Interaction between the human T-cell lymphotropic virus type III_B envelope glycoprotein gp120 and the surface antigen CD4: Role of carbohydrate in binding and cell fusion

(acquired immunodeficiency syndrome/retrovirus)

THOMAS J. MATTHEWS*, KENT J. WEINHOLD*, H. KIM LYERLY*, ALPHONSE J. LANGLOIS*, HANS WIGZELL†, AND DANI P. BOLOGNESI*

*Department of Surgery, Duke University Medical Center, Durham, NC 27710; and †Department of Immunology, Karolinska Institutet, S-110401 Stockholm, Sweden

Communicated by Paul Zamecnik, March 30, 1987

ABSTRACT Interactions between retroviruses associated with acquired immunodeficiency syndrome and their receptors on lymphocytes represent the initial steps in the process of infection and are also involved in multinucleated giant cell formation, which is one form of virus-mediated cytopathology. The exterior envelope glycoprotein of the retrovirus has been identified as gp120, and we demonstrate here that purified gp120 binds directly to cells expressing the CD4 (T4) surface antigen at a site spatially related to that recognized by the OKT4A monoclonal antibody. The gp120 was also able to temporarily interfere with viral infection and to block the process of multinucleated giant cell formation. However, if the carbohydrate chains were removed from gp120 by enzymatic treatment, CD4 binding and blockade of cell fusion was reduced by about a factor of 50. The significance of these results in relation to preventive and interventive approaches for acquired immunodeficiency syndrome is discussed.

Human immunodeficiency virus is the generic name for a family of lymphotropic retroviruses (1) whose primary target is the helper/inducer subset of T-lymphocytes bearing the CD4 cell surface marker (2-6). The actual mechanism by which the virus infects susceptible target cells is beginning to be understood. Quite remarkably, monoclonal antibodies to certain epitopes of CD4 can block infection (7-9) and can immunoprecipitate complexes of CD4 and gp120, the major envelope glycoprotein of the infecting virus (10). These results suggest that a key interaction between gp120 and CD4 initiates the infectious process.

One consequence of virus infection is the formation of multinucleated giant cells resulting from cell-cell fusion events (7). Studies by Lifson *et al.* (11) concluded that the CD4 antigen is an essential component in the reaction in which virus-negative cells bearing CD4 can fuse with infected cells. Recently, two studies (12, 13) have further delineated the requirements for cell fusion and have shown that the essential components can be limited to the expression of CD4 and virus envelope gene products on the respective cell surfaces.

These results, highlighting the importance of virus-receptor interactions in biological processes associated with virus infection, prompted us to examine more closely the nature of the association between gp120 and CD4. By using purified gp120, we have studied various aspects of its binding to appropriate target cells and addressed the role of its carbohydrate residues in these processes.

MATERIALS AND METHODS

Proteins. Endoglycosidase F (endo F; preparation 878740) was from Boehringer Mannheim. Monoclonal antibody 96-16 to human T-cell lymphotropic virus type III (HTLV-III) gp120 was provided by R. Ting (Biotech, Rockville, MD). Antibodies OKT4 and OKT4A were from Ortho Diagnostics, and fluorescein isothiocyanate-conjugated F(ab')₂ goat anti-mouse IgG was from Cooper Biomedical (Malvern, PA).

Cells. The CEM cells (14) were obtained from M. Herschfield, Department of Medicine, Duke University Medical Center, and were maintained in RPMI 1640/10% FCS. CEM cells chronically infected with either HTLV-III_B or HTLV-III_{RF} were derived from acutely infected cells and represent a subpopulation resistant to the cytopathological effects of the virus. Each of these cultures produced infectious virus over a 6-month observation period, and their level of cell surface gp120 as measured by anti-gp120 and cytofluorographic analysis was several fold that of their H9 counterparts (data not shown).

Virus. The HTLV-III_B and HTLV-III_{RF} virus isolates have been described (2, 15). The virus stocks were maintained and titrated for infectivity in the H9 cell line as described (16).

