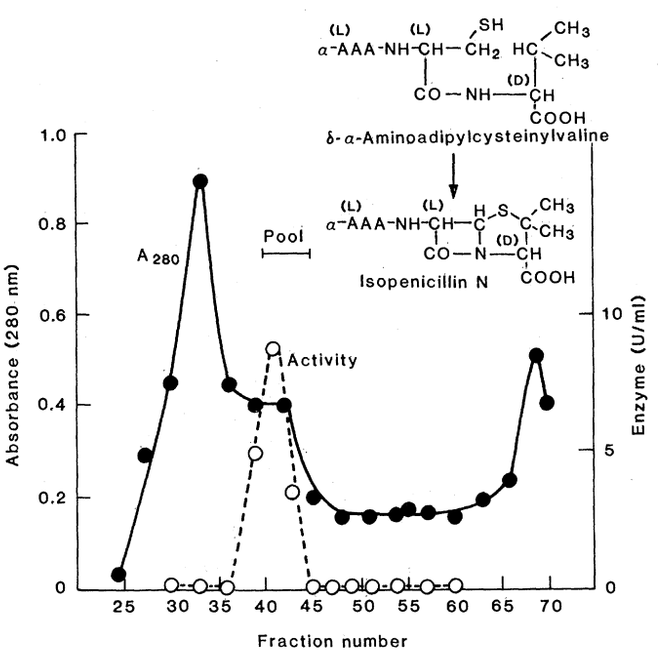
Abstract. *Isopenicillin N synthetase (cyclase) has been purified to homogeneity from* Cephalosporium acremonium *strain C-10. The enzyme has a molecular weight of 40,000 to 42,000 and yields a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The enzyme was purified in 10 percent yield by a combination of protamine sulfate and ammonium sulfate precipitations, gel filtration, and ion-exchange high-performance liquid chromatography. The purified enzyme can be stabilized with sucrose and stored at −20°C for several weeks without any loss in activity.*

None of the enzymes in the pathway from penicillin N to cephalosporin C (*1*) has been completely purified from any organism producing β-lactam antibiotics. We now describe our purification of a penicillin-forming enzyme isopenicillin N synthetase (cyclase) that converts the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine to isopenicillin N in the presence of O_2, Fe^{2+}, and ascorbate. We have been able to purify the enzyme to a homogeneous protein as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (*2*).

Slant cultures of *Cephalosporium acremonium* strain C-10 (*3*) were prepared on CM medium (percent: sucrose, 2; yeast extract, 0.4; peptone, 0.4; $NaNO_3$, 0.3; K_2HPO_4, 0.05; KH_2PO_4, 0.05; KCl, 0.05; $MgSO_4 \cdot 7H_2O$, 0.05; $FeSO_4 \cdot 7H_2O$, 0.001; and agar, 2) and were incubated at 25°C with 60 ± 10 percent humidity for 7 days. Slant growth, suspended in water, was added to a 500-ml Erlenmeyer flask containing 50 ml of C′ seed medium [percent: cornstarch, 4; corn steep liquor, 3; soybean meal, 1; $(NH_4)_2SO_4$, 0.1; $CaCO_3$, 0.3; and methyl oleate, 4.8 by volume]; the *p*H of the medium was adjusted to 7.0, and the mixture was autoclaved 40 minutes. The seed flask was incubated on a rotary shaker (50-mm throw, 25°C, 60 ± 10 percent humidity) for 54 hours. The seed culture (1.2 ml) was then added to 500-ml flasks containing 30 ml of fermentation medium (percent: sucrose, 2; cornstarch, 3; beet molasses, 5; soybean meal, 6; ammonium acetate, 0.8; $CaSO_4$, 1.25; $CaCO_3$, 0.5; and methyl oleate, 3 by volume); the *p*H was adjusted to 6.4 before autoclaving for 40 minutes, and the flasks were incubated as above for 60 hours.

The mycelia in each flask were harvested by centrifugation (8000*g*, 10 minutes), washed twice with 20 ml of ice-cold distilled water, suspended in 10 ml of ice-cold buffer A (50 m*M* tris-HCl, 10 m*M* $MgSO_4$, 10 m*M* KCl; *p*H 7.4 at 25°C) and sonicated (*4*). Cell debris was removed by centrifugation. Phenylmethylsulfonyl fluoride (PMSF) was added to the supernatant (1 m*M*), and the solution was frozen at −70°C until used. Upon thawing, fresh PMSF was added (1 m*M*) and the suspension was centrifuged at 20,000*g* (10 minutes, 2°C) to remove proteins precipitated in the cold.

The crude extract (70 ml from seven flasks) was treated (*5*) with protamine sulfate to remove nucleic acids and with solid $(NH_4)_2SO_4$ (enzyme grade) between 40 and 60 percent of saturation to remove other proteins. The pellet was resuspended in 2 to 4 ml of buffer A, centrifuged (20,000*g*, 10 minutes, 0°C), and placed in an ice bath during collection of the supernatant by pipette (some oily material that did not dissolve remained as a pellet as long as the temperature was kept low). The supernatant was then applied to an LKB Ultrogel AcA 54 column (1.6 by 90 cm) in buffer A at 4°C. Fractions were collected in tubes to which 50 percent sucrose (enzyme



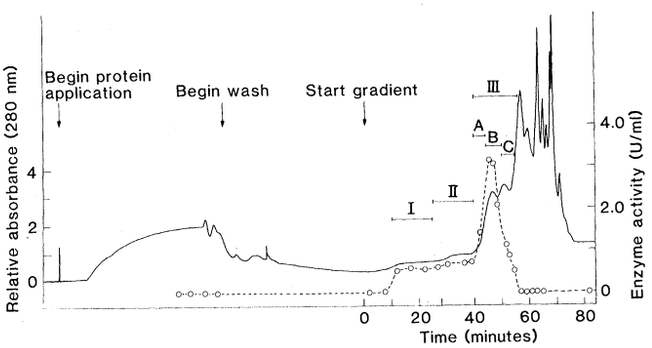


Fig. 1 (left). Gel filtration on LKB Ultrogel AcA 54. Fractions (40 drops = 2.3 ml) were collected at 10 ml/hour. Scheme depicting the reaction catalyzed by the enzyme is shown in the upper part of the figure. Fig. 2 (right). Ion exchange by HPLC (Waters Protein-Pak DEAE-5 PW). Diluted, *p*H-adjusted enzyme (40 ml) was applied at 1.0 ml/min. Detection of absorbency at 280 nm was by Waters model 440 absorbance detector set at 0.2 absorbance units full-scale. Enzyme was eluted by a 1-hour gradient (No. 10 on Waters model 660 solvent programmer).