Virus Antigens. Viral gp120 was isolated from HTLV-III_B-infected H9 cells lysed with 0.5% Triton X-100 by utilizing immunoaffinity chromatography in a fashion similar to that described (17) except for additional chromatography on lentil lectin resin (Pharmacia) and elution with 0.1 M methyl-D-mannopyranoside (Sigma). The viral p24 was purified from the fraction that did not bind to the lectin by phosphocellulose ion-exchange chromatography (Schleicher & Schuell).

Cell Fusion Assay. About 7.5×10^4 CEM cells were incubated with 5000 HTLV-III chronically infected CEM cells in 96-well plates (one-half area cluster plates; Costar, Cambridge, MA) in a final volume of 100 μ l of growth media for 24 hr at 37°C. Within this time period, giant cell formation could be seen evenly dispersed throughout the surface of each well. These cells had a diameter in excess of 5-fold that of CEM or chronically infected CEM, as previously indicated (18), and appeared in numbers that were proportional to the inoculation of infected cells. The efficiency of the process was such that 10-50 infected cells were required to score as a fusion event. It is likely that many more fusion events occurred but became obscured by the rapidly developing islands of giant cells, which appeared to grow by recruiting cells around them. The number of giant cells per well was estimated by microscopic examination at a $\times 40$ magnifica-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HTLV-III, human T-cell lymphotropic virus type III; endo F, endoglycosidase F.

FIG. 1. Analysis of endo F digestion products of gp120 by NaDodSO₄/PAGE. The gp120 purified from HTLV-III_B infected H9 cells is shown in lane 1. Antigen ($\approx 4 \mu\text{g}$) was digested with 0.05 unit of endo F in 0.05 M sodium acetate buffer (pH 5.5) for 2 hr at 37°C in the presence (lane 2) or absence (lane 3) of 0.025% NaDodSO₄. Molecular mass standards are shown in lane 4. The samples were electrophoresed in a 12% polyacrylamide gel.

tion, which allowed the entire well to be visualized in a single field.

RESULTS

Removal of Carbohydrate from gp120. Three forms of the viral gp120 representing various degrees of glycosylation were tested for biological activity. The electrophoretic profile of the intact gp120 isolated from Triton X-100-lysed cells is

shown in Fig. 1, lane 1. As we have reported earlier (18), treatment of the antigen with the enzyme preparation endo F (19, 20) in the presence of low levels of NaDodSO₄ (Fig. 1, lane 2) yielded faster migrating polypeptides with apparent molecular masses of 58–60 kDa (DG120). This size range is consistent with that estimated from the amino acid sequence of the protein backbone (21) and suggests that the bulk of the carbohydrate residues were removed under these conditions. Endo F digestion in the absence of detergent resulted in an apparently discrete molecule of about 97 kDa (Fig. 1, lane 3), suggesting a specific cleavage of only a portion of the carbohydrate side chains.

Requirement of Glycosylation of gp120 for CD4 Binding.

The native and deglycosylated forms of gp120 were then examined for their ability to bind to CD4 using several experimental approaches. The CEM T-lymphocyte cell line was used as a target cell because of its high level of CD4 expression at the cell surface (22). Using a monoclonal antibody to gp120 as well as monoclonal antibodies OKT4 and OKT4A in conjunction with the fluorescence activated cell sorter, it was possible to demonstrate direct binding of gp120 to CD4 on the surface of CEM cells in two ways: (i) by enabling the binding of monoclonal anti-gp120 to the cell surface and (ii) by preventing the binding of OKT4A to CD4. The results (Fig. 2) demonstrated, first, that attachment of monoclonal anti-gp120 to CEM occurred only following preincubation with gp120. A control viral polypeptide, p24, did not mediate binding (Fig. 2I). Moreover, the binding of gp120 to CEM specifically blocked the epitope on CD4 recognized by OKT4A but not OKT4 (compare Fig. 2 A and B to Fig. 2 G and H). The fully deglycosylated form of gp120 (DG120) lost a major portion of its binding capacity to CEM as evidenced by both the lack of binding of monoclonal anti-gp120 (Fig. 2 C and F) and its failure to block the binding

FIG. 2. Binding of gp120 and deglycosylated gp120 to uninfected CEM cells. CEM cells (3×10^6 per ml) were incubated for 2 hr at 37°C with mock-digested (no enzyme) gp120 at 5 $\mu\text{g}/\text{ml}$ in the presence of 0.025% NaDodSO₄ (A–C), with endo F-digested gp120 in 0.025% NaDodSO₄ (D–F), or with p24 at 35 $\mu\text{g}/\text{ml}$ (G–I). The cells were washed in 10 vol of phosphate-buffered saline (PBS)/2% fetal calf serum (FCS)/0.1% sodium azide. Cells (1×10^6) were resuspended in PBS/FCS/azide containing a 1:20 dilution of OKT4 (A, D, and G), OKT4A (B, E, and H), or anti-gp120 monoclonal antibody 96-16 (C, F, and I) ($\approx 50 \mu\text{g}/\text{ml}$). Following a 1-hr incubation on ice, cells were washed in 40 vol of PBS/FCS/azide, and the cell pellets were resuspended in 100 μl of a 1:20 dilution of fluorescein isothiocyanate-conjugated F(ab')₂ goat anti-mouse IgG and were incubated on ice for 1 hour. Finally, cells were washed in ≈ 200 vol of PBS/FCS/azide and were resuspended to a concentration of 10^6 cells per ml. Cytofluorographic analysis was performed using an EPICS model 753 fluorescence-activated cell sorter (Coulter). The results are plotted as relative cell number (y-axis) vs. fluorescence intensity on a linear scale (x-axis). The numbers in the upper right portion of each panel indicate the percentage of positive cells/mean fluorescent channel.

of OKT4A (Fig. 2 *B* and *E*). This is in marked contrast to the partially digested molecule (Fig. 1, lane 3), which essentially retained its binding capability (not shown and see below). In other assays (not shown) the monoclonal anti-gp120 was found to bind DG120 with the same efficacy as the native molecule.

To further demonstrate these features, we took advantage of the fact that 125 I-labeled gp120 (Fig. 3, lanes 1 and 2) retains a high avidity for the CD4 molecule. In contrast, following treatment of the iodinated antigen with endo F, the major digestion product (again about 60 kDa as shown in Fig. 3, lane 3) displayed a dramatic reduction in binding strength (Fig. 3, lane 4). What did bind, however, was a minor component of the radiolabeled digest represented in a 97-kDa band (Fig. 3, lane 3), which is analogous to the partially digested unlabeled antigen noted in Fig. 1, lane 3. The possibility that the observed minimal binding of the fully deglycosylated molecule (Fig. 3, lane 4) to CD4 is caused by nonspecific factors associated with enzymatic treatment was excluded by the observation that OKT4A antibodies were able to block its residual binding (data not shown).

Group Specific Blockade of Fusion and Infection with HTLV-III gp120. We next investigated the ability of gp120 to interfere with the processes of virus infection and virus-mediated cell fusion. A sensitive and quantitative assay for cell fusion was made possible through the development of a stable line of CEM cells infected with HTLV-III_B. When infected CEM were mixed with their uninfected counterparts, a rapid formation of multinucleated giant cells occurred, which could be easily visualized and enumerated within a 24-hr period (18). Although purified gp120 alone was not able to mediate cell fusion when added to uninfected CEM, it was a very effective inhibitor of the fusion mediated by infected CEM cells. Its blocking effect was complete and titratable such that as little as 500 ng of gp120 per ml completely inhibited the process under the assay conditions used (Fig. 4). The partially deglycosylated antigen (gp97) showed a similar level of activity. In contrast, the fully deglycosylated form was approximately 50 times less effective. These results are consistent with the binding activities noted for each form of the antigen and suggest that some but not all of the carbohydrate present on gp120 is necessary for binding and biological activity. In a recent report, endo-

FIG. 3. Impact of deglycosylation on the binding of 125 I-labeled gp120 to CD4. Purified HTLV-III_B gp120 labeled with 125 I (Enzymobeads; Bio-Rad) was treated with endo F in a fashion similar to that described in Fig. 1. Control gp120 received identical treatment as endo F-treated gp120 except that the enzyme was omitted. The radiolabeled antigen preparations (50 μ l of gp120 at $\approx$ 50 ng/ml and 2×10^6 cpm/ml) were allowed to bind 1×10^6 CD4⁺ Jurkat T-leukemia cells. The cells were washed, and bound material was analyzed by NaDodSO₄/PAGE (K. Lundin and H.W., unpublished data). The iodinated gp120 preparation is shown in lane 1 and the portion of the sample that specifically bound to cells is shown in lane 2, while the enzymatically digested antigen is shown in lane 3 and the corresponding cell binding fraction is shown in lane 4.

FIG. 4. Test of the ability of gp120 and deglycosylated forms of gp120 to block multinucleated giant cell formation mediated by chronically infected CEM cells. Dilutions of test antigens were incubated with 7.5×10^4 uninfected CEM cells for 30 min at 37°C in a volume of 50 μ l. About 5000 CEM cells chronically infected with HTLV-III_B were added to each well, and the number of giant cells per well was estimated 24 hr later. Average counts in duplicate wells are shown. The four antigens tested were HTLV-III_B p24 (●), gp120 digested with endo F in the presence of NaDodSO₄ (■), mock digested (no enzyme) gp120 with the same level of NaDodSO₄ (○), and gp120 digested with endo F in the absence of NaDodSO₄ (□).

glycosidase digestion of intact virus in the presence of NaDodSO₄ had no effect on virus binding to CD4⁺ cells (23). Under the conditions utilized, however, the major envelope digestion product was apparently 85–92 kDa and thus similar to the intermediate 97-kDa binding form observed in this report. It is possible that complete digestion to the 58–60 kDa form is sterically prevented when the glycoprotein is attached to the virus surface.

It was also of interest to test the ability of the HTLV-III_B gp120 to prevent the fusion of CEM cells infected with other virus isolates. A stable CEM cell line infected with HTLV-III_{RF}, which exhibits a 20% divergence in the amino acid sequence of the gp120 portion of the envelope gene (24) was used for this purpose. As shown in Table 1, the gp120 of HTLV-III_B was equally effective in its ability to prevent cell fusion mediated by HTLV-III_B- and HTLV-III_{RF}-infected CEM. In a similar manner, gp120 of HTLV-III_B interfered with infection of CEM by both the HTLV-III_B and HTLV-III_{RF}, although the degree of interference relative to cell fusion was not as pronounced (Table 2). This difference in relative blocking efficacy of the two processes is analogous to that reported for anti-CD4 antibodies in the inhibition of syncytia formation versus infection by virus pseudotypes (7). At first hand, this observation raises the question of infection by mechanisms not requiring prior attachment to CD4. However, it is also likely that continued synthesis and expression of CD4 on the target cell eventually produces unencumbered receptors that allow virus to infect. It is also

Table 1. Generic blockade of cell fusion with HTLV-III_B gp120

Day	gp120	Giant cells, no.	
		CEM/III _B	CEM/III _{RF}
2	–	73	64
	+	0	0
4	–	55	85
	+	0	0

About 5000 CEM cells chronically infected with either HTLV-III_B or HTLV-III_{RF} were added to duplicate wells containing 7.5×10^4 uninfected CEM cells in the absence or presence of HTLV-III_B gp120 (5 μ g/ml). The average number of giant cells in duplicate wells was enumerated.

Table 2. Delay of infection with HTLV-III_B gp120

Day	gp120	p24, ng/ml	
		HTLV-III _B	HTLV-III _{RF}
6	—	30	1
	+	3	0
8	—	100	15
	+	74	1
9	—	ND	60
	+	ND	11
14	—	150	160
	+	170	110

About 7.5×10^4 CEM cells were mixed with 50 infectious units of either HTLV-III_B or HTLV-III_{RF} in the presence or absence of HTLV-III_B gp120 (5 μ g/ml) in a volume of 0.1 ml. The volume of each culture was doubled every 24 hr by addition of media. Virus infection was based on the level of viral p24 antigen detected in 100- μ l samples taken from the supernatant of the culture using a competition radioimmunoassay developed by DuPont. ND, not done.

conceivable that fewer free receptors are required for virus attachment and penetration than are needed to mediate the fusion event.

DISCUSSION

These studies allow us to make the following general conclusions about the processes of gp120-CD4 binding and human immunodeficiency virus-mediated cell fusion. First, the fusion assays described herein can be used effectively as models to study the interrelationship between gp120 and the CD4 molecule. We conclude, as others have suggested, that the interaction of these two entities is very specific. A gp120 concentration of only 2–4 nM was required to completely block the fusion process, which also suggests a high affinity for the CD4 antigen. This is in marked contrast to the deglycosylated form of gp120 as well as bacterially expressed recombinant peptides (18), which also failed to interact with CD4.

A second point of interest is the relationship between the domains on gp120 required for CD4 binding and those that are responsible for neutralization of virus infectivity by antibody. As shown here, glycosylation has a strong influence on the binding domains, but in other studies (18) carbohydrate played no detectable role in determining epitopes involved in virus neutralization. Furthermore, neutralizing antibodies thus far raised against gp120 or recombinant envelope glycoprotein have exhibited virus isolate specificity (16, 25). In contrast, the interference of fusion by gp120 (Table 1) was generic since CEM infected with HTLV-III_B and the distantly related HTLV-III_{RF} failed to fuse with normal CEM in the presence of purified gp120 from HTLV-III_B. Likewise, HTLV-III_B gp120 interfered with infection of both the homologous virus and the HTLV-III_{RF} (Table 2). Thus, the binding of gp120 to CD4 may occur through conserved regions of the molecule as has also been suggested in a recent study using monoclonal antibodies (9). Our results further indicate that the conformation of this binding site is strongly influenced by the integrity of carbohydrate side chains. On the other hand, the type-specific epitopes for neutralization might reside in more variable domains whose immunogenicity is independent of glycosylation. One way to rationalize these observations is based on the elegant picornaviral models (26, 27) in which conserved receptor binding sites reside in depressions (pits or canyons) inaccessible to antibody but rimmed by hypervariable sequences that are accessible to neutralizing antibody. If this model applies to the human immunodeficiency virus, the broad neutralizing activity noted in human sera (16, 25) might be explained by

exposure to multiple genomic variants introduced exogenously or generated over time within a single individual.

Additional investigations should be aimed at a more precise definition of the gp120 amino acid and sugar residues responsible for receptor binding, virus neutralization by antibody, and recognition by the cellular arm of the immune system. The availability of native and deglycosylated gp120 along with recombinant fragments and synthetic peptides should provide some clarification of these important processes in the pathogenesis of acquired immunodeficiency syndrome. It is also becoming more evident that the very nature of the gp120-CD4 interactions may present opportunities for development of both preventive and interventive approaches to the disease.

We thank R. Gallo for virus, L. Arthur for infected cells used for gp120 isolation, G. Robey for helpful discussions, and C. McDanal for technical assistance. This work was supported by Grant 1P01-CA-A143447-01 from the National Cancer Institute and the Swedish Cancer Society.

1. Coffin, J., Haase, A., Levy, J. A., Montagnier, L., Oroszlan, S., Teich, N., Temin, H., Toyoshima, K., Jarmus, H., Vogt, P. & Weiss, R. (1986) *Science* **232**, 697.
2. Popovic, M., Sarngadharan, M. C., Read, E. & Gallo, R. C. (1984) *Science* **224**, 497–500.
3. Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) *Science* **220**, 868–871.
4. Schupbach, J., Popovic, M., Gilden, R. V., Gonda, M. A., Sarngadharan, M. G. & Gallo, R. C. (1984) *Science* **224**, 503–505.
5. Klatzmann, D., Barre-Sinoussi, F., Nugeyre, M. T., Dauguet, C., Vilmer, E., Griscelli, C., Brun-Vezinet, F., Rouzioux, C., Gluckman, J. C., Chermann, J. & Montagnier, L. (1984) *Science* **225**, 59–63.
6. McDougal, J. S., Mawle, A., Cort, S. P., Nicholson, J. K., Cross, G. D., Scheppeler-Campbell, J. A., Hicks, D. & Sligh, J. (1985) *J. Immunol.* **135**, 3151–3162.
7. Dalglish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984) *Nature (London)* **312**, 763–766.
8. Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J. & Montagnier, L. (1984) *Nature (London)* **312**, 767–768.
9. Sattentau, Q. J., Dalglish, A. G., Weiss, R. A. & Beverley, P. C. (1986) *Science* **234**, 1120–1123.
10. McDougal, J. S., Kennedy, M. S., Sligh, J. M., Cort, S. P., Mawle, A. & Nicholson, J. K. A. (1986) *Science* **231**, 382–385.
11. Lifson, J. D., Reyes, G. R., McGrath, M. S., Stein, B. S. & Engleman, E. G. (1986) *Science* **232**, 1123–1127.
12. Sodroski, J., Groh, W. C., Rosen, C., Campbell, K. & Haseltine, W. A. (1986) *Nature (London)* **322**, 470–474.
13. Lifson, J. D., Feinberg, M. B., Reyes, G. R., Rabin, L., Banapour, B., Chakrabarti, S., Moss, B., Wong-Staal, F., Steimer, K. S. & Engleman, E. G. (1986) *Nature (London)* **323**, 725–728.
14. Hershfield, M. S., Fetter, J. E., Small, W. C., Bagnara, A. S., Williams, S. R., Ullman, B., Martin, D. W., Jr., Wasson, D. B. & Carson, D. A. (1982) *J. Biol. Chem.* **257**, 6380–6386.
15. Wong-Staal, F., Shaw, G. M., Hahn, B. H., Salahuddin, S. Z., Popovic, M., Markham, P., Redfield, R. & Gallo, R. C. (1985) *Science* **229**, 759–762.
16. Matthews, T. J., Langlois, A. J., Robey, W. G., Chang, N. T., Gallo, R. C., Fischinger, P. J. & Bolognesi, D. P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9709–9714.
17. Robey, W. G., Arthur, L. O., Matthews, T. J., Langlois, A., Copeland, T. D., Oroszlan, S., Bolognesi, D. P., Gilden, R. V. & Fischinger, P. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7023–7027.
18. Putney, S., Matthews, T. J., Robey, W. G., Lynn, D. L., Robert-Guroff, M., Mueller, W. T., Langlois, A. J., Ghayeb, J., Petteway, S. R., Elder, J., Fischinger, P. J., Wong-Staal, F., Gallo, R. C. & Bolognesi, D. P. (1986) *Science* **234**, 1392–1395.

19. Elder, J. H. & Alexander, S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4540–4544.
20. Plummer, T. H., Elder, J. H., Alexander, S., Phelan, A. W. & Tarentino, A. L. (1984) *J. Biol. Chem.* **259**, 10700–10704.
21. Allan, J. S., Coligan, J. E., Barin, F., McLane, M. F., Sodroski, J. G., Rosen, C. A., Haseltine, W. A., Lee, T. H. & Essex, M. (1985) *Science* **228**, 1091–1094.
22. Hoxie, J. A., Alpers, J. D., Rackowski, J. L., Huebner, K., Haggarty, B. S., Cedarbaum, A. J. & Reed, J. C. (1986) *Science* **234**, 1123–1127.
23. McDougal, J. S., Nicholson, K. A., Cross, G. D., Cort, S. P., Kennedy, M. S. & Mawle, A. C. (1986) *J. Immunol.* **137**, 2937–2944.
24. Starcich, B. R., Hahn, B. H., Shaw, G. M., McNeely, P. D., Modrow, S., Wolf, H., Parks, E. S., Parks, W. P., Josephs, S. F., Gallo, R. C. & Wong-Staal, F. (1986) *Cell* **45**, 637–648.
25. Weiss, R. A., Clapham, P. R., Weber, J. N., Dalglish, A. G., Lasky, L. A. & Berman, P. W. (1986) *Nature (London)* **324**, 572–575.
26. Rossmann, M. G., Arnold, E., Erickson, J. W., Frankenberger, E. A., Griffith, J. P., Hecht, H. J., Johnson, J. E., Kamer, G., Luo, M., Mosser, A. G., Rueckert, R. R., Sherry, B. & Vriend, G. (1985) *Nature (London)* **317**, 145–153.
27. Luo, M., Vriend, G., Kamer, G., Minor, I., Arnold, E., Rossmann, M. G., Boege, U., Scraba, D. G., Duke, G. M. & Palmenberg, A. C. (1987) *Science* **235**, 182–191.